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MINISYMPOSIUM

# Modified LDL – trigger of atherosclerosis and inflammation in the arterial intima

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Atherosclerosis is characterized by chronic inflammation of an injured intima. The pathological processes are initiated by accumulation of morphologically distinct, modified forms of LDL, and followed by cellular infiltration and foam cell formation. Activated intimal cells secrete enzymes and agents capable of modifying LDL, and the modified lipids of LDL, in turn, are able to activate intimal cells and to trigger various inflammatory signals. These processes can initiate and maintain a vicious circle in the intima and lead to lesion progression. In this review, we focus on the LDL modifications relevant to the initial lipid accumulation and discuss their pro-inflammatory effects.

**Keywords:** atherosclerosis, inflammation, LDL, lipoproteins.

## Introduction

Atherosclerosis is an inflammatory disease of the arterial inner layer, wrote Rudolf Virchow, the creator of modern cellular pathology, more than 100 years ago [1]. This was also the last message of the giant of vascular biology of our times, Russell Ross, in his will for the coming millennium [2]. Early in this century, Sir William Osler also said that acute infection is one aetiological factor of atherosclerosis. Decade after decade, the major infectious agent of the particular time has been proposed, starting from bacterial infections (diphtheria, pneumonia, tuberculosis, typhoid fever), and then followed by suggestions of a viral aetiology (herpes simplex virus, cytomegalovirus) [3]. The latest and, at the moment, the most promising candidate is *Chlamydia pneumoniae* [4].

At the beginning of the century, however, the outstanding results obtained with cholesterol-fed rabbits shifted the major interest in the field of atherosclerosis research to the role of cholesterol [5]. The price paid was that the inflammatory and infectious hypotheses of atherosclerosis were pretty much ignored. Yet, of all the approaches in this field of research, the cholesterol era, which has lasted the entire century, has been, both diagnostically and therapeutically, the most successful [6]. Indeed, in atherosclerosis, in contrast to the various forms of arteritides, cholesterol accumulates in the intima; this is the most characteristic feature of atherosclerosis, and without cholesterol there is no atherosclerosis [1, 7].

Today, we appreciate the importance of all three components – cholesterol, infection and inflammation – in the genesis of atherosclerosis. But we are still ignorant of their interrelations, and we do not know which of the three comes first. However,

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atherosclerosis is a disease which progresses slowly, and in its course all these three components are likely to be present.

Low-density lipoprotein (LDL) particles are the carriers of cholesterol into the arterial wall. For LDL particles to become atherogenic, they have to be modified [8, 9]. Atherosclerosis typically develops at certain predilection sites. These sites are the branching points of arteries that are subject to turbulent flow. Inflammation develops as a response to the haemodynamic stress and other types of injury [10], and in such an inflammatory environment the LDL-modifying cellular processes become activated. Because modified LDL particles and the products released from them are pro-inflammatory, they themselves may have profound cell-activating effects and worsen the inflammation. Finally, it has been suggested that blood monocytes carry *Chlamydia pneumoniae* bacteria into atherosclerotic lesions [11]. Since monocytes preferentially adhere to the endothelium covering inflamed tissue sites, infection of the lesions may be a secondary phenomenon. Then again, infection of an inflamed tissue can worsen the inflammation and so accelerate LDL modification (Fig. 1). Accordingly, at any time point after the initial events, it is difficult to decide which of the three components of atherogenesis has a primary and which a secondary nature. In this regard, it is of particular interest that modified LDL has been observed in aortic samples from human fetuses even before signs of inflammation (e.g. macrophage infiltration) were visible [12]. Thus, LDL modification appears to be a very early event in the pathogenesis of atherosclerosis.

## LDL particles: the carriers of cholesterol into tissues

The bulk of the cholesterol circulates in LDL particles in the blood plasma. The LDL particles are spherical, consisting of a polar surface and an apolar core. The surface is composed of unesterified cholesterol, the phospholipids phosphatidylcholine and sphingomyelin, and a single polypeptide, the apolipoprotein B-100 (apoB-100) [13, 14].

