Macrophage Differentiation to Foam Cells

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Abstract: Foam cell formation from macrophages with subsequent fatty streak formation plays a key role in early atherogenesis. Foam cell formation is thought to be induced by Low Density Lipoproteins (LDL), including oxidized LDL (OxLDL) or minimally modified LDL (mmLDL). Understanding the molecular mechanisms involved in OxLDL- and mmLDL-induced foam cell formation is of fundamental importance for atherosclerosis and cardiovascular disease. The expression of many genes is likely modulated during macrophage transformation into a foam cell. In this mini-review we describe functional consequences of modulation of three groups of genes: Scavenger Receptors (SR-A, CLA-1/SR-BI, CD36, CD68, LOX-1, and SR-PSOX), the PPAR family of nuclear receptors, and a number of genes involved in eicosanoid biosynthesis, including lipoxygenases and leukotriene receptors. Scavenger receptors appear to play a key role in uptake of OxLDL, while mmLDL appears to interact with CD14/TLR4. The regulation of scavenger receptors is, in part, mediated by the PPAR family of nuclear receptors. PPARα and PPARγ agonists, such as thiazolidinediones and fibrates, and PPARδ agonists were tested as atheroprotective drugs and showed some beneficial effects. Eicosanoids are naturally occuring agonists for PPARs. Recent observations indicate a role of the components of the eicosanoid cascade, such as 5-lipoxygenase, 15-lipoxygenase and the leukotriene receptors in foam cell formation. Selective inhibitors of lipoxygenases and leukotriene receptors could be useful in the treatment of atherosclerosis by preventing or reducing foam cell formation.

Key Words: human, monocytes/macrophages, foam cells, LDL, scavenger receptors, PPAR, lipoxygenase, leukotrienes.

INTRODUCTION

Cardiovascular disease affects more than 58 million Americans and remains the most common cause of death in the U.S. and all over the world. Atherosclerosis accounts for the majority of these deaths [1-3], but the factors involved in early atherosclerosis and progression of this disease are still not completely defined. A critical event in atherogenesis is the focal accumulation of lipid-laden foam cells (FC) derived from macrophages (MΦ), smooth muscle cells, and other vascular cells with subsequent fatty streak formation. We focus on MΦ-derived foam cells, because MΦ play a central role in the atherogenic process as modulators of both lipid metabolism and the immune response [4, 5].

There are several excellent reviews evaluating various aspects of foam cell formation and discussing pharmacological agents capable of modulating FC formation [5-9]. We concentrate here on the aspects modulated by scavenger receptors, by the PPAR family of nuclear receptors, and by the families of lipoxygenases and leukotriene receptors. These pathways appear to be interrelated and affect each other.

The initiating effect in atherosclerosis may be the accumulation of LDL in the subendothelial matrix [1]. Accumulation is greater when levels of circulating LDL are raised, and both the transport and retention of LDL are increased in the sites of lesion formation. LDL trapped in the subendothelial matrix may also aggregate and LDL aggregation, like chemical modification of LDL particles, mediated by enzymes such as sphingomyelinase [10] may contribute to FC formation [1]. Modified LDL is avidly taken up by MΦ and leads to generation of FC [11], but it is unclear how LDL is modified in the vessel wall. Modified LDL is a collective term for various modifications of native LDL, including oxidation, glycation or acetylation. In this mini-review, we mainly concentrate on minimally modified LDL (mmLDL) and oxidized LDL (OxLDL), which represent naturally occuring products of LDL with various degree of oxidation. This variable degree of oxidation strongly affects the functional behavior of modified LDL. The uptake and further conversion of fully oxidized OxLDL is mediated by a relatively large family of scavenger receptors [5, 12-17]. OxLDL, as well as oxidized fatty acids, induce the expression of some scavenger receptors such as SR-A and CD36 [18], which may provide a positive feedback mechanism that could amplify FC formation. OxLDL also inhibits the production of NO, a compound with antiatherogenic properties [1], and can initiate apoptosis of cultured vascular cells [16].

There are several definitions for minimally modified LDL [19, 20]. LDL can be mildly (or enzymatically) modified, for instance, with xanthine/xanthine oxidase, Fe^{++}, 15-lipoxygenase (15-LO) alone or in combination with secretory PLA_2 ($sPLA_2$) [20-23]. Whether all these "minimally modified" varieties of LDL possess the same properties is

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not known. Here, we concentrate on the proposed mechanism of action of mmLDL prepared by 15-LO treatment. Such mmLDL mainly interacts with CD14/toll-like receptor 4 (TLR4) and promotes expression of SRs such as CD36 [24]. The binding of mmLDL thus may lead to increased uptake of OxLDL as well as to the inhibition of phagocytosis of apoptotic cells [24].

In mice and humans, peripheral blood monocytes represent a heterogenous population [25, 26]. In humans, two major subsets are defined as $CD14^{+}CD16^{-}$ and $CD14^{low}$ $CD16^{+}$, which have distinct migratory properties. CD14-positive monocytes are supposed to be more "inflammatory" cells, which easily migrate to sites of inflammation [25]. Only CD14-expressing monocytes bind mmLDL [20], thus the balance between CD14- and CD16-positive monocytes could be one of determining factors in FC formation, at least in the early stages of the atherosclerotic process.

