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Review article

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Apolipoprotein B-containing lipoproteins in atherogenesis

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Abstract

Apolipoprotein B (apoB) is the main structural protein of LDLs, triglyceride-rich lipoproteins and lipoprotein(a), and is crucial for their formation, metabolism and atherogenic properties. In this Review, we present insights into the role of apoB-containing lipoproteins in atherogenesis, with an emphasis on the mechanisms leading to plaque initiation and growth. LDL, the most abundant cholesterol-rich lipoprotein in plasma, is causally linked to atherosclerosis. LDL enters the artery wall by transcytosis and, in vulnerable regions, is retained in the subendothelial space by binding to proteoglycans via specific sites on apoB. A maladaptive response ensues. This response involves modification of LDL particles, which promotes LDL retention and the release of bioactive lipid products that trigger inflammatory responses in vascular cells, as well as adaptive immune responses. Resident and recruited macrophages take up modified LDL, leading to foam cell formation and ultimately cell death due to inadequate cellular lipid handling. Accumulation of dead cells and cholesterol crystallization are hallmarks of the necrotic core of atherosclerotic plaques. Other apoB-containing lipoproteins, although less abundant, have substantially greater atherogenicity per particle than LDL. These lipoproteins probably contribute to atherogenesis in a similar way to LDL but might also induce additional pathogenic mechanisms. Several targets for intervention to reduce the rate of atherosclerotic lesion initiation and progression have now been identified, including lowering plasma lipoprotein levels and modulating the maladaptive responses in the artery wall.

Sections

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Key points

- LDL is the main carrier of cholesterol in the blood and of circulating cholesterol into the artery wall.
- LDL is a proven causative factor for atherosclerotic cardiovascular disease, and reducing the plasma levels of LDL substantially reduces cardiovascular risk.
- Subendothelial retention of LDL and other apolipoprotein B-containing lipoproteins is the primary trigger for the development of atherosclerosis.
- Retained LDL becomes modified in the artery wall, and the focal accumulation of modified lipoproteins triggers the recruitment of monocytes and macrophages.
- Damage-associated molecular patterns, formed when retained LDL is modified, induce a maladaptive immune response.
- Different species of apolipoprotein B-containing lipoproteins are not equally atherogenic; triglyceride-rich lipoproteins and their remnants and lipoprotein(a) are markedly more atherogenic than LDL.

Introduction

Cardiovascular disease is the leading cause of death worldwide, causing more than 19 million deaths annually[1]. Atherosclerosis is the major disease process underlying approximately 85% of these deaths[1]. Elevated plasma concentrations of LDL are causally associated with atherosclerotic cardiovascular disease (ASCVD), and lowering LDL levels reduces ASCVD events in humans[2,3]. Although the role of LDL as the prime causative agent of atherosclerosis has been questioned[3], and competing hypotheses have been proposed to explain the early stages of atherogenesis, the accumulation of LDL and other apolipoprotein B (apoB)-containing lipoproteins in the intima of the artery wall is now established as the primary trigger for the development of atherosclerosis[3–8]. These trapped lipoproteins and their by-products induce maladaptive local responses that lead to atherosclerotic plaque initiation, progression and maturation.

ApoB, the major structural apolipoprotein of LDL and other atherogenic lipoproteins, is required for their formation and metabolism. ApoB exists in two isoforms: apoB100, consisting of 4,536 amino acids, and apoB48, a truncated version containing the first 2,152 amino acids[3]. Both proteins are encoded by the *APOB* gene, from which a single mRNA is transcribed. ApoB48 is generated by RNA editing, which introduces a stop codon at residue 2,153. In humans, apoB100 is expressed in the liver and secreted on VLDL, whereas apoB48 is expressed in the intestine and found on chylomicrons[3]. As chylomicrons and VLDL circulate through the bloodstream, their triglycerides are hydrolysed by lipoprotein lipase located on the endothelial surface of blood vessels. Remnants are formed that are either cleared from the circulation by the liver or, in the case of VLDL, converted to LDL, the main carrier of cholesterol.