### *Tissues with blood capillaries and lymphatic capillaries*

The physiological function of LDL particles is to provide cells with the cholesterol they need [6]. A fraction of the particles find their way via the bloodstream across the capillary endothelium to various extrahepatic tissues. The particles either cross the endothelium in transcytotic vesicles or pass between the endothelial cells [15–17]. After crossing the capillary endothelium, some of them bind to LDL receptors on tissue cells via a special sequence of the apoB-100. The particles are then endocytosed by the cells [18]. Within lysosomes, acidic proteases hydrolyse the apoB-100 and acidic lipases hydrolyse lipids, the particles being completely broken down.

The cholesterol released from the particles crosses the lysosomal membrane and is incorporated into the cellular membranes where cholesterol is needed. This receptor-mediated uptake is tightly regulated: once the cell has obtained the cholesterol it needs, it shuts down LDL receptor synthesis and so prevents intracellular accumulation of cholesterol [6]. Any excess of LDL particles not taken up by cells is rapidly removed by the lymphatic system which prevents extracellular accumulation of LDL particles. Accordingly, the concentration of LDL in the interstitial fluids of the various extrahepatic tissues is only one-tenth of that in the blood plasma [19]. Thus, in extrahepatic tissues, the regulated LDL receptor system and the lymphatic system ensure that neither intracellular nor extracellular accumulation of LDL cholesterol or other lipids takes place.

### *The arterial intima: a tissue without blood capillaries or lymphatic capillaries*

The fate of LDL particles that enter the arterial intima differs from that in other extrahepatic tissues: in the intima there is retention, modification and both intra- and extracellular accumulation of LDL particles and of the lipids they contain [20]. The most remarkable histological difference from other tissues is the lack of lymphatic vessels [21]. Accordingly, to reach the nearest lymphatic vessels which are located in the medial layer, the LDL particles have to pass through the intima. However, this passage is at least partly blocked by an elastic layer situated between the intima and the media. Thus, the passage of LDL particles is, at best, slow, and more LDL particles enter the intimal fluid than are removed from it. This imbalance leads to a progressive increase in the concentration of LDL in the intimal fluid, until it reaches, or even surpasses, that in the blood plasma [22]. Thus, during a steady

**Fig. 1** Injury, inflammation, LDL modification and infection in the development of atherosclerotic lesions.

state, the intimal fluid has an LDL concentration $\approx$ 10-fold higher than the interstitial fluids of other tissues [22]. This high concentration downregulates LDL receptors and so closes the physiological exit of LDL particles into cells.

## LDL and the extracellular matrix

The extracellular matrix of the arterial intima is composed of a tight negatively charged proteoglycan network [23]. The apoB-100 of LDL has specific sequences which contain clusters of positively charged amino acids lysine and arginine [24]. These sequences, called heparin-binding domains, interact with the negatively charged sulphate groups of the glycosaminoglycan chains of the proteoglycans, the binding resulting in extracellular retention of LDL particles in the arterial intima.

In the subendothelial space, the LDL particles appear to be associated with surface structures on collagen [25, 26]. Indeed, the collagen in the arterial intima is decorated by a small-molecular-weight proteoglycan [27], and our *in vitro* experiments have shown that the collagen-binding proteoglycan decorin binds LDL and so anchors it to the collagen [28]. Moreover, the extracellular matrix contains large chondroitin sulphate proteoglycans, either in monomeric form or as aggregates bound to

hyaluronic acid [23]. During their long stay in the extracellular intimal space, the LDL particles become exposed to many agents that can modify them. Some of the modifications increase, and others decrease the strength of binding of LDL to the matrix. Since LDL is constantly bound to and released from the intimal extracellular matrix components (starting at the basement membrane of the endothelium), it is likely that modifications take place both when bound and when not bound to the extracellular matrix. The interaction between LDL and these proteoglycans has been the topic of pioneering studies by German Camejo and coworkers for over 20 years [29].

Taken together, it is evident that LDL particles interact primarily with the various forms of proteoglycans in the subendothelial space, and in fact this interaction appears to be crucial for the development of atherosclerosis [20]. Very recently, the importance of LDL binding to the matrix was highlighted in experiments performed by Borén *et al.* [30]. These workers engineered human LDL with mutations of all the basic amino acids (arginines to serines and lysines to alanines) in a proteoglycan-binding region of apoB-100, and observed that such recombinant LDL particles failed to bind to heparin proteoglycans. When expressed in mice, the recombinant LDL caused only a mild degree of atherosclerosis despite strong hypercholesterolaemia [31].