The TLR4 signaling pathway was recognized as a potentially important modulator of cardiovascular disease [27]. Polymorphisms in the TLR4 and CD14 genes are associated with the incidence of atherosclerosis [28]. In a mouse model of atherosclerosis induced by a null mutation in the gene encoding for apolipoprotein E ($ApoE^{-/-}$) on a proatherogenic diet, added deficiency in TLR4 [29] or MyD88 [30], a molecule involved in TLR signaling, significantly protected against atherosclerosis. The atherosclerosis-resistant C3H/HeJ mice [31, 32] have defective TLR4 [33].

Scavenger Receptors

Several species of scavenger receptors (SR) have so far been identified. However, it still remains unclear which receptors are crucial for foam cell formation and progression. Class A scavenger receptors (SR-AI, SR-AII, SR-AIII), class B SR (CLA-1/SR-BI, SR-BII, CD36), and the class D receptor CD68, are thought to be most important during foam cell formation followed by uptake of modified LDL. Recently, several new members of the SR family have been discovered, namely, SR-PSOX (also known as CXCL16 [34]) and Lectin-Like Oxidized LDL Receptor-1 (LOX-1). Expression of SR-PSOX was shown in human atherosclerotic lesions [35]. LOX-1, which is mainly expressed in endothelial cells, but is inducibly expressed in macrophages, is highly upregulated in atherosclerotic lesions in humans [36].

Blocking of both class A and class B receptors, including CD36, significantly reduces OxLDL uptake, but doesn't prevent it completely [17, 37]. SR-A and CD36 account for 75-90% of uptake of modified LDL. Cholesteryl esters derived from modified lipoproteins fail to accumulate in macrophages isolated from the SR-A and CD36 double null mice [17]. Another recent study also suggests a significant role for CD36 as well as SR-BI and CD68, but not SR-A or LOX-1, in FC formation during OxLDL treatment of the macrophage-like THP-1 cells [38]. Like other investigators [18], we found upregulation of CD36, whereas SR-PSOX was downregulated by prolonged exposure of isolated human monocytes to OxLDL (Table **1**). Different SRs may play differential roles during FC formation: SR with broad ligand specificity, such as class A and class B scavenger receptors, can be upregulated by modified LDL and be positive modulators of FC formation; receptors that are more specific for OxLDL like SR-PSOX and LOX-1, can be down-regulated during FC formation. Thus, increased amounts of SR-PSOX and LOX-1 in atherosclerotic lesions in humans may represent defensive mechanisms which may be protective against excessive accumulation of cholesteryl esters. High activity of SRs in general may contribute towards *resistance* to atherosclerosis if accompanied by adequate amounts of Apo-E for cholesterol removal [39]. At this time, it is unclear whether scavenger receptors are pro- or anti-atherogenic. Most likely, different scavenger receptors have specific roles.

CD14

Bacterial lipopolysacharide (LPS) and mmLDL bind to the membrane glycoprotein CD14 [20, 40]. Polymorphisms in the promoter of the CD14 gene were shown to be associated with an altered risk of atherosclerosis, coronary artery disease and myocardial infarction [41-44]. The C(-260)→T polymorphism is associated with upregulation of CD14 and increased atherosclerosis [41].

We found significant upregulation of CD14 antigen after incubation of human monocyte-derived macrophages with OxLDL (Table **1**). Upregulation for CD14 was even more profound than that of CD36, a well-recognized receptor for OxLDL [45]. Upregulation of CD14 by OxLDL was previously observed [46, 47], however this process was shown to be related to monocyte differentiation (discussed in [48]). However, we did not detect any difference in expression of the markers of differentiation, such as mannose receptor, in OxLDL-treated vs. untreated MΦ (51±16% vs. 49±11% positive cells by flow cytometry, respectively).

Modified LDL (OxLDL and AcLDL)

FC formation is thought to be mediated by modified LDL. The formation of foam cells *in vitro* can be induced by OxLDL and acetylated LDL (AcLDL). The FC-inducing effect of AcLDL is much stronger than that of OxLDL [49-51]. Both AcLDL and OxLDL can induce proliferation of peripheral macrophages *in vitro* [52, 53]. However, the

Table 1. Protein Expression of CD14 and SRs during Foam Cell Formation. Percent of Positive Cells as Determined by Flow Cytometry. Mean±SE from at Least 3 Separate Experiments

	CD14	CD36	SR-PSOX	CD68 (total expression)
Control	31±7	60±10	65±9	63±18
+OxLDL (2-3 d)	60±13	78±9	56±11	62±27

growth-inducing activity of AcLDL was significantly inhibited by IL-4 and IL-10, whereas macrophage growth-stimulating activity of OxLDL, in contrast to that of AcLDL, was refractory to these suppressive cytokines [52]. OxLDL, but not AcLDL, induces PPARγ expression [48].

Numerous cellular effects of OxLDL have been described [15, 16, 18, 54]. It is hard to compare all the results obtained after application of OxLDL because, like mmLDL, it probably does not represent a uniform molecule and various preparations of OxLDL, various endotoxin levels, and other factors could influence the results. Even stratification to the same index of LDL oxidation as measured by thiobarbituric acid reactive substances (TBARS) cannot resolve this problem, because commonly used additives to the growing media, such as FCS, could provide significant variations in incubation conditions.

Possible cascades leading to FC formation are presented in Fig. **1**.

