



The Bidirectional Relationship between Cholesterol and Macrophage Polarization

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Abstract

Whether an atherosclerotic plaque progresses and eventually ruptures is heavily influenced by the function of macrophages. However, it is clear that a spectrum of macrophage phenotypes is present in the plaque, with some exhibiting stabilizing functions. While macrophages expressing characteristic M1 and M2 markers are evident, the disparate microenvironments of the plaque, such as regions of hemorrhage, promote other distinct macrophage phenotypes. Crucial to plaque development and progression is macrophage exposure to accumulated modified low density lipoproteins that leads to foam cell formation and development of the necrotic core. There are a range of biologically active compounds in low density lipoprotein (LDL) each having some bearing on which macrophage surface receptors are engaged and what cellular response ensues. Understanding the bidirectional interplay between 'cholesterol' and macrophage phenotype will provide valuable insight into key pathways to target which may possibly promote plaque stability by modulating macrophage function.

Keywords: Macrophage; Polarization; Cholesterol; Atherosclerosis; Review

Abbreviations

IL: Interleukin; iNOS: Inducible Nitric Oxide Synthase; IPH: Intra Plaque Haemorrhage; KLF: Krüppel Like Factor; LDL: Low Density Lipoprotein; LPS: Lipopolysaccharide; LXR: Liver X Receptor; M1: Type 1 Macrophage; M2: Type 2 Macrophage; M4: CXCL4 Derived Macrophage; M-Mac: M-CSF Derived Macrophage; MCP-1: Monocyte Chemotactic Protein 1; M-CSF: Macrophage Colony Stimulating Factor; MERTK: MER proto-oncogene, Tyrosine Kinase; Mhem: Heme Directed Macrophage; MIP-2: Macrophage Inflammatory Protein-2; mmLDL: Minimally Modified Oxidized LDL; Mox: Oxidised Phospholipid Derived Macrophages; MR: Mannose Receptor; NLRP3: NLR Family, Pyrin Domain containing 3; Nrf2: Nuclear Factor (erythroid-derived 2)-like 2; oxLDL: Oxidised LDL; PPAR γ : Peroxisome Proliferator-Activated Receptor Gamma; STAT: Signal Transducer and Activator of Transcription; TNF: Tumour Necrosis Factor

Introduction

Macrophages are a major cell type in the atherosclerotic plaque which play a key role in plaque development, progression and ultimately, rupture. An early and ongoing process in plaque development is macrophage uptake of modified LDL (a major carrier of cholesterol) that has accumulated in the sub-endothelial space [1,2]. This ingestion of retained lipoprotein transforms the macrophages into foam cells [3] and an inflammatory response ensues. However, this response is maladapted as the macrophage foam cells do not leave but are retained within the vessel wall [2]. Foam cell accumulation, their apoptosis and subsequent necrosis leads to development of the necrotic core - a major contributor to plaque instability [4,5]. It is thus

important to understand the processes involved in, and outcome of, macrophage lipoprotein uptake. This review will cover lipoprotein uptake by the different macrophage phenotypes identified in the plaque and, in addition, as cholesterol uptake or efflux can further modify macrophage function, the effect of LDL and HDL on macrophage polarization is also discussed.

Macrophage phenotypes in the atherosclerotic plaque

While macrophage polarization suggests two extremes of phenotype, and indeed the terms M1 and M2 predominate in the literature, it is well appreciated that there is a spectrum of macrophage phenotypes that can be adopted with considerable plasticity between them [6]. This is especially apparent in atherosclerosis where monocytes, and subsequently macrophages, are exposed to an array of factors throughout plaque initiation and progression and in the advanced plaque. Monocyte differentiation into macrophages is promoted by growth and survival factors, including M-CSF, GM-CSF [7] and CXCL4 [8], with all three of these factors present in atherosclerotic plaques [9-11]. Factors such as IFN- γ , IL-4 and IL-10, which are known to promote M1 and M2 macrophage polarization [12] are also evident in the plaque [13,14] and, as such, macrophages expressing a range of M1 and M2 markers have been identified in both murine and human plaques [15-19]. Furthermore, the advanced plaque is complex and heterogeneous in nature, often containing regions of intra-plaque hemorrhage (IPH). A unique phenotype of macrophage, the Mhem (M(Hb) or HA-Mac) macrophage, forms in these regions [20-22]. Aside from these identified forms, a range of intermediate phenotypes may also be present. In addition, other monocyte-derived cells (which share overlapping functions with macrophages) are also present in the plaque, such as dendritic cells [23,24] and fibrocytes [25].