Although extensive modification of LDL takes place only in the arterial intima, it is possible that LDL circulating in the bloodstream is already mildly modified, which may enhance the modification in the arterial intima. Thus, there is evidence for the presence of mildly oxidized or desialylated LDL circulating in the blood plasma [32, 33]. Although the fraction of the plasma LDL particles that are modified is very small, the pool may have a rapid turnover, which, in the course of time, would mean production of large quantities of modified particles within the circulation. How many of these particles find their way to the intimal areas which are the future sites of atherosclerosis is not known. Interestingly, it has recently been suggested that the particles modified in plasma could accumulate in the arterial intima in preference to native LDL particles [34].

Circulating LDL can also damage endothelial cells and cause endothelial dysfunction [35]. Whether this damage is due to the small modified (oxidized) fraction or the bulk of unmodified LDL is not known. Such dysfunctional endothelium, again, could modify (oxidize) LDL at an enhanced rate. Thus, regarding interactions between LDL and the endothelium, the end result may be enhanced influx of modified (oxidized) LDL into the arterial intima. Other types of endothelial damage, e.g. of bacterial or viral origin, could also activate endothelial cells to modify the LDL particles that are passing by or through them [36].

## Formation of lipid droplets: the first morphological sign of LDL modification

The first indication of rapid modification of LDL particles in the subendothelial space came from Fogelman's laboratory, where the astonishing observation was made that subendothelial LDL particles were enlarged only 2 h after injection of large amounts of human LDL into a normal rabbit [25]. Spontaneous development of similar kind of particles was previously observed in aortas of cholesterol-fed and WHHL rabbits [37, 38], and very recently also in the apoE knockout mice [26].

Based on the above observations, it appears that the initial morphological sign of atherosclerosis is the appearance of enlarged LDL particles in the subendothelial space [25, 26, 38]. Interestingly, decades ago Elspeth Smith [39], in her pioneering human studies, also noted that the first morphological sign of lipid accumulation in the arterial intima is the appearance of small perifibrous (pericollagenous) lipid droplets in the subendothelium. Guyton *et al.* [40–42] have also observed lipid droplets and lipid vesicles, in both the superficial and deep layers of the arterial intima and at all stages of atherogenesis. This suggests that extracellular lipid droplets and vesicles have an important role in the formation of intimal lipid deposits. Recently, Pasquinelli *et al.* [43] also observed initially small lipid droplets in the extracellular space of the superficial (subendothelial) intimal layer of human carotid arteries. Later, in the fatty streak stage with the typical cholesteryl ester-filled foam cells, no extracellular droplets were visible, which strongly suggests that they had been ingested by the macrophages. This sequence of events fully agrees with the *in vitro* data, which have convincingly demonstrated that macrophages ingest modified LDL particles by endocytosis or phagocytosis, by mechanisms that are either scavenger

receptor-dependent or -independent [8]. This ingestion is not regulated by the incoming cholesterol and so allows the macrophages to become filled with LDL-derived cholesteryl esters, i.e. to be converted into foam cells [44]. We have approached the question of the initial modifications of LDL leading to enlargement of the particles by performing a systematic series of *in vitro* experiments in which LDL particles have been modified by various agents and their fusion detected by several different methods [45–47].

## Proteolytically modified LDL particles: degradation of apoB-100

Proteolytic fusion of LDL was originally discovered on the surface of granule remnants in experiments with exocytosed granules of rat serosal mast cells, to which both the proteases and the LDL particles were bound [48, 49]. The granule remnants are small cytoplasmic cell organelles composed of heparin proteoglycans and two types of neutral proteases bound to them. These neutral proteases are chymase, a chymotrypsin-like endopeptidase, and carboxypeptidase A (CPA), an exopeptidase. Chymase degrades apoB-100 extensively and, from the many peptides formed, CPA then cleaves off the C-terminal amino acids. The role of the heparin proteoglycan matrix was highlighted by the finding that heparin proteoglycan-bound chymase preferentially proteolysed LDL particles that were also heparin-bound [50]. The particle-stabilising function of apoB-100 was emphasized by the experiments, since it turned out that the degradation and loss of apoB-100 due to proteolysis by chymase led to instability of the modified particles and ultimately to their fusion [50]. Later, when studying LDL proteolysis in the absence of heparin proteoglycans, we found that loss of apoB-100 peptides from the surface of the proteolysed LDL particles is, in fact, a prerequisite for their fusion [51]. This kind of release of apoB-100 peptides occurs after treatment of the particles with broad-spectrum endopeptidases such as chymotrypsin, trypsin or pronase [51]. In contrast, cleavage of apoB-100 by plasmin, kallikrein or thrombin, which led to formation of only a few large peptide fragments that remained bound to the particles, did not render these particles sufficiently unstable to induce their fusion. Interestingly, later studies showed that human arterial proteoglycans increase the rate of LDL fusion triggered by α-chymotrypsin [46].

