

# Heterogeneity of Macrophages in Atherosclerosis

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## Abstract

### Keywords

- ▶ atherosclerosis
- ▶ macrophage
- ▶ monocyte
- ▶ inflammation
- ▶ therapy

Atherosclerosis is a prevalent inflammatory condition and a frequent cause of morbidity and mortality worldwide. Macrophages are among the key immune cells driving lesion formation in the arterial wall. They have therefore evolved as potential targets for therapeutic strategies. Understanding of the different macrophage phenotypes and functions seems to be of pivotal importance for the development of treatments to target these immune cells. This review highlights the complexity of the mononuclear phagocyte system and summarizes important features of macrophage biology contributing to atherosclerosis.

## Introduction

Atherosclerosis is a chronic inflammatory disease of the arteries resulting from metabolic dysregulation and a maladaptive immune response of the vessel wall. This leads to atherosclerotic plaque formation with narrowing of the arterial lumen and plaque rupture causing myocardial infarction and stroke. Due to their high prevalence, atherosclerosis-related pathologies are nowadays among the most important diseases worldwide.<sup>1</sup> This review focuses on the biology of macrophages therein.

## Origin and Phenotype of Lesional Macrophages—Moving Targets

Among all leukocytes in the arterial wall, macrophages account for about 30 to 50% of total CD45<sup>+</sup> cells. Their high abundance already suggests some relevance in the context of atherosclerosis.<sup>2–4</sup> Where do they originate? It has been text book knowledge for decades that bone marrow (BM)-derived monocytes extravasate at sites of atherosclerotic lesions where they differentiate to macrophages and phagocytose-modified low-density lipoprotein (LDL) particles. However, recent publications have illustrated a vast heterogeneity within macro-

phage populations, which is at least in part associated with differences in cell ontogeny, resulting in potentially distinct functions in atherosclerosis.<sup>4–6</sup> Due to comparable morphology and similarities in the expression of common phagocyte markers, an unequivocal identification of these subpopulations has remained challenging and so far required specific lineage tracing and fate mapping analysis<sup>7</sup> (▶ **Fig. 1**). By these means, several studies over the past years have shown that tissue-resident macrophages originate in relevant amounts from erythro-myeloid progenitors that arise in the yolk sac (YS)—an early endodermal embryonic structure.<sup>8–10</sup> Shortly after initiation of YS hematopoiesis, hematopoietic stem cells (HSCs) are also generated in the dorsal aorta and seed the fetal liver. At later stages of fetal development, hematopoiesis shifts to the BM compartment, giving rise to circulating monocytes and eventually tissue macrophages.<sup>6,11,12</sup> In mouse arteries, macrophages have a mixed origin from fetal and BM hematopoiesis.<sup>6</sup> However, the quantitative contribution in the arterial wall as well as their response to inflammatory cues, particularly in the process of atherosclerosis, has remained unclear. Further, potential functional consequences of this observation have remained unknown.

Due to early lineage specification, macrophages are provided with tissue-specific signatures already during fetal

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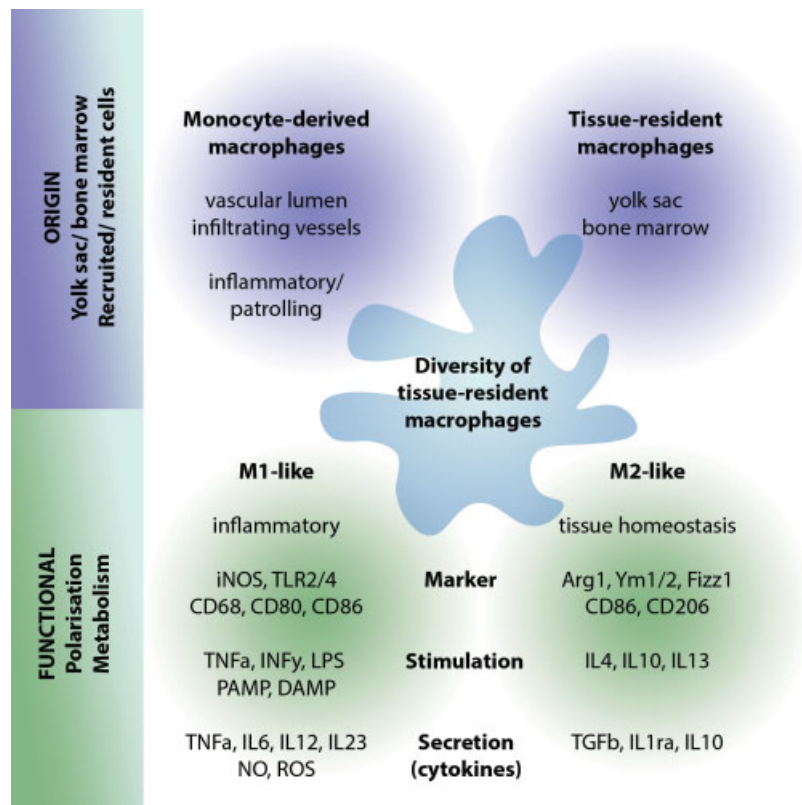
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**Fig. 1** Diversity of tissue-resident macrophages. Depicted are cellular origins and functional markers as well as stimulatory and secreted molecules of different macrophage subpopulations.