These various 'polarized' macrophage forms differ greatly in their ability to take up oxLDL. However, oxLDL uptake itself can also alter macrophage phenotype. The numerous bioactive compounds present in LDL, and their modified forms, exert specific effects. Oxidized phospholipids, for example, promote formation of the Mox macrophage, which is distinct from the M1 and M2 macrophage forms [26]. Cholesterol is also present in the plaque in crystalline form. Cholesterol crystals activate different and distinct pathways in the macrophages. Aside from cholesterol uptake, its efflux also influences macrophage phenotype, with emerging studies addressing how HDL may impact on the M1/M2 nature of macrophages in the plaque.

Effect of Macrophage Phenotype on 'Cholesterol' Uptake

M-Macs/GM-Macs and the M4 macrophage

Monocyte to macrophage differentiation is promoted by exposure of monocytes to macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF) or CXCL4 [7,8] and the macrophages formed have been described as M-Mac, GM-Mac [27] and M4 [28], respectively. It should be noted, however, that GM-CSF and M-CSF cultured macrophages have also been called M1 or M2 [29], but such designation is not recommended in the current nomenclature guidelines [30]. It is noted, though, that M-CSF stimulation induces expression of a substantial portion of the M2 transcriptome [31]. Furthermore, M-CSF differentiated macrophages have also been called M0 or resting macrophages [32]. *In vitro*, upon LPS stimulation, GM-CSF derived macrophages produce higher levels of inflammatory cytokines than their M-CSF derived counterparts [29,33]. Furthermore, M-CSF derived macrophages express higher levels of the M2 marker CD163 and the anti-inflammatory factor Krüppel-like factor (KLF)2 [29]. Though the GM-CSF derived macrophages are more inflammatory in nature [33], they accumulate less oxLDL than the M-CSF derived macrophages [29]. This may be attributed to M-CSF upregulation of CD36 [29], a receptor for oxLDL [34,35]. Conversely, GM-CSF has been found to upregulate expression of genes that promote reverse cholesterol transport (PPAR γ , LXR- α and ABCG1 [27]). M-Macs retain CD14, while this is down-regulated in GM-Macs, which exclusively express 25F9 [27]. In human coronary atherosclerotic plaques, CD68⁺ macrophages staining both with, and without, CD14 are evident with CD14⁺ CD68⁺ macrophages prevalent in the lesion, whereas the CD14⁻ CD68⁺ macrophages were found in areas devoid of disease [27].

CXCL4 derived macrophages (M4) lack CD163. Indeed, macrophages lacking CD163 are evident in the plaque [16], though these could also be M1 macrophages. The suppression of CD163 by CXCL4 could not be recovered by subsequent incubation with M-CSF or IL-10. Furthermore, the loss of CD163 was accompanied by an inability of hemoglobin-haptoglobin (Hb:Hp) to induce hemoxygenase 1 (HO-1) expression [16]. Transcriptome analysis and functional studies show that M4 macrophages are distinct from M-CSF differentiated as well as M1 and M2 polarized macrophages [36]. CD36 expression is lower on M4 macrophages than M-CSF differentiated macrophages and, accordingly, they have less intracellular cholesterol upon incubation with oxLDL [36]. The increased ABCG1 gene expression suggests that this may be through both reduced lipid uptake and increased efflux [36]. It is not clear whether M4 macrophages are predominantly pro- or anti-atherogenic [36]. However, as CXCL4 deficiency results in decreased

atherosclerotic plaque burden [37], M4 macrophages may play a pro-atherosclerotic role [16].