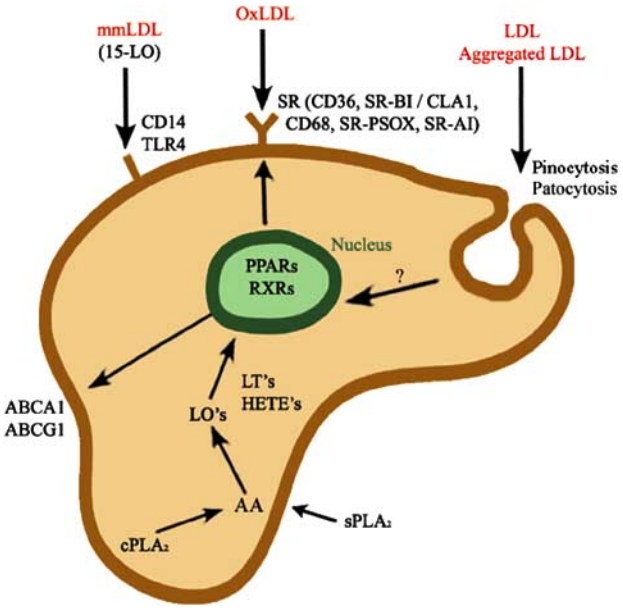

Fig. (1). Different mechanisms of foam cell formation.

15-lipoxygenase (15-LO) inititates formation of mmLDL, which binds CD14/TLR4. Extensively oxidized LDL binds scavenger receptors (SR). Further signaling is mediated by PPARs/RXRs, which can either promote FC formation *via* upregulation of CD36, or prevent FC formation by upregulation of reverse cholesterol transport (ABCA-1/ABCG-1). Uptake of aggregated LDL is facilitated by pinocytosis (actin-independent uptake) and by phagocytosis or patocytosis (actin-dependent uptake), shown only for PMA-stimulated human macrophages.

$sPLA_2$ = secreted phospholipase A_2, $cPLA_2$ = cytosolic phospholipase A_2, AA = arachidonic acid, LO's = lipoxygenases, HETE's = hydroxyeicosatetraenoic acids, LT's = leukotrienes.

First, 15-lipoxygenase inititates formation of mmLDL, which interacts with CD14/TLR4. Some components of minimally oxidized low density lipoprotein, oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine, epoxyisoprostane and epoxycyclopentenone phospholipids are potent activators of peroxisome proliferator-activated receptor (PPAR) alpha [55]. Extensive oxidation of LDL could be mediated by various enzymes, including sphingomyelinase and myeloperoxydase [1, 56]. Next, OxLDL may react with SRs. At least two different structural elements of OxLDL have direct effects on PPARγ: 13-hydroxyoctadecadienoic acid (13-HODE) (or 9-HODE) and oxidized phosphatidylcholine (hexadecyl azelaoyl phosphatidylcholine), which is considered to be the most potent natural ligand of PPARγ [57, 58].

Native LDL

Macrophage can also accumulate cholesterol via fluid phase endocytosis [59]. This model suggests that FC formation could be mediated not only by modified LDL, but also by native LDL taken up by activated macrophages [59]. Aggregation of LDL leads to sequestration of aggregated LDL by MΦ and promotes LDL uptake [60]. The macrophage activator and CD14/TLR4 ligand, microbial-derived LPS, can also stimulate macrophage LDL uptake [59]. MΦ uptake of aggregated LDL may be mediated by both actin-independent and actin-dependent endocytic pathways such as pinocytosis and patocytosis [60, 61]. Patocytosis can be reversed by MΦ exposure to plasminogen [60]. MΦ can release the stored aggregated LDL as a result of conversion of plasminogen to active plasmin mediated by MΦ urokinase plasminogen activator. This mechanism can evidently protect the cell from excessive accumulation of cholesterol and, consequently, from formation of FC. Thus, the prevention of LDL aggregation as well as inhibition of fluid phase endocytosis or macropinocytosis specifically in macrophages could have anti-atherosclerotic effects. Interestingly, elevated circulating levels of the plasminogen activator inhibitor (PAI-1), and its upregulation in atherosclerotic tissues have been demonstrated [62, 63], and deletion of the PAI-1 gene reduced neointimal growth after injury in $ApoE^{-/-}$ mice despite the persistence of hypercholesterolemia [64]. Possibly, increased PAI-1 may prevent the release of aggregated LDL and thus may promote FC formation which stimulates atherogenesis and coronary artery disease. PAI-1 is the principal inhibitor of urokinase type plasminogen activator and tissue-type plasminogen activator, and reduction of its circulating levels could be favorable in the treatment of atherosclerosis. However, this potential treatment has limitations, because PAI-1 deficiency in mice leads to increased fibrinolysis and bleeding [65].

ENZYMES OF THE EICOSANOID CASCADE

Arachidonic acid (AA) is a common polyunsaturated fatty acid (PUFA) in cell membrane phospholipids. It can be derived from phospholipids by action of phospholipase A_2 [66] (Fig. **2**).

PLA_2 activity can be detected in vascular disease and appears to play a role in atherogenesis [67, 68]. Two major types of PLA_2 are present in monocytes/MΦ: a cytosolic type ($cPLA_2$) and secretory type PLA_2 ($sPLA_2$). $cPLA_2$ activity plays an important role in both $O_2^{\cdot-}$-production and optimal LDL lipid oxidation by activated human monocytes [8, 69].