development.<sup>13</sup> These findings support the notion that ontogeny contributes not only to macrophage heterogeneity but also to functional differences of tissue (arterial) macrophages. However, the tissue environment continuously shapes the cellular identity of resident macrophages.<sup>14,15</sup> Thus, it is unknown to date whether cell ontogeny has functional consequences for macrophage biology in later life.

Besides these differences in macrophage origin, also the phenotypic diversity of macrophages within arteries has been greater than expected. Several pioneering studies recently identified three to five subsets of macrophages by ribonucleic acid (RNA) sequencing or mass cytometry.<sup>2–4</sup> We will discuss some of the molecular markers in the course of this review.

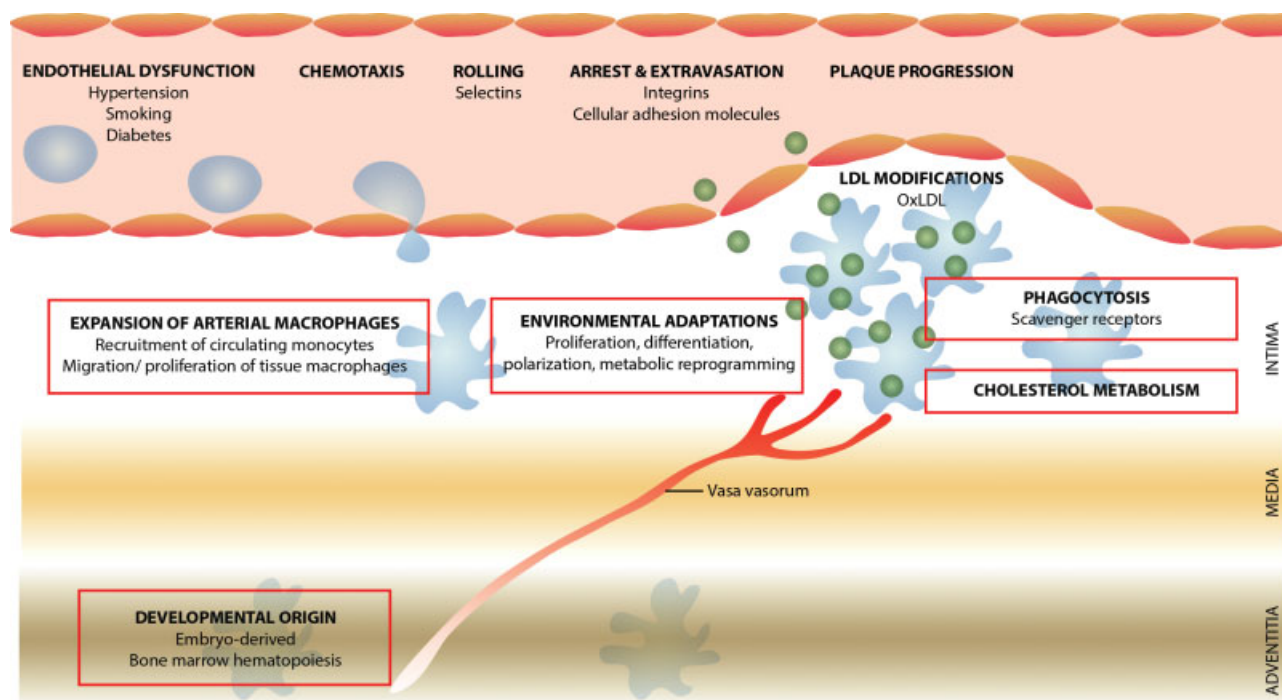
### Monocyte-Derived Macrophage Recruitment from the Circulation