M1 and M2 macrophages

After macrophage differentiation, exposure to cytokines such as IFN- γ and IL-4 primes the cells to adopt classical (M1) and alternative (M2) phenotypes, respectively [12]. The NF- κ B pathway and signal transducer and activator of transcription (STAT) 1 direct M1 polarization [38-40], while the transcription factors Krüppel-like factor, peroxisome proliferator activated receptor- γ (PPAR γ) and STAT6 [38-40] drive M2a macrophages. Exposure to IL-10 promotes M2c macrophages through STAT3 [41].

The lipid handling capacities of the M1 and M2 macrophages have been examined *in vitro* and the presence of M1 and M2 foam cells examined in both mouse and human plaques. Though M2a macrophages take up less lipid than resting macrophages [18], when M2a (IL-4), M2b(IC) and M2c (IL-10) were compared with M1 macrophages (M-CSF with LPS plus IFN- γ) they were found to take up more lipid [42]. (Although it is noted that monocytes in this later study were from obese subjects with diabetes). That said, IL-4, IL-10 and immunocomplex upregulate expression of CD36 and SR-A1 relative to IFN- γ [42] and separately, IFN- γ has been shown to reduce CD36 expression [43] which is consistent with the greater lipid uptake by M2, compared to M1, macrophages. As M2 (a,b and c) macrophages do not differ in apolipoprotein A1 (ApoA1) or HDL-stimulated cholesterol efflux compared with M1 macrophages (M-CSF with LPS plus IFN- γ), then the net increase in foam cell formation may primarily be due to cholesterol uptake [42]. However, in healthy controls, ApoA1- and HDL₃-cholesterol efflux was found to be lower in M2a compared to resting macrophages [18]. Furthermore, the level of expression of both the ABCA1 and ApoE genes was lower in M2a compared to M1 and resting macrophages [18], suggesting that lower cholesterol efflux does contribute to increased cholesterol accumulation in M2 macrophages. Consistent with this increased lipid uptake, mannose receptor (MR: an M2 marker) positive macrophages were found localized more centrally within the plaque, in the ApoE^{-/-} mouse, compared to M1 macrophages, and they exhibited a higher level of ADRP (a marker of lipid uptake) expression [42].

In human plaques, although the presence of inflammatory macrophages has long been recognized, the first identification of M2 macrophages was by Bouhrel in 2007 [15]. In contrast to the murine model, human M2 macrophages (MR⁺) have been found to be present in more stable regions of the plaque distant from the core [15,18]. MR⁺ foam cells that did form were reported to contain smaller lipid droplets than M1 foam cells [18] and, as such, foam cells are thought to primarily be M1 derived [18]. Of note, we have found that expression of the M2 markers MR (also known as CD206) and CD163 differs in carotid plaques, with many sections lacking MR but containing CD163 and then at a level comparable to that of the M1 markers, CD64 and CD86 [19]. Furthermore, when the M2 markers (CD163, MR) were present they, not just M1 (CD64 and CD86) markers, could be found on foam cells associated with the core. In addition, both M1 and M2 markers were found in the cap, primarily on spindle shaped cells [19].

The discrepancies between the murine and human data may reflect a difference in the stage of atherosclerosis examined, for although M2 foam cells have been found to predominate in young ApoE^{-/-} mice, M1 foam cells are more prevalent in older mice [17]. Whether M2 macrophages and their foam cell forms predominate in the early

human atherosclerotic plaque is not clear, but with M-CSF promoting expression of M2 related genes [31], then M2 macrophages (and subsequently M2 derived foam cells) may arise early in plaque development. Cholesterol uptake promotes ER stress which triggers the unfolded protein response [42,44]. Since M2 (IL-13 derived) foam cells are more sensitive to the unfolded protein response than other forms of macrophages [45], the lack of M2 macrophages in advanced human plaque may, in part, stem from increased cell death. Furthermore, during plaque progression the switch from an M2 to an M1 promoting environment may both impede apoptotic cell clearance (as M2 cells have greater capacity for efferocytosis) [46] and promote the formation of M1 macrophages/foam cells.