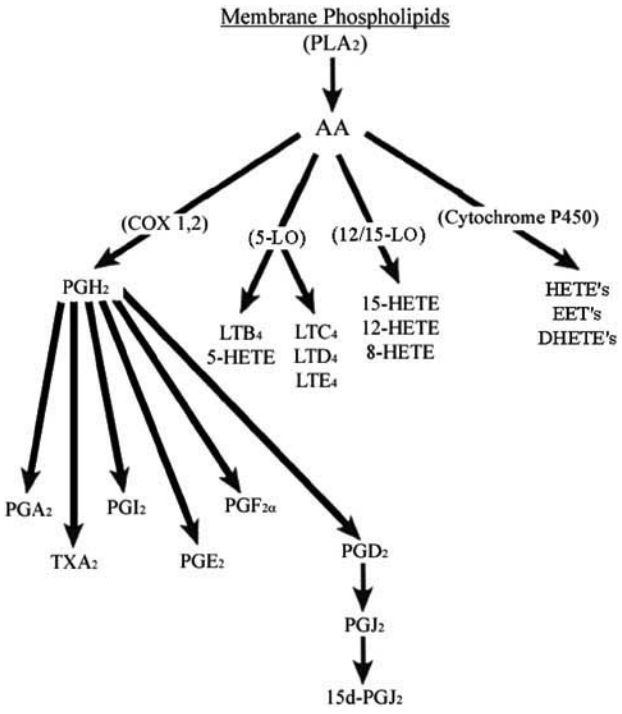

Fig. (2). Eicosanoid cascade.

PLA_2 = phospholipase A_2, AA = arachidonic acid, COX 1,2 = cyclooxygenase 1 and 2, LO = lipoxygenases, PG = prostaglandins, TXA_2 = thromboxane A_2, LT = leukotrienes, HETE's = hydroxyeicosatetraenoic acids, EET's = epoxyeicosatetraenoic acids, DHETE's = dihydroxyeicosatetraenoic acids.

Among the cytosolic isoforms of PLA_2, the 85 kDa Ca^{++}-dependent $cPLA_2$ ($cPLA_2$-α or $cPLA_2$-IV), but not the Ca^{++}-independent cytosolic PLA_2 ($iPLA_2$-β or $iPLA_2$-VI) has been shown to mediate the OxLDL-induced AA release in mouse peritoneal macrophages [70]. However, $iPLA_2$-β has been considered a target for the treatment of inflammatory diseases, including atherosclerosis, because it plays an important role in driving acute inflammation and is responsible for induction of $cPLA_2$-α [71].

Several secretory isozymes of PLA_2, in particular, $sPLA_2$-IIA, $sPLA_2$-X and $sPLA_2$-V, are now considered potential proatherosclerotic factors [72].

Arachidonic acid can be converted into a huge range of compounds termed eicosanoids, which includes leukotrienes, hydroxyeicosatetraenoic acids (HETE), and prostaglandins (PG). Eicosanoid metabolism is regulated by a series of enzymes including cytochrome P450-like enzymes, cyclooxygenases and lipoxygenases (Fig. **2**), some of which are related to atherogenesis [73-76]. $cPLA_2$–α in monocytes was shown to provide substrate preferentially utilized by cyclooxygenase(s), while $sPLA_2$ provides substrate preferentially utilized through the lipoxygenase (LO) cascade [71, 77]. Both cyclooxygenase- and lipoxygenase-derived metabolites may play a role in atherogenesis.

Cyclooxygenase (COX) products, prostaglandins, have multiple effects on atherogenesis [76, 78]. Aspirin, an inhibitor of cyclooxygenase(s), is an effective drug for the prevention of the complications of atherosclerotic cardiovascular disease in humans [79, 80] and was shown to have an antiatherogenic effect in mice [81, 82]. Aspirin treatment reduces the number of FC within atherosclerotic lesions in the LDL-Receptor knockout model of atherosclerosis in mice [82]. The beneficial effect of aspirin is thought to be mediated by a decrease in platelet aggregation through the inhibition of production of thromboxane A_2 (TXA_2) by plateletes [83]. Thus, TXA_2, a potent inducer of platelet adhesion and of vasoconstriction, is considered as "proatherogenic"eicosanoid [84]. Thromboxane receptor antagonist S18886 inhibited atherogenesis in ApoE-deficient mice [85]. The same authors failed to detect any effect of aspirin on atherogenesis, which suggests a role of compound(s) other than TxA_2 in promoting atherogenesis by its action at thromboxane receptors [85]. In summary, the inhibition of thromboxane synthesis or its receptor may protect against atherosclerosis.