The process of monocyte recruitment to inflamed arteries is facilitated by multiple mechanisms and is fundamental to the process of atherosclerosis.<sup>16,17</sup> On the one hand, oxidized LDL (oxLDL) stimulates the endothelium to express adhesion molecules, secrete macrophage colony stimulation factor (M-CSF), as well as granulocyte macrophage (GM)-CSF, and reduce the synthesis of nitric oxide (NO).<sup>18,19</sup> Monocytes, on the other hand, upregulate the expression of adhesion molecules, mainly involving different heterodimers of  $\beta$ 1 integrins, like very late antigen 4, and  $\beta$ 2 integrins, like lymphocyte function-associated antigen 1 and macrophage-1 antigen (Mac1)<sup>20</sup> (**► Fig. 2**).

The process of monocyte extravasation starts with P- and E-selectin-mediated rolling along the endothelium followed by intercellular cell adhesion molecule-, vascular cell adhesion molecule (VCAM)-, and integrin-dependent firm adhesion.<sup>21–23</sup> Subsequent transendothelial migration requires C-C motif chemokine receptor (CCR) 2 which interacts with monocyte chemoattractant protein 1 (MCP1/CCL2). Additionally, CCR5 and CX3C chemokine receptor (CX3CR) 1 are involved. Therefore, it is not surprising that deficiency of these receptors has been associated with a significant reduction in atherosclerosis.<sup>24</sup> Within the intimal layer, BM-derived monocytes differentiate to macrophages under the influence of M-CSF/GM-CSF, where they proliferate and sustain the sterile inflammation.<sup>25</sup> An additional recruitment of monocytes through plaque-infiltrating blood vessels from the adventitial side is currently under investigation. Notably, especially human atherosclerotic plaques are characterized by a dense vascularization<sup>26,27</sup> (**► Fig. 2**).

It is of fundamental importance that not only monocyte-derived but also resident macrophages in the vascular wall, which include macrophages of fetal origin, are recruited to sites of intimal oxLDL accumulation. Being present in the vessel wall already in steady state, they can proliferate upon activation to provide an effective machinery for cholesterol phagocytosis.<sup>28,29</sup> As foam cells become increasingly immobile, the inflammatory process is locally enhanced.<sup>30</sup>

Unfortunately, cellular markers of mononuclear phagocytes are often overlapping and make an unequivocal identification of different macrophage subclasses challenging.



**Fig. 2** Macrophages in the context of atherosclerosis. Depicted are major steps during the pathogenesis of atherosclerosis which involve macrophages. Key aspects of this review are highlighted in red.

This becomes increasingly difficult as vascular smooth muscle cells (VSMCs) and pericyte-like cells display various degrees of plasticity and can upregulate markers on their surface that are commonly related to the macrophage lineage.<sup>31</sup> There is significant evidence of VSMC-to-macrophage transdifferentiation *in vivo*, which is associated with clonal expansion and downregulation of VSMC markers.<sup>32</sup> Further, VSMCs and also endothelial cells are capable of phagocytosing some modified LDL particles.<sup>33</sup> Recent data on the transcriptional landscape of aortic macrophages supports this heterogeneity and points to yet undefined subpopulations.<sup>4</sup> A detailed understanding of the phenotype and function of all cells contributing to the mononuclear phagocyte lineage in the arterial wall will be essential for a precise understanding of atherogenesis and the development of targeted therapies.

### Adhesion Receptors as Potential Therapeutic Targets

An obvious approach to limit foam cell formation and development of atherosclerotic plaques would be the inhibition of monocyte/macrophage adhesion and extravasation. In animal studies, an inhibition of the adhesion receptor VCAM reduced atherosclerosis.<sup>34,35</sup> However, this did not translate well into humans as demonstrated in the Aggressive Reduction of Inflammation Stops Events study which aimed to reduce major cardiac events by a VCAM inhibitor.<sup>36,37</sup> In a similar vein, targeting selectins or integrins was not applicable to humans as selective therapy for atherosclerosis. Interestingly, therapeutic approaches aiming at cytokine-mediated pathways which have been linked to certain macrophage subpopulations

have shown some promising results. For example, CCR2 is typically expressed in BM-derived monocytes and is essential for monocyte extravasation in atherosclerotic lesions. In this regard, small interfering RNA-mediated silencing of CCR2 reduced monocyte infiltration in atherosclerotic plaques.<sup>38</sup> Potentially, a specific treatment of macrophage subpopulations might represent a useful approach to limit atherosclerosis while maintaining other macrophage functions.