M1 macrophages are thought to be detrimental to plaque stability as aside from contributing to formation of the necrotic core, they contribute to thinning of the fibrous cap. M1 macrophages are found in the rupture-prone shoulder regions of the plaque [47] and there is an inverse relationship between the level of M1 (CD86), but not M2 (CD163), with carotid plaque cap thickness [19]. Similarly, levels of CD68 and CD11c (M1) are higher, while levels of the M2 markers (CD163 and MR) are lower in symptomatic patients compared to asymptomatic patients [48]. These findings are consistent with known role of M1 macrophages in tissue destruction [6]. M2 macrophages are considered athero-protective as they (CD163⁺ and CD206⁺ macrophages) produce collagen I in the carotid plaque [19]. This is consistent with their known role in tissue repair.

Mhem macrophages

Intraplaque hemorrhage is a common feature in the advanced plaque and its presence is associated with plaque progression [49]. Red blood cells have cholesterol enriched membranes which can result in increased cholesterol deposition and subsequent enlargement of the core [50]. Furthermore, RBC lysis releases hemoglobin. Heme and hemoglobin are strong oxidizers which could potentially increase lipid oxidation [51]. Hemoglobin is bound by haptoglobin to form the Hb:Hp complex [52] and macrophages scavenge this complex through CD163-the Hb:Hp receptor [52]. This results in the release of IL-10, which forms an autocrine loop, stimulating further expression of CD163 [22]. Such IPH localized macrophages are known as Mhem macrophages [20]). They are CD68^{pos}CD163^{high}HLADR^{low} and thus distinct from conventional macrophage foam cells which are CD68^{pos}CD163^{low}HLADR^{high} [22]. *In vitro* studies show that Hb:Hp driven adoption of the Mhem phenotype is IL-10 dependent [22] which suggests that the Mhem macrophages are related to the M2c form. While transcriptome analysis shows that Mhem are distinguishable from M2 (and M1) macrophages by the expression of ATF [20], this comparison was made between IL-4 stimulated (M2a) but not IL-10 stimulated macrophages. However, exposure to Hb:Hp (or heme [53]) will no doubt trigger upregulation of specific genes which would allow distinction of these cells from M2c macrophages.

Despite iron loading (as seen by the presence of CD163/Perls double stained cells in the plaque), the Mhem macrophages were negative for 8oxoG, a marker of oxidant stress, rather they stained strongly for HO-1 [22]. HO-1 breaks down hemoglobin to carbon monoxide, biliverdin (which is rapidly converted to bilirubin) and free iron [54] which is either used by the cell or bound by ferritin and exported out of the cell via the iron exporter ferroportin [21]. ATF-1 induced upregulation of HO-1 enables the safe handling of iron. At the same time, ATF-1 also promotes iron cholesterol efflux through a LXR β \rightarrow LXR α \rightarrow ABCA1 pathway [20]. As such, Mhem macrophages

are resistant to lipid loading as they have lower expression of genes associated with lipid uptake, but higher expression of genes involved in reverse cholesterol transport [20,21]. In the plaque, Mhem macrophages are found distant from the core and do not take up lipid [20-22]. Indeed, CD163⁺ macrophages are reported to be absent from lesions that have a lipid core but no hemorrhage [22].

The anti-inflammatory functions of the Mhem form are athero-protective. Their occurrence in IPH, which is associated with plaque progression, is thought to be a case of an adaptive response which is 'too little too late' [55]. Moreover, formation of the full Mhem state may be inhibited in the plaque as IFN- γ and LPS can prevent Mhem formation [22]. Furthermore, it has been shown in thrombi from acute coronary syndrome (ACS) patients, with diabetes or insulin resistance, that IL-10 production is impaired. This would further limit the ability of the Mhem to stabilize the plaque [56].