ApoB differs from other apolipoproteins in that it does not transfer between different lipoproteins[3] owing to its size and the presence of antiparallel β-sheets that form robust lipid-binding structures[9,10]. Given that all apoB-containing lipoproteins contain a single apoB100 protein, plasma apoB concentration is a direct measure of the number of circulating atherogenic lipoproteins[2,3,11]. Although all apoB-containing lipoproteins are thought to contribute to the formation and progression of atherosclerotic plaques, the main focus to date has been on LDL because it is by far the most abundant atherogenic lipoprotein species[2,3]. In this Review, we present new insights into the role of apoB-containing lipoproteins in atherogenesis, with an emphasis on the mechanisms that lead to atherosclerotic plaque initiation and growth. First, we describe in detail the causative role that LDL has in the early stages of atherogenesis, and then we widen the discussion to explore emerging concepts of the atherogenicity of other apoB-containing lipoproteins. A large amount of the evidence presented comes from animal models and in vitro studies, and care has to be exercised in translating the findings to human atherosclerosis. When possible, key experimental findings are linked to clinical observations.

Initiation of atherosclerosis: role of LDL retention

Influx of LDL into the artery wall

To enter the artery wall, lipoproteins must cross the vascular endothelium. Genetic evidence has disproved the idea that LDL diffuses passively across the endothelium and instead shows that active transcytosis occurs (Fig. 1), mediated by a regulated vesicular pathway involving caveolae[12–14], scavenger receptor class B member 1 (SR-B1)[15] and activin receptor-like kinase 1 (ref. 16). These results explain the studies showing that the greatest lipid accumulation and intimal thickening in rabbit aortas occurred in areas where the endothelium had regenerated after de-endothelialization, rather than in adjacent denuded areas (that is, areas of the intima without an endothelial lining)[17,18]. SR-B1 seems to have a key role in LDL transcytosis by interacting with the guanine nucleotide exchange factor dedicator of cytokinesis protein 4 (DOCK4)[19,20]. Interestingly, expression of SR-B1 and DOCK4 is higher in human atherosclerotic arteries than in unaffected arteries[20]. Oestrogens inhibit LDL transcytosis by reducing endothelial SR-B1 levels through the G protein-coupled oestrogen receptor[21,22]. This observation might help to explain the lower risk of ASCVD in premenopausal women[23]. Conversely, factors such as activation of the NACHT, LRR and PYD domain-containing protein 3 (NLRP3) inflammasome or hyperglycaemia increase LDL transcytosis[24,25], which might contribute, at least in part, to the accelerated atherosclerosis observed in people with type 2 diabetes mellitus[26]. Studies have shown that rapid correction of hypercholesterolaemia in mice improves endothelial barrier function, limiting LDL ingress[27]. However, the underlying mechanisms are unclear and need to be validated in humans.

Endothelial glycocalyx. The luminal surface of all vascular endothelial cells is covered by the endothelial glycocalyx, which comprises membrane-bound, negatively charged proteoglycans, glycoproteins, glycolipids and glycosaminoglycans[28]. The endothelial glycocalyx has been proposed to function as a regulator of LDL transendothelial transport; when through the glycocalyx, LDL can cross the endothelium via transcytosis. Studies on the coronary arteries of cholesterol-fed pigeons, and subsequent studies on the carotid arteries of hypercholesterolaemic mice, have shown that the thickness of the glycocalyx is reduced in the regions containing atherosclerotic plaque[29–32]. However, experimental evidence is still lacking as to the wider pathogenic relevance of this phenomenon, and the exact nature of the interaction between LDL and the glycocalyx remains to be elucidated.