### Macrophage Polarization in the Context of Atherosclerosis

Macrophages can not only be distinguished by their origin, but also by their functional differentiation pattern, which is also referred to as macrophage polarization. In analogy to T1 and T2 helper cells, macrophages have been classified in M1 and M2 subpopulations. Today, this classification seems to be oversimplified, since macrophage characteristics vary on a continuum between the classical M1 and M2 phenotypes and they are significantly influenced by the local microenvironment. Still, this conventional classification helps to summarize different macrophage characteristics and macrophage subclasses which are nowadays referred to as M1- or M2-like<sup>39</sup> (→ Fig. 1).

M1-like macrophages are classically activated by tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interferon  $\gamma$ , and lipopolysaccharides (LPS) as well as other pathogen-associated molecular pattern (PAMP) and danger-associated molecular pattern (DAMP) signaling cascades. Upon activation, these cells secrete TNF $\alpha$ , interleukin (IL) 1 $\beta$ , IL6, IL12, IL23, NO, and reactive oxygen species (ROS) as well as multiple chemokines.<sup>40</sup> M1-like macrophages are characterized by the expression of inducible NO synthase, Toll-like receptor (TLR) 2 and 4, as well as CD68,

CD80, and CD86. These characteristics of M1-like macrophages are in line with their predominant role in pathogen defense including phagocytosis and destruction of foreign bodies as well as antigen presentation to T cells, triggering an adaptive immune response<sup>41–43</sup> (► **Fig. 1**).

M2-like alternatively activated macrophages are stimulated mainly by IL4, IL10, and IL13. They are characterized by the expression of arginase 1 (Arg1), mannose receptor C type 1 (CD206), chitinase-like protein 3 (Chil3, Ym1), Chil4 (Ym2), and resistin-like alpha (Fizz1) as well as CD86. Arg1 and Fizz1 expression are dependent on signal transducer and transcription activator 6 (STAT6) signaling.<sup>44</sup> Major secreted molecules are IL1 receptor antagonist, IL10, transforming growth factor  $\beta$  (TGF $\beta$ ), as well as multiple chemokines including C-C motif chemokine 17 (CCL17), CCL18, CCL22, and CCL24.<sup>43</sup> M2-like macrophages can be further subdivided in several subclasses with partially overlapping functions. In principle, all M2-like macrophages are characterized by a rather immunosuppressive phenotype as well as predominant functions in tissue repair and homeostasis.<sup>41–43</sup> M2 polarization has been associated with expression of the triggering receptor expressed on myeloid cells-2 (TREM-2).<sup>45</sup> In recent studies, using single-cell RNA sequencing, TREM-2<sup>high</sup> macrophages were linked to the population of foamy macrophages<sup>4,46</sup> (► **Fig. 1**).

M1-like macrophages are rather inflammatory and their appearance is associated with high-risk plaques, while M2-like macrophages seem to be predominantly present in stable plaques and the adventitia.<sup>40,47</sup> Expression of STAT6 has been described to cause a shift from macrophages with an inflammatory M1-like to a more stable M2-like phenotype.<sup>44</sup> Also, environmental circumstances are capable of influencing the equilibrium between M1- and M2-like activated macrophages since incubation with LDL causes a proinflammatory shift in macrophage subpopulations.<sup>48,49</sup> Kadl et al even described an entirely new macrophage subpopulation upon oxLDL exposure in mice—the so-called Mox macrophages. These cells are proatherogenic and characterized by a decreased phagocytic and chemotactic capacity in a nuclear factor like 2-dependent manner.<sup>50</sup> Moreover, also MhB macrophages have been described in atherosclerotic lesions as they develop upon exposure to the heme products emerging from intraplaque hemorrhage of infiltrating blood vessels.<sup>51,52</sup> They express high levels of CD163 as well as CD206 and they are resistant to cholesterol accumulation due to increased expression of cholesterol efflux receptors like the adenosine triphosphate (ATP)-binding cassette transporters (ABC), ABCA1 and ABCG1.<sup>53</sup> Additionally, heme-dependent macrophage subpopulations have also been associated with efficient phagocytosis of extravasated erythrocytes (erythrophagocytosis).<sup>54</sup>