Effect of 'Cholesterol' on Macrophage Polarization

While variation in lipid handling by different macrophage phenotypes is evident, the reverse is also apparent, that lipid handling alters macrophage polarization. LDL is the major carrier of cholesterol, and its modification and uptake by macrophages leads to foam cell formation in the atherosclerotic plaque. Efflux of cholesterol from macrophages, i.e. reverse cholesterol transport, is mediated by HDL or its apolipoproteins, in particular, ApoA1 [57]. Aside from their role in cholesterol handling, the pro- and anti-inflammatory impacts of LDL and HDL on macrophages in the plaque may significantly influence macrophage contribution to overall plaque stability.

LDL

LDL is a major risk factor for atherosclerosis. Its accumulation and oxidation within the vessel wall are crucial events in plaque formation [2]. The importance of LDL in atherosclerosis development is clearly evident in Familial Hypercholesterolemia (FH) where the absence (homozygous FH) or reduction (heterozygous FH) of the LDL receptor (which removes LDL from the circulation) or its functions results in accelerated atherosclerosis development [58]. Strong evidence from many studies has demonstrated that the reduction of LDL by statin therapy is associated with reduced occurrence of vascular events [59,60]. However, despite the known detrimental role of oxLDL in atherosclerosis, it has been described to have both pro- and anti-inflammatory effects, including on macrophages [61-66]. Such discrepancies may arise from the heterogeneous nature of the modified LDL preparations used in culture. The effect of LDL uptake on macrophage phenotype depends on the degree, and form of, LDL modification [67]. For example, Miller et al. demonstrated that minimally modified LDL (mmLDL), but not LDL or oxLDL, stimulation of macrophages induced early expression of mRNA for macrophage inflammatory protein-2 (MIP-2), MCP-1, TNF- α and IL-6 [68]. MmLDL, is not sufficiently modified to be taken up by scavenger receptors but is recognized by LDL receptors and TLR4 [67,69]. Moderately oxidized LDL is taken up by Lox 1 and CD36, with more extensive oxidation required for SRA-1 engagement [67]. There are numerous biologically active compounds present in modified LDL such as modification in phospholipids, sphingolipid and free fatty acid products, oxysterols, and ApoB [67]. Their modification impacts which receptors are engaged and what cellular response occurs. For example, CD36 recognizes oxidized phospholipids; TLR-4 recognizes oxidized cholesteryl esters and SRAI/II recognizes modifications of the ApoB protein [67]. For a comprehensive review see Levitan [67]. The

pro-inflammatory effects of oxLDL are mediated by NF- κ B, AP-1, STAT1/, NFAT, SP-1 and HF-1[67] as well as a down regulation of KLF2 [29]. The anti-inflammatory effects of oxLDL arise from inhibition of NF- κ B [6,65] and stimulation of PPARs and Nrf2 [42,67].

The cellular response of macrophages to oxLDL may also be dependent, in part, on the phenotype of the macrophage prior to oxLDL interaction. While GM-CSF derived macrophages produce higher levels of IL-6 and MCP-1 than M-CSF derived macrophages upon LPS stimulation, pre-exposure to oxLDL (prior to LPS stimulation) resulted in M-CSF derived macrophages producing higher levels of IL-6, IL-8 and MCP-1 (equivalent to that produced by LPS stimulated GM-CSF macrophages) and lower production of IL-10 compared to M-CSF alone. In contrast, no change in cytokine expression for GM-CSF derived macrophages was seen [29]. In a separate study, GM-CSF induced human macrophages exposed to oxLDL were shown to have an inhibited IL-1 and TNF α response to LPS [63]. Furthermore, M1, but not M2, macrophages exposed to oxLDL upregulated growth factor mediated NF- κ B signaling pathways [70].