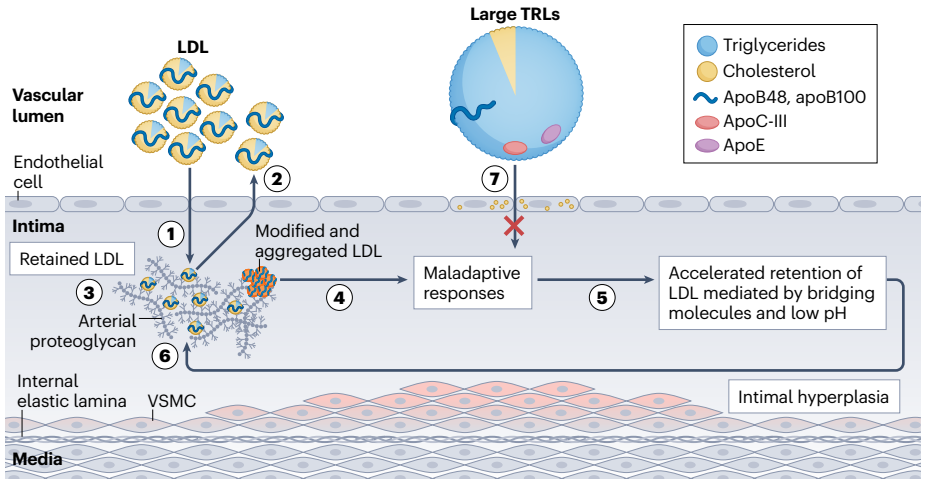

Fig. 1 | Early stages of atherogenesis. (1) To enter the arterial wall, lipoproteins must cross the endothelium by transcytosis. The transport vesicles are approximately 100 nm in diameter, so transcytosis is restricted to lipoproteins <70 nm in diameter (LDL and smaller triglyceride-rich lipoprotein (TRL) remnants). (2) Most lipoproteins that enter the arterial wall flow back into the circulation. (3) However, a fraction of LDL is trapped in the arterial wall by binding to the extracellular matrix. This binding is mediated by ionic interactions between positively charged amino acid residues in apolipoprotein B-100 (apoB100) and negatively charged sulfate or glycosaminoglycan chains of artery wall proteoglycans. (4) Modification of the retained LDL by secretory sphingomyelinase (produced by the endothelium and atherosclerotic plaque macrophages) and phospholipases leads to loss of LDL stability and aggregation. (5) The modified LDL triggers maladaptive responses that accelerate atherosclerotic lesion development. For example, activated macrophages release bridging molecules, including lipoprotein lipase, which facilitate accelerated binding of LDL to the artery extracellular matrix. The inflammatory process also leads to local acidification of extracellular fluids, which protonate histidine residues, increasing the net positive charge of the LDL particle. (6) Activated macrophages also stimulate transformation of vascular smooth muscle cells (VSMCs) from a contractile to a proliferative state, resulting in increased synthesis of LDL-binding proteoglycans. (7) Although large TRLs (chylomicrons and $VLDL_1$) are too large to enter the artery wall by transcytosis, a novel mechanistic link has been revealed between large TRLs and vascular disease, mediated by the induction of lipid droplets in the endothelium[191,193]. The mechanism seems to involve activation of nuclear factor-κB and upregulation of vascular cell adhesion molecule, which promotes atherogenesis and plaque inflammation[191–193].

Electrostatic interactions between apoB100 and artery wall proteoglycans

The interaction between LDL in the subendothelial space and artery wall components is mediated by electrostatic attraction between negatively charged sulfate or carbohydrate groups of artery wall proteoglycans and positively charged amino acid residues in apoB. In 1949, Faber reported on an association between intimal mucopolysaccharides and cholesterol deposition in the artery wall[33]. This seminal work inspired further investigation, including the early studies of Camejo and colleagues[8,34] that led to the identification of eight clusters of positively charged amino acids in apoB100 that bind to proteoglycans in human isolated artery[35–38].

To determine which of these clusters of positively charged amino acids are functional in intact lipoproteins, we generated transgenic mice expressing mutant forms of human apoB100 (ref. 39). We identified residues 3359 to 3369 (termed site B) in apoB100 as the primary proteoglycan-binding site and, in particular, the essential role of positively charged arginine and lysine residues in this site for the interaction between LDL and artery wall proteoglycans[39,40]. Subsequent experiments in mice with mutations in site B confirmed the crucial link between LDL atherogenicity and the proteoglycan-binding activity of apoB100 (ref. 4), thereby demonstrating that vascular retention of apoB100-containing lipoproteins is an initiating event in atherogenesis. Site B is located in the carboxy-terminal half of apoB100 and coincides with the LDL receptor-binding site[41]. Site B is, therefore, not present in apoB48. However, we also identified a proteoglycan-binding site in the amino terminus (residues 84 to 94) of apoB48 (ref. 42). In apoB100, this site is masked by the C-terminal portion of apoB100 and is, therefore, not functional.

Subsequent studies confirmed the existence of a third functional binding site in apoB100, termed site A (residues 3148 to 3158)[43], which was originally proposed by Camejo and colleagues[44]. In vitro, site A becomes functional when LDL is modified by secretory group IIA phospholipase A_2 ($sPLA_2$), which catalyses the hydrolysis of surface phospholipids on LDL, resulting in the formation of smaller and denser particles that have increased affinity for proteoglycans[45]. Elevated plasma levels of $sPLA_2$ and another phospholipase, lipoprotein-associated phospholipase A_2 (Lp-PLA_2), have been identified as important risk factors for ASCVD[46,47]. One might hypothesize that an association between elevated levels of small, dense LDL and an increased risk of ASCVD might be, at least partly, due to the exposure of site A on apoB100, secondary to the action of these enzymes[48,49]. However, in clinical trials involving individuals at high risk of a cardiovascular event, treatment with varespladib (an inhibitor of $sPLA_2$) or darapladib (an inhibitor of Lp-PLA_2) was not associated with a reduced risk of ASCVD events compared with placebo[50,51]. Similarly, in Mendelian randomization studies, gene variants that lowered Lp-PLA_2 activity were not associated with a decreased in ASCVD events[51]. These results indicate that $sPLA_2$ and Lp-PLA_2 are unlikely to be causal risk factors for ASCVD.