In the context of atherosclerosis, another new subpopulation was named M4-like macrophages as their phenotype is somewhere in between the continuum of M1 and M2 characteristics and since they are stimulated by platelet-derived CXCL4. They express TNF $\alpha$ , IL6, matrix metalloproteinase (MMP) 7, and MMP12 as well as S100 calcium-binding protein A8.<sup>55,56</sup> Interestingly, the expression of CD163 is irreversibly lost in these cells leading to a proatherogenic phenotype and advanced plaque morphology.<sup>40,55,57</sup>

## Novel Approaches to Characterize Macrophage Subpopulations

The enormous complexity in today's macrophage populations have been summarized by specialists in the field in order to provide a clear overview with all different subclasses.<sup>58</sup> Several studies now try to specifically address macrophages subpopulations.<sup>59</sup> Moreover, novel transcriptome-based experiments strive to further characterize and classify these diverse populations<sup>2,4,60</sup> (► **Fig. 1**). Cole et al identified five different macrophage subpopulations in atherosclerotic aortas by mass cytometry,<sup>2</sup> whereas Cochain et al distinguish three subpopulations identified by single-cell RNA sequencing.<sup>4</sup> Both groups reported a shift towards inflammatory monocyte-macrophage populations under high fat diet.<sup>2,4</sup> In a similar approach, using single-cell RNA sequencing and mass cytometry Winkels et al identified one macrophage population by RNA sequencing and three populations by mass cytometry. Interestingly, lymphocyte antigen 6 complex positive inflammatory monocytes seemed to be decreased in disease, while the populations of macrophages increased by 110%.<sup>3</sup> It is still unclear if infiltrating monocytes, which differentiate to macrophages, or local proliferation of tissue resident macrophages, is the major trigger for cell pool expansion.<sup>61</sup>

All three studies identified a predominant group of macrophage clusters, which is found under physiological conditions and usually referred to as resident macrophages. These cells express high levels of lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1)<sup>3,4</sup> and CD206<sup>2,4</sup> when compared to macrophage clusters in atherosclerotic lesions. Interestingly, also several previous studies have attributed these markers to tissue-resident macrophages.<sup>6,62,63</sup> M-CSF 1 receptor (CSF1R) has been shown to interact with LYVE1 to regulate arterial stiffness by controlling collagen expression in VSMCs.<sup>63</sup> Moreover, an inhibition of CSF1R significantly reduced the number of resident macrophages without affecting monocytes in the blood or aorta.<sup>63,64</sup> M-CSF and CSF1R seem to selectively influence and control tissue-resident macrophages without affecting inflammatory reactions mediated by monocytes. This therapeutic approach might therefore be useful for targeted interventions and is even discussed for cancer therapies.<sup>65</sup>

In contrast, macrophage populations in atherosclerotic lesions are characterized by a shift towards inflammatory characteristics, which have previously been described as M1-like.<sup>2,4</sup> Interesting candidates characterizing these clusters are CD14, nucleotide oligomerization domain-like receptor family, pyrin domain containing 3 (NLRP3), and IL1 $\beta$ .<sup>2,4</sup> However, the quantitative contribution of expanding resident macrophages versus infiltrating inflammatory cells remains unclear, and is even contradictory in the three latest phenotypical screens as Winkels et al observed a decrease of inflammatory monocytes in response to western diet, while the two other studies described an increase of this respective immune cell population.<sup>2–4</sup>

Future therapeutic approaches might specifically target macrophage plasticity and polarization. Markers identified

by phenotypical clustering using RNA sequencing and mass cytometry show clear analogies to conventional M1- and M2-like macrophages.<sup>2–4</sup> Potential interventions could be designed to modulate the shift between M1- and M2-like polarization states. Further, targeting of specific macrophage subpopulations may offer to intervene with lesion formation and avoiding adverse effects on immunological defense mechanisms (e.g. against invading pathogens).

### Macrophage Immunometabolism—An Emerging Target

Recent evidence supports the notion that macrophage functions are closely intertwined with their metabolic features.<sup>66,67</sup> In a simplified perspective, M1-like macrophages utilize glycolytic metabolism while M2-like macrophages feature mitochondrial oxidative phosphorylation and fatty acid metabolism. Consequently, glycolysis inhibition blocks classical activation of primary monocytes and monocytic cell lines.<sup>68</sup> Thus, specific macrophage effector functions could be regulated through distinct metabolic pathways.<sup>69</sup> However, drug targeting of metabolic pathways has been challenging, even *in vitro*, due to off-target effects and challenges of dosing.<sup>70</sup> Thus, the overlay of immunometabolism with macrophage polarization and function requires further research.