Most of our understanding of the effect of LDL/oxLDL on macrophage polarization comes from *in vitro* studies. The degree and form of LDL modification *in vivo* is not clear [71]. Interestingly, foam cell formation in peritoneal macrophages of an LDLR^{-/-} mouse fed a high cholesterol/high fat diet was associated with a suppression rather than activation of inflammatory gene expression [72], suggesting that macrophage polarization to an M1 phenotype in the plaque arises from extrinsic pro-inflammatory signals. For example, foam cell necrosis is one of the factors that can stimulate an inflammatory response [5]. In addition, the necrotic core of atherosclerotic plaques is hypoxic which would be expected to promote an M1 phenotype as hypoxia switches the metabolism of macrophages to an anaerobic glycolytic pathway [73], the pathway used by M1 macrophages. Furthermore, succinate (a Krebs cycle intermediate) induces HIF1 α expression which promotes expression of pro-inflammatory genes [74]. However, a separate study, using the Reversa mouse (a mouse in which hypercholesterolemia can be conditionally reversed [75]), suggests that LDL may stimulate M1 polarization, as a reduction of LDL resulted in stabilization of the plaque with decreased total macrophages (CD68 and Moma⁺) but increased gene expression of M2 markers such as Arg1, MR, CD163, C-lectin and FIZZ1 [76]. Whether this relates to M1 to M2 skewing, or merely an efflux of M1 macrophages is not clear.

Mox Macrophages

Of the different components of oxLDL, the effect of oxidized phospholipids on macrophage polarization has been specifically examined. Oxidized phospholipids are major contributors to oxLDL binding to scavenger receptors [77], in particular CD36 [78]. Incubation of M1 or M2 macrophages with oxidized phospholipids results in the formation of macrophages that are different from both the M1 and M2 phenotypes. This distinct phenotype has been termed the Mox macrophage [26]. It lacks CD163 which is characteristic of M2 and Mhem macrophages, however, like the Mhem macrophages, Mox express HO-1 [26]. Mox marker gene expression is largely mediated by Nrf2 (a redox-sensitive transcription factor). Whether the Mox macrophage is inflammatory/pro-atherosclerotic is not completely clear. While macrophage (MSCF or GM-CSF derived) incubation with oxidized phospholipids results in the upregulation of IL-1 β , the incubation of M1 macrophages with oxidized phospholipids

results in the down regulation of IL-1 β , iNOS, TNF α and MCP-1 [26]. Furthermore, HO-1 is athero-protective as discussed above. However, Mox macrophages derived from M2 macrophages have reduced arginase 1 (Arg1: M2 marker) expression [26]. The ability of oxidized phospholipids to stimulate or inhibit inflammation is known to be dependent on the biological situation [79] and this appears to be the case with different macrophage phenotypes. Though Mox macrophages have been identified in the mouse, their identification in human plaques is yet to be determined.

Cholesterol crystals

Cholesterol crystals are a major feature of advanced plaques where they are readily identified by the presence of cholesterol clefts (left after tissue processing). However, their formation starts early in the plaque, even in fatty streaks [80]. Minute crystals are evident within two weeks of high cholesterol feeding in the ApoE^{-/-} mouse which coincides with the appearance of inflammatory cells [81]. Although cholesterol crystals are primarily evident as extracellular deposits in the necrotic core, where they are proposed to arise from lipid deposition or RBC death, they can also form within macrophages themselves [81,82]. Cholesterol crystals induce NLRP3 inflammasome activation leading to the release of the inflammatory cytokines IL-1 β and IL-18 in both human [83] and murine [81] macrophages.