What is more important: the rate of LDL influx into the artery wall or subendothelial retention of LDL?

Early research by Schwenke and Carew in cholesterol-fed rabbits showed that whereas the rate of LDL ingress did not differ significantly

between areas of normal artery that were deemed either 'susceptible' or 'resistant' to subsequent atherosclerotic plaque development, the susceptible regions had greater LDL accumulation[52]. These findings suggest that mechanisms that facilitate selective retention of LDL determine the degree of local accumulation of the lipoprotein. Tran-Lundmark and colleagues confirmed this notion in molecular studies that involved crossing mice expressing heparan sulfate (HS)-deficient perlecan (also known as HSPG2) with hypercholesterolaemic *Apoe*$^{-/-}$ mice[53]. Compared with *Apoe*$^{-/-}$ control mice, the influx of LDL into the aorta wall was increased, whereas subendothelial retention of LDL was significantly reduced in *Apoe*$^{-/-}$ mice with HS-deficient HSPG2 (ref. 53). Importantly, atherosclerotic lesions were also markedly reduced in these *Apoe*$^{-/-}$, HS-deficient HSPG2 mice[53], underscoring the importance of LDL retention over the degree of endothelial permeability to LDL as the main pathogenic mechanism. Of note, only a small amount of LDL is retained in the artery wall by proteoglycan binding; most particles that enter subsequently leave without contributing to atherosclerotic lesion growth[53].

Determinants of LDL retention by artery wall proteoglycans

LDL composition. The protein composition of apoB-containing lipoproteins affects their interaction with artery wall proteoglycans. For example, apoB48-containing lipoproteins typically contain numerous apoE molecules, which have a proteoglycan-binding domain that is almost identical to that found in apoB100 (ref. 54). In addition, apoC-III, a small exchangeable apolipoprotein, increases the binding of LDL to proteoglycans while decreasing the hepatic uptake of apoB-containing lipoproteins, leading to increased lipoprotein accumulation in the vessel wall[55,56]. The mechanisms underlying this observation remain unclear because apoC-III lacks the positively charged domains that are necessary for binding to artery wall proteoglycans[55,56]. However, in vitro studies of LDL from individuals with type 2 diabetes have shown that an increased apoC-III to apoB molar ratio in LDL is associated with an altered lipid composition in LDL and increased proteoglycan binding[55]. This study suggested that a more fluid monolayer on the LDL particle surface facilitates the acquisition of additional apoC-III molecules, and consequent conformational changes in apoB100 led to an increase in its affinity for proteoglycans[55].

Other changes in LDL quality might also affect its binding to artery wall proteoglycans. Changes in core lipid composition, such as increased cholesterol or triglyceride content[57], might induce conformational changes in apoB that affect its interaction with proteoglycans[43]. Cholesterol enrichment of LDL correlates with increased affinity for proteoglycans[43]. This phenomenon is likely to be due to a conformational change in site B in apoB100. Likewise, triglyceride-rich LDL is less atherogenic than cholesteryl ester-enriched LDL in *Apoe*$^{-/-}$ mice[58]. This result is in accordance with the findings of an earlier study showing a reciprocal relationship between the triglyceride content of LDL and the number of exposed lysine amino groups in apoB100 (ref. 59). In addition, LDL isolated from patients with type 1 diabetes was retained in the artery wall of diabetic mice to a greater extent than LDL from healthy control individuals[60]. Although glycation of LDL is one potential modification that might increase retention in the artery wall, other as-yet-unidentified mechanisms might also be involved[60].

Artery wall constituents. Although most proteoglycans show affinity for LDL, proteoglycans with elongated glycosaminoglycan chains seem to have a crucial role in binding to LDL. Histology studies of human arteries have shown strong co-localization between LDL and the proteoglycans biglycan, decorin and versican core protein[61,62]. The composition of proteoglycans in arteries varies between animal species, and HSPG2 seems to be the primary proteoglycan that binds to lipoproteins in the mouse aorta[53].