### Initiation of Lesion Formation: Lipid Uptake Alters Macrophage Functions

Based on the basic concept of plaque development by Ross and Glomset<sup>71</sup> and Goldstein and Brown,<sup>72</sup> LDL preferentially enters the endothelium in areas of vascular branches where endothelial cells partially lose their definite orientation and barrier structure. Along with turbulent flow and endothelial dysfunction, these areas are predisposed to LDL extravasation by passive diffusion through endothelial cell junctions.<sup>73</sup> Apolipoprotein B (ApoB)—the major component of LDL particles—is linked to matrix proteoglycans in the intima and mediates the local accumulation of lipids which are subsequently phagocytosed by macrophages<sup>74</sup> (► **Fig. 2**). Lipid uptake together with alterations of the tissue microenvironment imposes significant changes in macrophage phenotype and functions, representing a major driver of lesion formation.<sup>75</sup> In the following, we will summarize the process of lipid uptake and its consequences in more detail.

### Macrophages Phagocytose Modified LDL

LDL modifications in the vascular intima most importantly involve oxidation processes by ROS, metal ions, and local enzymes like lipoxygenases.<sup>76</sup> OxLDL is engulfed by macrophages where it accumulates and leads to changes in macrophage morphology (foam cells—a central component of atherosclerotic plaques) and polarization towards an inflammatory phenotype<sup>75</sup> (► **Fig. 2**). Therapeutic interventions addressing LDL oxidation might be promising to reduce

atherosclerosis in the future. For example, lipoxygenase-12 is an enzyme that generates bioactive lipids from different polyunsaturated fatty acids. Mice deficient in this enzyme develop significantly less atherosclerotic lesions.<sup>77</sup>

Beyond its important function in removing LDL from peripheral tissues, high-density lipoprotein (HDL) inhibits the process of LDL oxidation via esterases contained in HDL particles.<sup>78,79</sup> Moreover, HDL weakens the proinflammatory function of mononuclear phagocytes.<sup>80</sup> Yet, also proinflammatory effects of HDL have been identified as it leads to lipid raft disruption by passive cholesterol depletion and increased inflammatory cytokine expression.<sup>81</sup>

An important defense mechanism conserved between humans and other species is characterized by the recognition of distinct molecular structures—so-called pattern recognition receptors (PRRs). These PRRs are primarily known for their ability to recognize pathogens. However, under certain circumstances like tissue damage, organisms are also required to phagocytose their own molecular structures—so-called DAMPs. Heat shock proteins, ATP, high mobility group box 1, and also oxLDL are important DAMPs triggering macrophage responses. The uptake of oxLDL particles is mediated by scavenger receptors with their most prominent representatives being the class B receptor CD36, CD68, lectin-like oxLDL receptor 1 (LOX1), as well as different class A scavenger receptors (SR-A).<sup>82–85</sup> Interestingly, downregulation of CD36 by the activation of Mac1—an integrin adhesion receptor—prevents the formation of inflammatory macrophages and foam cells.<sup>86</sup> Beyond macrophages, also endothelial cells and VSMCs are capable of oxLDL phagocytosis in atherosclerotic lesions.<sup>33</sup> Upon receptor-mediated endocytosis, TLR complexes assemble and enhance nuclear factor  $\kappa$  light chain enhancer of activated B cells (NF $\kappa$ B) signaling.<sup>87,88</sup>

Intracellular lipid turnover involves hydrolysis, esterification, and efflux. The major mediators of lipid efflux are ABCA1 and ABCG1.<sup>89</sup> Interestingly, large genome-wide association studies have shown that the isoform ABCG8 is associated with a decreased risk for atherosclerosis.<sup>90</sup> Similar associations have been identified for ApoE, especially its isoform ApoE  $\epsilon$ 2, which is involved in cholesterol removal and thereby reduces plaque burden.<sup>90,91</sup> In macrophages, accumulating lipids are sensed by liver X receptors (LXRs), which can reduce scavenger receptor-mediated endocytosis and enhance cholesterol efflux. By these and others processes, LXRs mediate anti-inflammatory effects in mouse models of atherosclerosis.<sup>92,93</sup> In a similar vein, lipophagy—a special form of autophagy—also reduces lipid accumulation and prevents the local inflammatory response.<sup>94,95</sup> However, intracellular lipid accumulation leads to activation of the inflammasome complex involving NF $\kappa$ B signaling.<sup>95,96</sup> Associated proteins like NLRP3 and caspase 1 are upregulated with subsequent release of major inflammatory mediators like IL1 $\beta$  and IL18, which in turn induced IL6 and TNF $\alpha$  production.<sup>97</sup> Thus, excessive phagocytosis of modified LDL particles in atherosclerotic lesions sustains the vascular inflammatory response in the absence of pathogens—a so-called sterile inflammation<sup>5,98</sup> (► **Fig. 2**). In addition to oxLDL, interactions with cholesterol crystals induce an