In the human arterial intima, chymase-containing mast cells are present, and extracellularly located granule remnants are visible by microscopic examination [52]. Such exocytosed mast cell granule remnants have also been found to bind LDL particles in the intima, suggesting that chymase is one intimal enzyme capable of proteolysing apoB-100 [53]. Interestingly, the apoB-100 in a fraction of LDL particles isolated from the atherosclerotic arterial intima is fragmented and the fragmentation pattern is similar to that found in LDL particles after they have been proteolysed by heparin proteoglycan-bound chymase *in vitro* [54–57]. However, the fragmentation pattern is not specific, and other neutral proteases also have to be considered as agents potentially capable of modifying LDL in the intima. Interestingly, recent immunostaining experiments by Torzewski *et al.* [58] with monoclonal antibodies against modified LDL have provided evidence for the presence of proteolytically (trypsin) modified LDL in human atherosclerotic intima. It should be kept in mind, however, that fragmentation of apoB-100 can also result from oxidative modification of LDL [56, 59]. Accordingly, a detailed analysis of the apoB-100 cleavage sites in the LDL particles isolated from lesions is necessary for identification of the various proteases and oxidative agents that may modify LDL *in vivo*.

## Phospholipolytic modification of LDL particles

A second possible type of modification of LDL particles is lipolysis. LDL particles isolated from atherosclerotic lesions and extracellular lipid droplets are relatively enriched in sphingomyelin and lysophosphatidylcholine, a finding suggesting hydrolysis of the phosphatidylcholine of LDL [55]. Indeed, in the human arterial intima, notably in inflamed areas, a phospholipase of secretory type (the secretory type II phospholipase $A_2$), capable of hydrolysing LDL phosphatidylcholine, has been found [60–62]. In addition, aggregated LDL isolated from atherosclerotic lesions contains ceramide, the lipolytic product of sphingomyelinase, revealing hydrolysis of sphingomyelin [63]. Furthermore, hydrolysis of sphingomyelin could be demonstrated in LDL particles retained in strips of human aorta

when such strips were incubated with LDL *in vitro* [63]. Interestingly, for human secretory sphingomyelinase to act on LDL particles, the particle surface has first to be slightly altered, e.g. by the action of phospholipase $A_2$ or by lipid peroxidation [64]. These findings, together with the fact that both secretory sphingomyelinase and secretory phospholipase $A_2$ have been found in the arterial intima, support the view that these two lipolytic enzymes play roles in the modification of LDL during atherogenesis. We found that, in the fluid phase, sphingomyelinase induced both aggregation and fusion, but that phospholipase $A_2$ caused only aggregation of LDL [65]. However, if heparin was added to the incubation medium, phospholipase $A_2$ also induced fusion of LDL particles [66], similar to the effect of sphingomyelinase treatment of heparin proteoglycan-bound LDL particles [67]. Taken together, it appears that the conditions favouring lipolytic fusion include several lipases acting in concert and a proteoglycan-containing environment.