Production of TXA_2 is mediated by constitutively expressed COX-1, whereas both COX-1 and inducible COX-2 mediate biosynthesis of prostacyclin PGI_2, which is a vasodilator, inhibits platelet activation and can be considered as "antiatherogenic" molecule [84]. COX-2 is widely expressed by macrophages, including FC [86, 87], and may stimulate production of another proinflammatory eicosanois, such as PGE_2. Activation of inducible cycloxygenase-2 and PGE_2 production mediates upregulation of monocyte-derived matrix metalloproteinase-1 by OxLDL, which contributes to vascular remodeling and plaque rupture [54]. Therefore, expression of COX-2 might play a role in atherosclerosis and a perspective of using specific inhibitors of this isozyme to treat atherosclerosis and cardiovascular events is discussed [84, 88-91], but the recent removal of COX-2 inhibitors from the market has called this idea into question. LDL-mediated chemotaxis of U937 cells is mediated by cyclooxygenase products [92]. In mouse peritoneal macrophages, OxLDL stimulates PGE_2 synthesis [93]. However, OxLDL suppressed COX-2 in human monocytes/MΦ [94]. Arachidonate metabolism in macrophages after foam cell transformation was found to be impaired (mainly, due to decreased phospholipase activity and changes in fatty acid composition) [95]. Interaction of prostanoids with their specific G-protein coupled membrane receptors (Table **2**) and nuclear receptors of the PPAR family (see below) may provide some explanation of their effects on atherogenesis, although the mechanisms are still not completely resolved [8].

A growing number of publications reflects an important role of the lipoxygenases in FC formation and development of atherosclerosis. There are several isoforms of LO: 12-LO, 15-LO (closely related), 8-LO and 5-LO [96]. One of the enzymes responsible for "minimal modification" of LDL is 12/15-LO [97]. 12/15-LO inserts molecular oxygen into polyenic fatty acids, producing H(P)ETE and H(P)ODE, which are likely transposed across the cell membrane to "seed" the extracellular LDL [98]. Deletion of 12/15-LO reduces atherogenesis in ApoE-knockout mice [99]. This study initiated a number of investigations on the proatherogenic function of 12-LO and its products [100-103]. The human isoenzyme 15-LO type 1 (15-LO-1) appears to be a human analog of murine 12/15-LO and is considered to

Table 2. Eicosanoid Receptors [112, 113, 122, 185-188]

	Eicosanoid	Eicosanoid receptor
Cyclooxygenase-derived	PGE_2	EP_1, EP_2, EP_3, EP_4
	PGF_{2a}	FP
	PGD_2	DP_1, DP_2
	PGI_2	IP
	TXA_2	TP
Lipoxygenase-derived	LTB_4	BLT_1, BLT_2
	Cysteinyl LT's	$CysLT_1$, $CysLT_2$

contribute to the formation of oxidized lipids in atherosclerotic lesions. Early observations demonstrated that 15-LO-1 is present in atherosclerotic lesions and colocalizes with macrophages [104]. However, a recent report failed to detect 15-LO-1 in human monocytes, FC, and advanced atherosclerotic lesions, thus raising a question whether this isoenzyme plays a significant role in human atherogenesis [105]. Instead, these more recent data point to the importance of another LO isozyme: 5-LO (Alox 5; EC 1.13.11.34). Indeed, OxLDL stimulates LTC_4 synthesis, which is mediated by 5-LO, in mouse peritoneal MΦ [89]. OxLDL was found to increase production of 5-HETE in monocytic cell lines, although it did not affect 5-LO mRNA in U937 cells [106]. By contrast, AcLDL, but not OxLDL, stimulates 12-HETE synthesis in mouse peritoneal macrophages [107].

The 5-LO gene maps to a mouse chromosome locus that confers almost total resistance to atherogenesis. Even heterozygous 5-$LO^{+/-}$ mice showed a dramatic decrease in aortic lesion development [73]. Enhanced expression of 5-LO in FC was first observed by Spanbroek *et al*. [105]. Using a customized microarray technology, we confirmed up-regulation of the 5-LO mRNA expression in a human monocyte-derived macrophage model of FC formation by 50% (unpublished observation). Based upon these data, it looks promising to use 5-LO inhibitors in the treatment and prevention of atherosclerosis. In contrast to 12/15-LO, 5-LO action is not associated with LDL oxidation [108]. Current concepts suggest that 5-LO cascade may generate pro-inflammatory mediators during critical stages of atherogenesis and that the 5-LO pathway may modulate the adaptive immune response [105, 109]. The precise molecular mechanisms by which 5-LO and its products promote atherogenesis are still not completely understood.

5-LO is a rate-limiting enzyme in the synthesis of leukotrienes and 5-hydroxyeicosatetraenoic acid (5-HETE) [110]. Activation of monocytes and MΦ is associated with enhanced production of a chemoattractant 5-LO product, leukotriene B_4 (LTB_4) [111]. Specific receptors for LTB_4, BLTR1 and BLTR2, are widely expressed on monocytes [112, 113]. BLTR2 can also bind 12-H(P)ETE and 15-HETE [114]. Consistent with the idea that products of 5-LO, including leukotriene B_4, could be of importance in atherogenesis, LTB_4 accumulates in human atherosclerotic plaques [115]. Observations on the role of the 5-LO products in atherogenesis are summarized in [116]. Interestingly, leukotriene B_4 receptor antagonism reduced monocyte-derived foam cell formation in mice [117]. LTB_4 is one of the most potent chemoattractants produced in atherosclerotic lesions and may be pro-atherogenic because of its ability to promote chemotaxis and adhesion of leukocytes [117]. LTB_4 antagonism had no significant effect on lesion size in MCP-$1^{-/-}$ApoE$^{-/-}$ mice, suggesting that MCP-1 and LTB_4 may either interact or exert their effects by a common mechanism [117]. MCP-1, a CC chemokine, is implicated in recruiting monocytes in atherogenesis [118, 119].