inflammatory macrophage phenotype by activation of the NLRP3 inflammasome, which enhances atherosclerotic lesion formation.<sup>99</sup>

It should be noted that lipid metabolism exerts a multitude of effects on the organism, including the hematopoietic system. Disturbance of cholesterol homeostasis results in a hematopoietic bias towards proinflammatory monocytes, which are known to promote atherosclerosis. These effects can be direct, for example, by affecting cholesterol metabolism in HSCs,<sup>100</sup> or indirect by disturbing lipid metabolism in tissues that signal to the BM compartment (e.g. adipose tissue).<sup>101</sup> Regarding this exciting topic, please refer to excellent review articles by Murphy and colleagues.<sup>102,103</sup>

To reduce the atherosclerotic burden, several steps in the process of cholesterol extravasation, oxidation, and subsequent uptake by macrophages could potentially be harnessed as therapeutic targets. Today's prevention strategies primarily address factors that minimize endothelial dysfunction as a prerequisite for cholesterol accumulation. Going one step further in the pathophysiological cascade, oxLDL uptake could potentially be targeted by the inhibition of scavenger receptors. However, eight different classes of receptors are summarized under the term scavenger receptors and partially overlapping functions as well as reciprocal compensation make targeted therapies very challenging.<sup>104,105</sup> Modification and genetic deletion of SR-A mostly resulted in reduction of atherosclerotic lesions<sup>83,105</sup> however, also opposite results have been reported.<sup>106</sup> Similarly, studies on CD36 showed controversial results, especially with respect to sex-dependent differences in mice.<sup>85,107</sup> Finally, knockout studies of LOX1 have provided evidence for a reduction in atherosclerosis. However, genetic deletion was not limited to macrophages and effected also the endothelium, limiting conclusions regarding the specific mechanism involved.<sup>108,109</sup> Beyond these partially controversial results, uptake of modified LDL particles is not limited to scavenger receptors since also acetyl-CoA acetyltransferase-1, a mitochondrial enzyme that catalyzes acetoacetyl-CoA formation, is involved in this process.<sup>110-112</sup> Further studies, including parallel targeting of different scavenger receptors, should enlighten this field in the future and might eventually provide new therapeutic approaches.

### Resolution of Inflammation: Efferocytosis Controls the Inflammatory Response

In order to maintain tissue homeostasis, the organism is capable of removing dead cells in an immunologically silent form—the so-called efferocytosis (Latin “*efferre*”: “to bury” or “to take to the grave”) or programmed cell removal.<sup>113,114</sup> Mononuclear phagocytes as well as nonspecialized cells with phagocytic capabilities like VSMCs or endothelial cells are capable of efferocytosis.<sup>115,116</sup> However, it was reported that the capacity of efferocytosis in atherosclerotic plaques is 20-fold less in comparison to other tissues.<sup>117</sup> Therefore, it is not surprising that atherosclerosis is a highly active immunological process in which multiple proinflammatory mediators including DAMPs are released during the necrotic lysis of dying

cells.<sup>118</sup> Efferocytosis can be divided in four major steps: First, “find me” signals help to attract cells capable of efferocytosis by chemotaxis (1). Most important representatives of these “find me” signals are lysophosphatidylcholine, CX3CL1, sphingosine 1 phosphate, and ATP or uridine triphosphate nucleotides<sup>119,120</sup> as well as different alarmins as secreted chemotactic agents.<sup>121</sup> Afterwards, different “eat me” and “don't eat me” signals trigger the decision of phagocytosis (2) which requires cellular reorganization for proper engulfment—a process mainly regulated by small GTPases like Ras homolog gene family member A, cell division control protein 42, and Ras-related C3 botulinum toxin substrate (3).<sup>122</sup> Defective “eat me” signals are highly associated with atherosclerotic progression and represent the most important players in the process of efferocytosis. Prominent representatives are LDL receptor-related protein 1, MER proto-oncogene tyrosine kinase (MerTK), and protein S, among others.<sup>123</sup> Finally, engulfed cells are digested and processed including the release of anti-inflammatory cytokines (4).