Other components of the artery wall can also influence the subendothelial retention of LDL. For example, the expression of chondroitin sulfate proteoglycan 4 (also known as NG2) is increased in early atherosclerotic lesions in *Apoe*$^{-/-}$ mice, and NG2-positive synthetic vascular smooth muscle cells (VSMCs) increase LDL binding and uptake by macrophages in the artery wall[63]. Interestingly, NG2 glycosaminoglycan chains are not essential for LDL binding to NG2 (ref. 63), highlighting the unique ability of NG2 among LDL-binding proteoglycans to bind LDL through hydrophobic interactions.

We have shown that the cytokine tumour necrosis factor ligand superfamily member 13 (also known as APRIL) confers atheroprotection by binding to HS chains on HSPG2 and thereby limits LDL retention, macrophage accumulation and necrotic core formation[64]. In light of these findings, interventions that increase the interaction between APRIL and HSPG2 could protect against atherosclerosis. Indeed, atherosclerosis was reduced in *Apoe*$^{-/-}$ mice treated with an anti-APRIL antibody that increased the APRIL–HSPG2 interaction[64]. Moreover, blocking sulfated glycosaminoglycans in the artery wall in *Apoe*$^{-/-}$ mice with monoclonal antibodies inhibits the interaction between LDL and proteoglycans[65].

Regional intimal hyperplasia. The focal pattern of atherogenesis is striking, with lesions developing in the vicinity of artery branches and along the inner curvature of arteries, while other segments of the artery network remain relatively unaffected. The reason for the selective accumulation of LDL in these vulnerable areas is unclear. However, sites prone to develop atherosclerosis often have intimal hyperplasia[62,66–70], a thickening of the innermost layer of the vessel wall due to the accumulation of VSMCs and artery wall proteoglycans[61,62] (Fig. 1). Interestingly, some vascular interventions (such as percutaneous coronary intervention and coronary artery bypass graft surgery) promote intimal hyperplasia and the accelerated retention of apoB-containing lipoproteins[71,72].

Findings by Nakashima and colleagues support the role of intimal hyperplasia in the pathogenesis of atherosclerosis[62]. The researchers examined lipid deposition and macrophage infiltration in the coronary arteries of people aged <40 years who had died from non-cardiovascular causes, and found that extracellular lipids accumulate in areas of diffuse intimal thickening[62]. The investigators also showed that the lipid accumulation precedes the infiltration of macrophages[62]. Further elegant studies by Bentzon and colleagues confirmed that LDL retention is the rate-limiting factor for LDL accumulation in the mouse aortic arch, and showed that LDL-retaining regions can be subdivided according to their capacity for continued LDL accumulation under hypercholesterolaemic conditions[73,74].

Lesion microenvironment. Atherosclerotic lesions are often hypoxic environments with elevated lactate levels and local acidification of extracellular fluids[75,76]. We demonstrated a profound influence of local pH on the binding of LDL to artery wall proteoglycans, highlighting the crucial role of positively charged histidine residues in increasing LDL binding to human coronary arteries[77]. Histidine residues remain uncharged at pH 7.4, but become protonated and positively charged at lower pH; the proportion of protonated histidine residues increases from approximately 4% at pH 7.4 to 25% at

pH 6.5 (ref. 77). Our results indicate that LDL binding can be inhibited at lower pH by blocking either site B or protonated histidine residues, suggesting that both components are required simultaneously for the increased affinity of LDL for artery wall proteoglycans at acidic pH[77]. Although apoB100 contains 114 histidine residues, they are not clustered in sufficient numbers to independently form a glycosaminoglycan-binding site. However, histidine residues can contribute positively charged groups to lysine–arginine clusters. One such cluster, site A (residues 3148–3158), contains two histidine residues and five lysine or arginine residues within an 11-amino acid stretch[77]. Consequently, site A might become functional at lower pH levels and, in this state, cooperates with site B to increase LDL binding to proteoglycans[77].

Before moving on to consider the next stage in the atherosclerotic disease process, it is worth noting that the large body of evidence summarized above supports the concept that lipid accumulation in the initial stages of atherosclerosis occurs independently of the presence of macrophages. Macrophages, either resident or from the circulation, congregate subsequently in lesions with substantial lipid deposition.