In addition to LTB_4, monocytes are able to synthesize from AA another type of leukotrienes, peptide or cysteinyl LT (LTC_4, LTD_4, LTE_4) [120, 121]. CysLT bind to specific CysLT receptors [122]. There is some evidence that a CysLT receptor may be involved in atherogenic processes: Allen *et al*. observed enhanced CysLT-induced contractility in vessels from atherosclerotic patients compared to normals [123, 124]. The authors concluded that atherosclerosis might be associated with expression of cysteinyl leukotriene receptor(s) capable of inducing hyperreactivity of human coronary arteries in response to peptide leukotrienes [124].

Of the two known receptors, CysLT1 and CysLT2 ([125] and Table **2**), MΦ predominantly express cysLTR1 [126]. Therapeutic antagonists of CysLT1 have been on the market since 1995 (pranlukast) with approved indications in the treatment of asthma [127]. Application of the receptor antagonists of peptide leukotrienes (montelukast, zafirlukast, pranlukast) as well of the inhibitors of BLTR may have clinical potential in the treatment or prevention of atherosclerosis.

A dual inhibitor of both 5-LOX and COX pathways, licofelone, is currently in stage III clinical trials for inflammatory disorders including osteoarthritis [128, 129]. This drug has been shown to possess analgesic, anti-inflammatory, antipyretic, antibronchocostrictory and antiplatelet-aggregating properties. Preliminary results of clinical studies of licofelone in osteoarthritis indicate that the drug has a comparable or slightly better efficacy than that of naproxen but possesses much better gastrointestinal safety [129]. The potential of this and similar drugs in the treatment of atherosclerosis remains to be established. Both thromboxane synthase activity and 5-lipoxygenase activity can be a target of pharmaceutical intervention. Dual inhibitors of both these activities such as CV6504 [130] may serve as a basis in the development of next generation of drugs against atherosclerosis, although they are not currently developed for this indication. In addition to the COX and LO pathway of AA metabolism, generation of non-enzymatic products of arachidonate termed isoprostanes is increased in both humans with atherosclerosis and in ApoE-knockout mice [131].

CHOLESTEROL EFFLUX PATHWAYS

Cholesterol efflux is as important for MΦ differentiation to FC as cholesterol accumulation. Bidirectional cholesterol efflux could occur via aqueous diffusion (inefficient pathway) and via SR-BI, while unidirectional efflux is mediated by ATP-binding cassette transporters (ABC), which encompasses seven distinct subfamilies (from A to F) [132-134]. Lipid efflux from MΦ or reverse cholesterol transport directs cholesterol to the liver and is mainly mediated by

high density lipoproteins (HDL). One of the originally discovered transporters, ABCA1, is involved in cholesterol and phospholipid transport in macrophages [135]. ABCA1 expression is highly regulated both at the transcriptional and translational levels [135]. It is of interest that unsaturated fatty acids force increased degradation of ABCA1 [136]. Mutations in the ABCA1 gene have been associated with familial high-density lipoprotein deficiency and Tangier disease characterized by low levels of HDL, lipid-poor apolipoproteins and hypertriglyceridemia [137, 138]. ABCA1 was considered as a new therapeutic target for treating cardiovascular disease [139]. However, the activity of ABCA1 is thought to mediate cholesterol efflux to lipid-poor apolipoproteins but not directly to HDL particles that constitute the bulk of plasma HDL [140]. Other ABC transporters, ABCG1 and ABCG4, as well as the scavenger receptor SR-BI, mediate cholesterol efflux to HDL but not to lipid-poor apolipoproteins [132, 140]. While ABCG4 mRNA levels are relatively low in both murine and human macrophages, ABCG1 is highly expressed in MΦ ([140] and our unpublished data). Thus, these transporters could play important roles in the protection against atherosclerosis and a search of genetic disorders predisposed to atherosclerosis may include a search for mutations in ABCG1 and ABCG4 genes and in other ABC transporters.

"METABOLIC RECEPTORS" AND THEIR ROLE IN FC FORMATION

MΦ cholesterol ester accumulation and consequently, FC formation, reflects a balance between SR-mediated cholesterol uptake and cholesterol efflux [134]. Thus, if SR expression could be generally considered as a pro-atherogenic factor, factors promoting lipid efflux from the lipid-loaded cell could be considered as "anti-atherogenic". A family of nuclear receptors called "metabolic receptors" includes peroxisome proliferator-activated receptors together with liver X receptors (LXRs) and farnesol-activated receptor [141]. All of them regulate gene expression in a heterodimeric complex with another nuclear hormone receptor, retinoic X receptor (RXR) [142-145]. Both PPARs and LXRs stimulate ABC-mediated cholesterol efflux, although they have many other effects.

PPAR Family of Nuclear Receptors

PPARs regulate the expression of various genes involved in lipid and carbohydrate metabolism and are implicated in metabolic disorders predisposing to atherosclerosis, such as diabetes and dyslipidemia [146]. There are 3 PPAR subtypes (α,γ,δ) that are often co-expressed [147]. PPARα and PPARγ are detected in macrophage-rich areas of atherosclerotic lesions. PPARδ is expressed in most tissues and has a less defined function, but it also may be related to atherogenesis because PPARδ activators promote reverse cholesterol transport [148]. PPARδ may control the inflammatory status of MΦ, because deletion of PPARδ from murine FC increased the availability of suppressors of inflammation, which led to decreased lesion formation in the LDL-$R^{-/-}$ mice model of atherosclerosis [149].