Defects in any of these steps lead to a significant exacerbation of atherosclerosis.<sup>124,125</sup> Interestingly, also clinically relevant molecules such as ApoE or HDL turned out to be positively associated with active efferocytosis and therefore prevent excessive atherosclerosis.<sup>126</sup> In case of insufficient efferocytosis, a necrotic core within the plaques is formed which maintains an inflammatory response and favors lesion progression.

A therapeutic approach to modulate efferocytosis could target the relation between “eat-me” and “don't eat me” signals in order to control the inflammatory response by effective efferocytosis. In this sense, stimulation of MerTK signaling might prevent plaque progression. For example, in LDL receptor deficient mice additional deletion of MerTK causes plaque progression by reduced efferocytosis.<sup>127,128</sup> Very similar findings were found in ApoE<sup>-/-</sup> MerTK<sup>-/-</sup> mice.<sup>125</sup> MerTK can be cleaved to produce inactive MerTK and soluble Mer which are both associated with advanced atherosclerosis.<sup>127,129</sup>

### Current and Future Clinical Interventions

Until today, numerous strategies have been attempted to reduce and prevent atherosclerosis. Therapeutic approaches targeted key pathophysiological mechanisms underlying different steps of lesion formation ranging from LDL regulation and prevention of oxLDL formation, to platelet inhibition, reduction of monocyte/macrophage recruitment, and immunosuppressive strategies. However, the large diversity of mononuclear phagocytes makes targeted interventions very challenging and we have only begun to unravel the enormous cellular complexity with respect to origin, polarization, and metabolism in a population which has so far been regarded as uniform. Moreover, the cellular identity of macrophages continuously adapts to the tissue of residence and can be reprogrammed by an altered (inflammatory) microenvironment.

Most successful in humans was the introduction of statins and related drugs leading to significant LDL

reduction as well as potential pleiotropic effects which are currently under investigation. This treatment limits the development of atherosclerosis and even reduces all-cause mortality.<sup>130</sup> Other important therapeutic approaches address smoking, the optimization of blood pressure, or glucose levels which contribute to endothelial dysfunction.

In an era of increasingly controlled cardiovascular risk factors, the histological features of vascular injuries are changing as superficial erosions become more and more relevant, while plaque rupture events decrease. This pathophysiological difference will need to be considered in the development of treatment strategies as discussed in a recent review by Libby et al.<sup>131</sup>

Novel therapeutic developments have increasingly focused on the inflammatory aspect of atherosclerosis. The CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) trial investigated canakinumab as a monoclonal antibody that targets IL1 $\beta$  in the setting of cardiovascular disease. Interestingly, this medical intervention in humans significantly reduced the rate of cardiovascular events including myocardial infarction.<sup>132</sup> A potential mechanism and the idea behind this treatment concept was the reduction of inflammatory activity in atherosclerosis. Similarly, an anti-inflammatory effect of statins beyond their effect on lowering cholesterol has been described.<sup>133–135</sup> The LoDoCo (Low Dose Colchicine for Secondary Prevention of Cardiovascular Disease) trial also provided some evidence that colchicine reduces cardiovascular events through its anti-inflammatory properties.<sup>136</sup> Interestingly, methotrexate treatment did not reduce the rate of cardiovascular events.<sup>137</sup> Ongoing studies now address the protective effect of colchicine and tocilizumab—an antibody against the IL6 receptor—in the context of atherosclerosis.<sup>138</sup>

## Conclusion

The complexity in the inflammatory process underlying atherosclerosis has made therapeutic interventions very challenging. The heterogeneity of macrophage populations, their differential contribution to distinct stages of lesion formation, as well as their precise targeting requires further research. Selective inhibition of proatherosclerotic macrophage subsets or a reduction in inflammatory monocyte recruitment, could potentially reduce the atherosclerotic burden without affecting basic protective macrophage functions. Beyond the conventional targeting of surface receptors, immunometabolism might serve as an effective switch to selectively address certain macrophage subpopulations in dependence of their predominant form of energy supply. These approaches are likely to provide new perspectives for the treatment and prevention of lesion formation through macrophage-directed immunotherapies.

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### Conflict of Interest

None declared.

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