Early stages of atherosclerosis: modification of retained LDL and cellular responses

Modification of LDL retained in the artery wall

When retained in the subendothelial space, LDL is susceptible to modification by various proteases and lipases that alter its structure[78] and to oxidation by non-enzymatic and enzymatic mechanisms, involving lipoxygenases, myeloperoxidase, NADPH oxidases and nitric oxide synthases, among others[79] (Fig. 2). The expression of these enzymes and the production of reactive oxygen species by vascular cells and, more importantly, by macrophages contribute to LDL modification. Oxidation of LDL generates a myriad of different oxidized lipids that have the capacity to trigger vascular inflammation. Both cholesterol and phospholipids in LDL can undergo oxidative modification, giving rise to oxidized cholesteryl esters and various oxidized phospholipids (oxPLs), as well as their many degradation products. For example, oxidation of the abundant phospholipid 1-palmitoyl-2-arachidonoyl-*sn*-phosphatidylcholine results in the generation of highly reactive breakdown products of the *sn*-2 polyunsaturated fatty acids, including malondialdehyde and 4-hydroxynonenal, which in turn form adducts with the ε-amino groups of (generally) lysine residues of apoB or the amino groups of other phospholipids such as phosphatidylethanolamine[80]. Similarly, the remaining core aldehyde carrying the phosphocholine head group can also modify apoB. Different types of oxPL product have been shown to activate endothelial cells and stimulate the expression of chemokines and adhesion molecules, promoting leukocyte recruitment to the artery wall[81]. In contrast to native LDL, oxidized LDL (oxLDL) is taken up by macrophages via the scavenger receptors CD36 and SR-A1, leading to foam cell formation[81].

OxPLs associated with LDL particles can also be detected in the circulation by an immunoassay that measures the number of moles of oxPL per apoB100-containing particle[82]. These particles can either be released in an oxidized form from atherosclerotic plaques or be oxidized in the periphery. Increased plasma levels of oxPL–apoB have been found to be a marker of the extent of atherosclerosis and to improve risk prediction for myocardial infarction, ischaemic stroke and peripheral artery disease, independent of apoB or LDL cholesterol levels[83–85]. Of note, oxPLs on lipoprotein(a) (Lp(a)) particles can also be assessed, as discussed below.

Mechanisms of accelerated subendothelial retention of LDL in atherosclerotic lesions

The presence of modified LDL that has been retained in the arterial wall triggers cellular responses that accelerate further LDL trapping. For example, oxLDL-induced release of cytokines and growth factors by inflammatory cells induces VSMCs to proliferate and produce proteoglycans with extended glycosaminoglycan chains, which have increased affinity for LDL[86,87]. Furthermore, infiltrating macrophages secrete bridging molecules, in particular lipoprotein lipase, which increases lipoprotein retention because these molecules have binding sites for both proteoglycans and lipoproteins[88–92]. In addition, macrophages release secretory sphingomyelinase (SMase), which increases lipoprotein retention and atherosclerosis in mouse models by promoting LDL aggregation[93–97]. Aggregation of LDL in the artery wall increases the affinity of the lipoprotein for proteoglycans[95,98] and accelerates the progression of atherosclerosis in humans[99]. Aggregated LDL is readily taken up by macrophages and induces, in contrast to oxLDL, foam cell formation and mild mitochondrial dysfunction in human macrophages, without triggering oxidative stress or endoplasmic reticulum stress[100]. Interestingly, macrophages engulf aggregated LDL and produce free cholesterol in an acidic, hydrolytic extracellular compartment known as the lysosomal synapse[101]. Through actin polymerization, macrophages create a tight seal around aggregated LDL, releasing lysosomal contents into this space in a process called digestive exophagy[101].

The augmented binding of LDL to artery proteoglycans that occurs in established atherosclerotic lesions (mediated by bridging molecules, aggregation and low pH) might provide a mechanistic explanation for a puzzling clinical observation; namely, a lifetime plasma LDL cholesterol level of ≤2 mmol/l is associated with a very low risk of developing atherosclerosis[102], whereas the same plasma level of LDL cholesterol is associated with atherosclerotic plaque progression in individuals with existing ASCVD who are receiving lipid-lowering treatment[103].

After LDL and other apoB-containing lipoproteins aggregate in the artery wall, their diffusion back into the plasma becomes nearly impossible owing to the substantial size of the aggregates and their greatly increased affinity as multivalent ligands for the arterial extracellular matrix. In addition, conformational changes occur that are likely to expose additional positively charged domains on apoB. Sneck and colleagues elucidated the mechanism behind SMase-induced aggregation of LDL by showing that SMase induces a substantial and widespread conformational change in apoB, exposing hydrophobic motifs that seem to stick digested LDL particles together[104]. Interestingly, the susceptibility of plasma LDL to aggregation after exposure to SMase ex vivo varies considerably between individuals, predicts future death from ASCVD and can be altered by diet[99]. This work adds to the growing body of evidence implicating SMase in human atherosclerosis[7,93].