## Oxidative modification of LDL

Some 10 years ago, evidence of the presence of oxidatively modified LDL particles in atherosclerotic lesions was presented [55], and since then its role in the pathogenesis of the disease has been under unceasing investigation. To date, it is known that oxidized LDL is present in atherosclerotic lesions, and evidence of multiple mechanisms of its generation *in vivo* has been presented. Whether the amount of oxidized LDL particles and the degree of oxidation of the particles are sufficient to trigger the various effects that have been assigned to LDL extensively modified *in vitro* is unclear [68]. It is interesting that even minor oxidative damage to the surface phospholipids of LDL has been shown to lead to the formation of biologically active lipids capable of triggering various pro-inflammatory signals, and, on the other hand, that the enzymes platelet-activating factor acylhydrolase (PAF-AH) and paraoxonase (PON), capable of breaking down these biologically active phospholipids, are carried with lipoproteins [69]. Interestingly, in the rat, a single injection of human LDL resulted in accumulation of LDL in the arterial wall, where it became oxidatively modified within 6 h [70]. The presence of oxidized LDL was associated with activation of the transcription factor nuclear factor κB (NFκB) in the endothelium, as well as with endothelial expression of intercellular adhesion molecule-1 (ICAM-1).

Oxidative modification, even when mild, alters the structure of the LDL particles and renders them capable for aggregation [56]. As the oxidation progresses, the particles undergo fusion which also generates vesicles and irregular pieces of membranous material [45, 71]. Thus, *in vitro* oxidation of LDL is, at least in theory, capable of creating lipid particles similar to those found in the arterial wall. However, it is unknown whether the high degree of oxidation required to produce these modified particles occurs *in vivo*, but it is likely that oxidation participates in rendering the extracellularly deposited LDL particles more labile and susceptible to enzymatic modifications than native LDL.

## Hydrolysis of the cholesteryl esters of LDL

Extracellular accumulation of unesterified cholesterol-rich liposomes in the arterial intima, both in the early developing lesion [41, 72] and in the atherosclerotic plaque [73, 74], has suggested that the cholesteryl esters of LDL are hydrolysed in the arterial intima. *In vitro* experiments have shown that fused particles and vesicles enriched in unesterified cholesterol can be formed after treatment of LDL with a fungal cholesterol esterase after limited trypsinization [75, 76]. Although cholesterol esterase activity in human, rabbit and rat aortas have been demonstrated [77, 78], solid demonstration of a potential enzyme responsible for the hydrolysis of the cholesteryl esters of LDL has been lacking. Interestingly, Shamir *et al.* [79] recently reported the presence of carboxyl ester lipase, a lysophospholipase and bile salt-activated cholesterol esterase, in human atherosclerotic aorta. This enzyme has been shown to be secreted by both endothelial cells [80] and macrophages [81] in culture. Whether this enzyme will turn out to be anti-atherogenic via its lysophopholipase activity or pro-atherogenic via its cholesterol esterase activity remains to be investigated.

## Effect of modification of LDL particles on their interaction with proteoglycans

We made the unexpected observation that the proteolysed, fused LDL particles that had lost

substantial quantities of their apoB-100 on the exocytosed rat mast cell granules were bound to the granule heparin proteoglycans more strongly than their native counterparts [82]. This observation could be extended to the interaction between proteolytically modified LDL particles and human aortic proteoglycans [83]. Thus, when LDL particles were incubated with chymotrypsin and then allowed to flow through a column in which human arterial proteoglycans had been immobilized, a fraction of the particles were bound more tightly to the proteoglycans than were the non-proteolysed particles. We were able to show that the particles which were bound tightly were larger in size than native particles, i.e. they had fused.

To study the mechanism of the increase in binding strength, we separated the fused from the non-fused particles by size-exclusion chromatography and estimated the number of so-called active lysine residues of apoB-100 by nuclear magnetic resonance spectroscopy in the two particle populations. These studies showed that the number of active lysines per fused particle was 10-fold higher than in the non-fused particles despite loss of apoB-100 fragments [83]. Thus, the loss of active lysines due to proteolysis must have been less than their gain due to addition of new apoB-100 fragments to the particles due to particle fusion. This gain may also be partially due to exposure of lysine-containing regions buried in structurally intact apoB-100 of native LDL.

We have, moreover, observed that, in the LDL particles that have undergone fusion after treatment with sphingomyelinase or phospholipase $A_2$, the strength of binding to proteoglycans is increased [65]. Taken together, fusion of LDL, whether induced by hydrolysis of apoB-100 or of the two major components of surface phospholipids, is accompanied by increased strength of particle binding to proteoglycans. Moreover, an increase in the number of active lysines was observed irrespective of the modification leading to fusion. Therefore, it is likely that this increase in the active lysines is a major factor contributing to the increased binding strength, since it would allow the particles to interact with a larger number of negatively charged groups of the glycosaminoglycans present in the proteoglycans.