PPARγ activators can upregulate CD36 [46, 48] (likely pro-atherogenic effect) and also induce the expression of ABCA1, a transporter that controls apoAI-mediated cholesterol efflux from macrophages [143, 150, 151], as well as ABCG1 [152] (both anti-atherogenic). PPARγ ligands therefore may both promote and prevent the development of cardiovascular disease [153]. Probably, the net effect depends upon which pathway prevails. Most modern reviews estimate the net effect of PPAR activation as anti-atherogenic, because it increases turnover of deleterious OxLDL, stimulating both OxLDL uptake and reverse cholesterol transport, thus both decreasing oxidized LDL and providing cholesterol for HDL [8, 154].

PPARα ligands increase LOX-1 expression in vascular endothelial cells [155]. PPARα deficiency reduces atherosclerosis in $ApoE^{-/-}$ mice [156]. However, fibrate drugs, PPARα ligands, also reduce the process of atherosclerotic lesion formation. In addition to its effect on reverse cholesterol transport, this may be partly explained by repression of MHC-II expression and subsequent inhibition of T-lymphocyte activation, an effect shared by PPARγ agonists [146]. Some observations show that PPARα and PPARγ do not influence AcLDL-induced FC formation of human macrophages and may just activate ABCA1-mediated cholesterol efflux [151]. Aldehyde-containing phosphatidylcholines, which are supposed to be crucial contributors to the biological response of mmLDL [19], are also considered putative ligands for PPARα [157] and promote the formation of the proinflammatory chemokines IL-8 and MCP-1 by endothelial cells [157].

Since the creation of the first class of PPARγ agonists, thiazolidinediones, novel classes of both full and partial agonists with varying activities have been discovered [9]. This led to the hypothesis that one ligand can activate (or deactivate) PPARγ depending on the tissue in which PPARγ is expressed and new families of compounds were designated "selective PPAR modulators" or SPPARMs [9, 158-160]. SPPARMs might be new potential drugs in the treatment of atherosclerosis.

However, possible beneficial effects of PPAR agonists such as the thiazolidinedione family (rosiglitazone, pioglitazone, triglitazone), SPPARMs or PPARα agonists (fibrates) in the treatment of atherosclerosis need to be further verified. PPARγ ligands can induce expression of adipocyte lipid binding protein (ALBP/aP2) [161]. ALBP/aP2 is a gene that is highly upregulated in foam cells in response to oxLDL. It can facilitate cholesterol ester accumulation and was shown to be highly expressed *in vivo* in macrophage/foam cells of human atherosclerotic plaques. Thus the clinical use of insulin-sensitizers such as thiazolidinediones for the prevention of atherosclerosis must await the outcome of clinical trials designed to address this issue [161].

LXRs and FC Formation

The queston of whether liver X receptors are theraupeutic targets in atherosclerosis is widely discussed in recent publications [134, 141]. LXRs are nuclear receptors activated by oxysterols [134]. LXRs form obligate heterodimers with the RXR and regulate the expression of target genes containing LXR response elements [141]. The LXR signaling pathway in MΦ is involved in both promotion of lipid metabolism and cholesterol efflux and repression of inflammatory genes [134]. LXR targets include the cholesterol

efflux transporters ABCA1, ABCG1, ABCG5 and ABCG8 [134]. LXR also controls both fatty acid as well as cholesterol metabolism and mediates the lipid-inducible expression of ApoE in MΦ [162]. LXR activity in MΦ is a strong determinant of susceptibility to atherosclerosis [134]: deletion of LXR in MΦ led to a significant increase in atherosclerotic lesion formation in both $ApoE^{-/-}$ and $LDL\text{-}R^{-/-}$ mice [163].

Joseph and Tontonoz suggested that induced transcription of LXR target genes may be a potential strategy to treat atherosclerosis [141]. Treatment of lipid-loaded cells with LXR ligand GW3965 led to a dose-dependent increase in expression of the genes responsible for reverse cholesterol transport [164]. It was noted that LXR-selective agonists might be useful in modulation of cholesterol efflux, although their lipogenic activity might be a significant limitation [141]. Administration of another LXR agonist, T-0901317, to $LDL\text{-}R^{-/-}$ mice on a Western diet inhibited the formation of atherosclerotic lesions and induced expression of ABCA1 in macrophages [165]. LXR agonists that specifically activate LXR in macrophages, but not in the liver, or specific LXRβ agonists, with selective activity on cholesterol reverse transport, but not on lipogenesis, might be useful [134, 166], but no clinical data exist at this time.

INTERRELATIONSHIP BETWEEN EICOSANOIDS, PPARS AND STIMULATION OF FC FORMATION AND ATHEROGENESIS BY MODIFIED LDL

PUFA belonging to both the ω-6 (linoleic acid, AA) and ω-3 family (eicosapentaenoic acid, docosahexaenoic acid) may modulate expression of CD36. Despite some variability in the results [167-169], the general conclusion is that specific modulation of CD36 by PUFA may be involved in the initiation and progression of atherogenesis [167].