Resident and recruited macrophages in atherosclerotic lesions

The focal accumulation of modified lipoproteins in vulnerable areas of artery walls leads to the migration of monocytes and macrophages into the area as part of the cellular response (Fig. 2). Single-cell analyses have identified substantial heterogeneity of macrophages in atherosclerotic plaques: 'resident-like', 'lipid-associated' and 'inflammatory' subtypes are all present[105–107]. Aortic intima-resident macrophages, which are sustained by local proliferation and are found at sites of intimal thickening, are the first macrophage subtype to take up oxLDL and aggregated LDL[108]. Indeed, early exposure to hypercholesterolaemia results in the depletion and reprogramming of resident macrophages in the intima

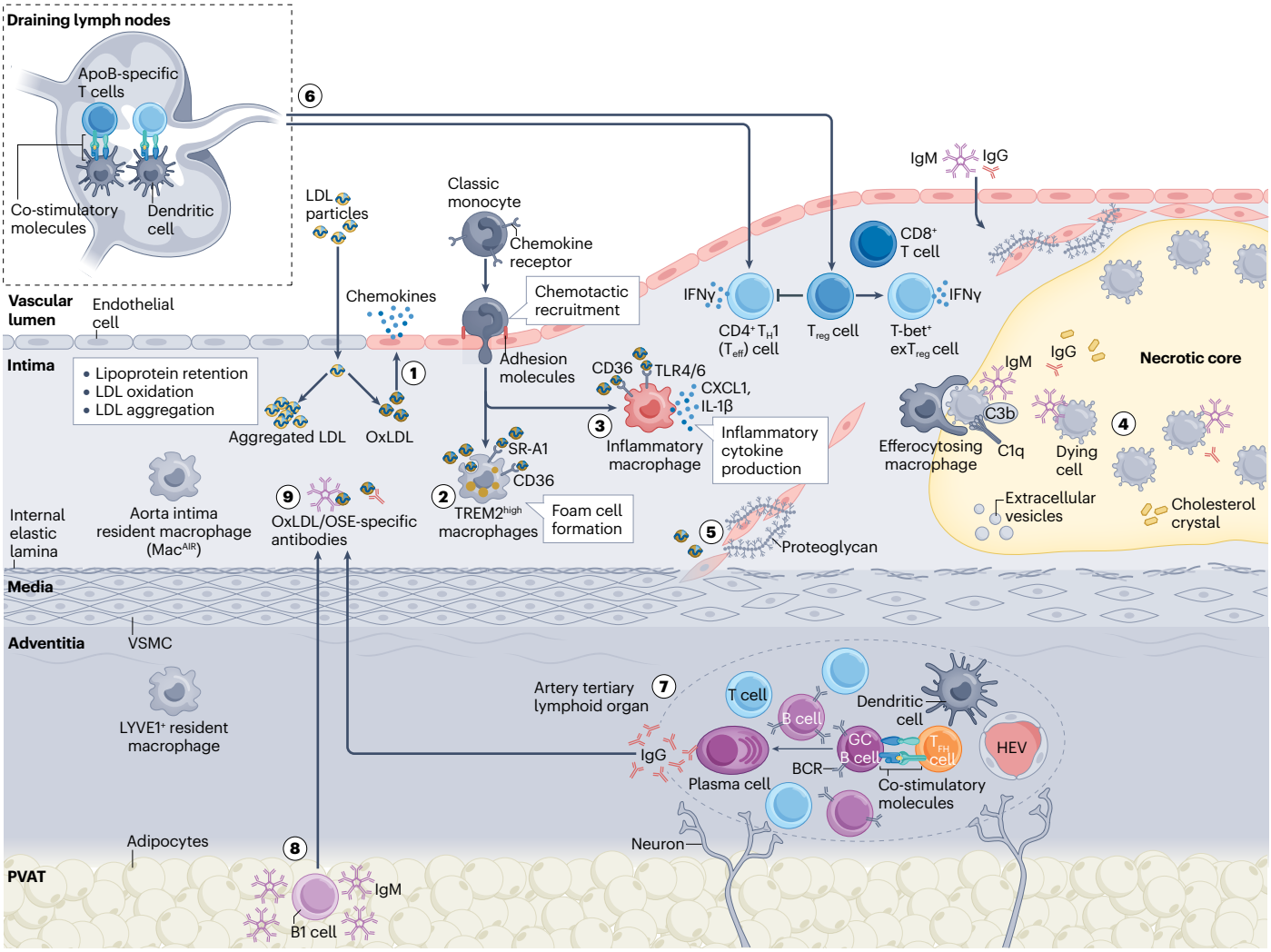

Fig. 2 | Cellular responses to LDL retention. (1) Retention of LDL exposes the lipoprotein to various modifications that alter the biological activity of LDL components, resulting in an array of cellular responses. Generation of oxidized LDL (oxLDL) leads to the formation of various oxidized lipid species that trigger inflammation. OxLDL and oxidized phospholipids in particular activate endothelial cells, inducing the expression of adhesion molecules and chemokines, which results in the recruitment of monocytes into the artery wall. (2) Resident and monocyte-derived macrophages take up oxidized and aggregated LDL, leading to the formation of foam cells. Aorta intima resident macrophages (Mac^{AIR}) and adventitial resident macrophages ($LYVE1^{+}$) are the earliest to respond to hypercholesterolaemia, resulting in their depletion and reprogramming, respectively. During progression of atherosclerosis, $TREM2^{high}$ macrophages make up the main foam cell subset and mediate protective homeostatic functions, including lipid handling and efferocytosis. (3) Inflammatory macrophages secrete pro-inflammatory cytokines and chemokines in response to damage-associated molecular patterns, such as oxidation-specific epitopes (OSEs) present on oxLDL. Cholesterol crystals taken up by or formed in macrophages trigger the activation of the NLRP3 inflammasome and the secretion of IL-1β and IL-18. (4) Impaired cellular cholesterol homeostasis results in cell death and the accumulation of dying cells, particularly during advanced disease stages when efferocytosis is impaired. (5) OxLDL also stimulates vascular smooth muscle cells (VSMCs) to proliferate and produce proteoglycans but can also trigger VSMC death. (6) Both