Oxidation of LDL inhibits rather than augments the interaction between LDL and proteoglycans [84]. Therefore, if LDL becomes oxidized before being bound to the proteoglycans, it is likely to remain in the intimal fluid. Alternatively, if LDL particles become proteoglycan-bound, and then oxidized, they are likely to be released from their binding sites [85].

## Effect of LDL modification on their interaction with cells: possible triggers of inflammation

The modified LDL particles can trigger various pro-inflammatory reactions mainly via the lipids, such as fatty acids, lysophosphatidylcholine, oxidatively modified phospholipids, ceramide and unesterified cholesterol. Lipolysis of LDL phospholipids by phospholipase $A_2$ [66, 86, 87] and lipolysis of the cholesteryl esters by carboxyl ester lipase [79] release fatty acids from LDL which may lower smooth muscle cell proliferation and induce secretion of extracellular matrix capable of trapping increasing amounts of LDL [88]. Lipolysis of LDL phospholipids by phospholipase $A_2$ and lipid peroxidation [89] generate lysophosphatidylcholine, which has potentially a multitude of pro-inflammatory effects in the arterial intima. Lysophosphatidylcholine and oxidized LDL can be chemoattractants for monocytes and T lymphocytes, induce expression of growth factors and adhesion molecules in endothelial cells, and be mitogenic for macrophages and smooth muscle cells [90, 91]. In addition, oxidized LDL is immunogenic, and autoantibodies are commonly found both in experimental animals and in human subjects with atherosclerotic lesions [91]. Even minimally oxidized LDL contains modified phospholipids that are chemotactic for monocytes [92]. Secretory sphingomyelinase can hydrolyse sphingomyelin in modified LDL to ceramide, which is a potent intracellular messenger molecule. It is possible that when intimal cells, particularly macrophages, ingest large amounts of sphingomyelinase-modified LDL, some of the ceramide escapes lysosomal hydrolysis and enters the signalling pathway [63].

Cholesterol esterase-treated LDL, if taken up by macrophages, induces secretion of macrophage chemotactic protein-1 (MCP-1) by these cells, a powerful cytokine capable of inducing influx of monocytes into inflammatory tissue sites, such as the atherosclerotic lesions [93]. Massive uptake of