Direct interaction of the lipoxygenase cascade, scavenger receptors and the PPAR family of nuclear receptors has also been demonstrated: 15-LO was shown to regulate PPARγ activity by providing activating ligands that ultimately lead to the induction of expression of CD36 [46, 48, 170]. MΦ uptake of OxLDL provides ligands for PPARγ, including 9-HODE and 13-HODE [46, 171]. The mechanism by which OxLDL upregulates CD36 appears to involve activation of PPARγ [172] and PPARα [173]. PPARγ is induced in human monocytes following exposure to OxLDL and is expressed at high levels in foam cells [48]. The ability to induce PPARγ expression was specific for OxLDL, because native or AcLDL had no effect [48]. PPARγ was shown to be a positive modulator of another SR, SR-A [13]. It was also suggested that OxLDL modulates the oxidative burst in macrophages via activation of PPARγ, which could be related to the progression of atherosclerosis [174].

Many fatty acids, such as palmitic, stearic, linoleic acid as well eicosanoids (leukotriene B_4, HETE) and OxLDL derivatives (9-HODE, 13-HODE) are PPAR activators [175]. The cyclooxygenase-2 metabolite 15-deoxy-Δ[12, 14]-prostaglandin J_2 (15d-PGJ2) was identified as a natural ligand for PPARγ [176] and was found in the cytoplasm of FC in human atherosclerotic plaques [177], although its PPARγ-independent actions have been also described [178]. PPARγ expressed in macrophages has been postulated as a negative regulator of inflammation and a positive regulator of their differentiation into foam cells. 15d-PGJ2 suppresses the lipopolysaccharide-induced expression of COX-2 in macrophage-like differentiated U937 cells [179]. Expression of COX-2 in macrophages was suggested to be regulated by a negative feedback loop mediated through PPARγ, which makes possible a dynamic production of PG [179]. On the other hand, Pontsler *et al*. demonstrated COX-2 induction by selective PPARγ agonists, which include oxidatevely fragmented phospholipids in OxLDL [180]. Obviously, this discrepancy could reflect differences in the specificity of the PPARγ agonists, but also suggests that this system is incompletely understood. PPARα agonists such as PUFA and prostaglandins could also promote COX-2 induction [181, 182].

OxLDL induces $cPLA_2$ activation, which contributes to the supply of fatty acids required for the cholesteryl esterification, probably through the acceleration by oxidized lipids of the catalytic action of $cPLA_2$ in macrophages [183]. $cPLA_2$-α plays a role in PPAR-mediated gene transcription in human hepatoma cells, and this effect is likely mediated by AA and prostaglandin E_2 [147]. Thus it is assumed that $cPLA_2$-α may represent another therapeutic target for treatment of atherosclerosis [147]. PUFA released by phospholipase A_2 could activate LXRα transcription through activation of PPARα [184]. Regulation of lipid metabolism by naturally occuring PUFA through nuclear receptors may shed a light on novel aspects of arising and persisting of disorders such as atherosclerosis.

CONCLUSION

Taken together, scavenger receptors, peroxisome proliferator-activated receptors and eicosanoids interact during foam cell formation, and thus could all be targets for therapeutic intervention. All these groups of molecules have both pro- and anti-atherogenic effects. While some of their effects in simple cellular systems like *in vitro*-generated foam cells are relatively clear, surprising outcomes in gene-targeted mice and during treatment with agonists and antagonists have been confusing. Any effects on foam cell formation should not be seen in isolation, but must be evaluated in the context of pro- and anti-inflammatory effects that represent independent risk factors for coronary artery disease and stroke.

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ABBREVIATIONS

AA = Arachidonic acid

ABC = ATP-binding cassette transporter

acLDL = Acetylated LDL

ALBP/aP2 = Adipocyte lipid binding protein

ApoE	=	Apolipoprotein E
COX	=	Cyclooxygenase
DHETE	=	Dihydroxyeicosatetraenoic acid
EET	=	Epoxyeicosatetraenoic acid
FC	=	Foam cell
FCS	=	Fetal calf serum
HDL	=	High density lipoproteins
H(P)ETE	=	Hydro(pero)xyeicosatetraenoic acid
H(P)ODE	=	Hydro(pero)xyoctadecadienoic acid
LDL	=	Low density lipoproteins
LO	=	Lipoxygenase
LOX-1	=	Lectin-like oxidized LDL receptor-1
LPS	=	Lipopolysacharide
LT	=	Leukotriene
LXR	=	Liver X receptor
mmLDL	=	Minimally modified LDL
MΦ	=	Macrophage
OxLDL	=	Oxidized LDL
PAI-1	=	Plasminogen activator inhibitor-1
PG	=	Prostaglandin
PLA_2	=	Phospholipase A_2
PPAR	=	Peroxisome proliferator- activated receptor
PUFA	=	Polyunsaturated fatty acid
RXR	=	Retinoic X receptor
SPPARM	=	Selective PPAR modulator
SR-PSOX	=	Scavenger receptor for phosphatidylserine and oxidized lipoprotein
TBARS	=	Thiobarbituric acid reactive substances
TLR4	=	Toll-like receptor 4
TXA_2	=	Thromboxane A_2
15d-PGJ2	=	15-deoxy-Δ[12, 14]-prostaglandin J_2

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