HDL

A low level of HDL, both as reduced ApoA1 and as HDL particle cholesterol content, is associated with an increased risk of cardiovascular diseases and associated clinical events [84]. As such, HDL is considered athero-protective [85]. Indeed, infusion of HDL or ApoA1 in mouse models leads to decreased plaque size [86]. HDL/ApoA1 are known to exert anti-inflammatory actions which, while initially attributed primarily to cholesterol transport, can also be direct effects on cells [87]. Injection of ApoA1 into the ApoE^{-/-} mouse leads to a decrease in total plaque macrophage content with a significant reduction in the expression of M1 markers (IL-1 β and MCP-1) and an increase in the expression of M2 markers (Arg1 and MR) [88]. Of note, the injected ApoA1 was almost completely incorporated into HDL. Aortic segments transplanted from HDL-deficient (ApoE^{-/-}) mice into mice with normal HDL levels (i.e. wild type mice) showed increased M2 markers (FIZZ1, Arg1, CD163, MR) and decreased inflammatory markers (MCP-1, TNF α) [89]. These findings suggest that HDL (and ApoA1) promote an M2 phenotype *in vivo*, but do not provide information regarding whether this is a direct effect of HDL on macrophages. To understand direct interactions, *in vitro* work has been conducted. Incubation of mouse bone marrow derived macrophages with HDL led to increased FIZZ1 and Arg1 expression and suppression of resting and IFN γ -induced iNOS, TNF α and IL-6, suggesting a direct effect of HDL in promoting an M2 phenotype [90]. This was proposed to be via JAK/STAT pathways, specifically JAK1 or JAK 2 interacting with STAT6 [90]. Macrophages incubated with ApoA1 had decreased LPS-stimulated production of IL-1 β , IL-6 and TNF α , with this anti-inflammatory effect attributed to the interaction between ApoA1 and ABCA1, which subsequently activated the JAK2/STAT3 pathway [91]. As well as altering macrophage polarization, HDL and ApoA1 have been shown to decrease TNF α -mediated adhesion of monocytes to endothelial cells *in vitro*, thus showing further anti-inflammatory effects [92].

In humans, administration of reconstituted HDL leads to improved plaque composition and even regression [93,94]. However,

examination of effects of HDL at the cellular level in humans becomes difficult as the plaque cannot be readily removed for analysis. As such, work examining the anti-inflammatory effects of HDL in humans predominantly consists of *ex vivo* and *in vitro* studies. Infusion of recombinant HDL led to decreases in inflammatory parameters such as reduced CD11b expression on monocytes [95]. Anti-inflammatory changes such as this suggest that high HDL promotes a shift towards an M2 macrophage phenotype and conversely, a shift away from an M2 phenotype with lower HDL levels. In contradiction to this, monocytes isolated from patients with low HDL were equally able to become M2 macrophages under IL-4 stimulation as those from patients with normal HDL levels, suggesting low HDL levels do not lead to reduced formation of M2 macrophages [96]. However, the *in vivo* environment is much more complex, with other factors influencing macrophage polarization coming into play, as such the possibility of low-HDL patients having reduced numbers of, or capacity to form, M2 macrophages cannot yet be ruled out. While human monocyte-derived macrophages incubated with HDL did not show increased gene expression of M2 polarisation markers (MR, CD200R, F13A1, Stabilin-1, IL1RA, CD163, IL-10, PPAR γ) [96], the HDL concentrations used were low and it is possible that more physiological concentrations of HDL could lead to changes in marker expression. More *in vitro* work, similar to that done for mice, may determine whether, and how, HDL and its components affect macrophage polarization and function. The mechanisms by which changes in macrophage polarization occur in mice may also be active in humans.

Conclusion

The microenvironment influences the phenotype of macrophages in the atherosclerotic plaque and, as such, a range of macrophage phenotypes are present. One key factor in their environment is lipid, in particular oxLDL with its bioactive components and cholesterol crystals. A bidirectional relationship exists between macrophages and cholesterol where the phenotype of the macrophage affects its ability to handle lipid. Conversely, oxLDL or interaction with cholesterol crystals influences macrophage phenotype. However, whether the response generated promotes or suppresses inflammation depends on the degree and form of LDL modification. This complex interplay, both pro- and anti-inflammatory, is clearly swayed (albeit by other factors in the milieu as well) towards a pro-atherogenic process in cardiovascular diseases. However, mouse models in particular demonstrate that the atherogenic balance can be switched to promote plaque regression accompanied by macrophage polarization to an M2 macrophage. This suggests that despite the complexity of the plaque, key figures such as HDL and PPARs may be able to delay and reverse atherosclerosis development, with this being, in part, through modulating macrophage polarization.

Author Contribution

Heather Medbury and Helen Williams drafted the manuscript. All authors read through and made suggestions and corrections to the manuscript. All authors approved the final manuscript.

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