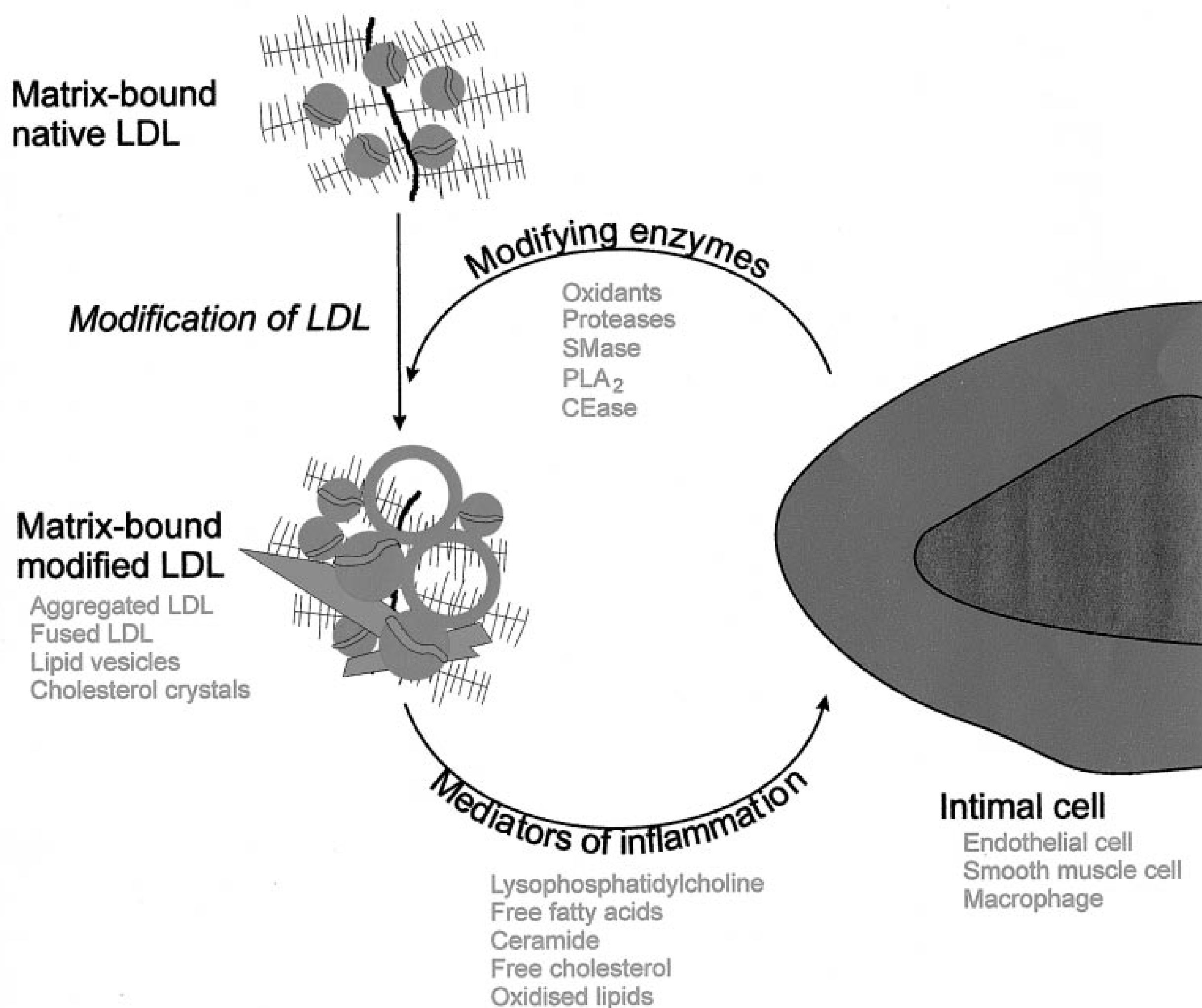


**Fig. 2** Vicious circle caused by LDL modification in the arterial intima. Modification of LDL entrapped in the arterial intima causes formation and release of potent inflammatory mediators. These mediators cause intimal cells to secrete increasing amounts of enzymes capable of modifying LDL and increasing amounts of proteoglycans capable of trapping LDL in the arterial intima. This can lead to a self-perpetuating circle which accelerates the development of atherosclerotic lesions. SMase, sphingomyelinase; $PLA_2$, phospholipase $A_2$; CEase, cholesterol esterase.

these particles could also result in loading of macrophages with unesterified cholesterol, which is highly cytotoxic [94]. Cholesterol esterase-treated LDL (like oxLDL) induces selective adhesion and transmigration of monocytes and T lymphocytes through endothelial cell monolayers [95]. In addition, cholesterol esterase-treated LDL can bind the complement and C-reactive protein (CRP) [96], and in this way may elicit various inflammatory responses in the arterial intima. The role of the complement in the development of atherosclerosis was recently highlighted by the experiments of Schmiedt *et al.* [97], who showed that genetic deficiency of the complement component C6 protects rabbits against diet-induced atherosclerosis.

## Conclusions

Modification of LDL particles appears to be a key event in the initiation of atherogenesis. Anchoring of LDL to extracellular matrix increases the residence time of LDL in the intima, and so allows extensive modification, possibly multiple types, to take place. It appears that in the arterial intima, LDL

modifications can trigger a vicious circle (Fig. 2). In this scheme, matrix-bound LDL particles are modified by enzymes secreted by intimal cells. The modified particles become unstable, and aggregate, fuse and form lipid vesicles and membranous material. The modified forms of LDL have many pro-inflammatory effects on intimal cells, which can stimulate the cells to secrete increasing amounts of enzymes capable of modifying LDL still further.

Since modification of LDL particles is likely to be critical for their atherogenicity, i.e. to cause accumulation of lipids in the arterial intima, inhibition of LDL modification could be a therapeutic goal. Perhaps the intimal cells could be prevented from becoming activated and thereby from secreting proteases, lipases and oxidizing agents. This takes us back to the all-important question of the initiation of inflammation and infection in the arterial intima. Until we know the answer, we should treat all three contributing components of atherogenesis – lipids, inflammation and infections – when present.

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