effector T (T_{eff}) cells and regulatory T (T_{reg}) cells are primed in secondary lymphoid organs and possibly in advanced artery tertiary lymphoid organs during advanced atherosclerosis. T_{eff} cells and T_{reg} cells are recruited to the intima where they mediate pro-atherogenic and anti-atherogenic effects, respectively. Some of these T cells have specificity for apolipoprotein B (apoB) peptides, and apoB-specific T_{reg} cells can convert to pro-inflammatory exT_{reg} cells during atherosclerotic disease progression. (7) Lymphoid organs, including artery tertiary lymphoid organs, also contain B cells that differentiate to plasma cells in germinal centre (GC) reactions to produce class-switched IgG antibodies against oxLDL or OSE. (8) By contrast, IgM against oxLDL or OSE are largely derived from B1 cells that are prominently found in perivascular adipose tissue (PVAT). (9) Antibodies, including those with specificity for oxLDL or OSE, are present in the intima of atherosclerotic lesions. IgM can inhibit the disease process by neutralizing oxLDL or extracellular vesicles and by increasing the clearance of dying cells by macrophages, whereas the function of IgG is less clear. BCR, B cell receptor; CX3CL1, CX3C-motif ligand 1; HEV, high endothelial venule; IFNγ, interferon-γ; SR-A1, scavenger receptor type A1; T_{FH}, T follicular helper; $T_{H}1$, T helper 1; TLR, Toll-like receptor.

and adventitia, respectively[109]. Subsequently, newly recruited inflammatory monocytes arrive and give rise to several macrophage subsets, including those that scavenge modified LDL[110]. Accordingly, intermittent hyperlipidaemia in mice during youth is associated with subsequent rapid progression of atherosclerosis[109]. This finding underscores the crucial need to manage abnormal plasma lipid levels effectively from an early age onwards.

Surprisingly, in vitro formation of lipid-loaded macrophages (foam cells) is linked to the suppression of inflammatory gene expression[111]. In agreement, the gene expression profile of TREM2^{high} macrophages, which have been identified as a major foam cell subset, points to a function in lipid metabolism rather than inflammation[112]. Indeed, our studies in mice demonstrated a protective function for TREM2^{+} macrophages in atherogenesis, by promoting efferocytosis and efficient lipid handling[113]. However, Monaco and colleagues identified PLIN2^{high}TREM1^{high} macrophages, which also express IL-1β, as Toll-like receptor (TLR)-dependent inflammatory lipid-associated macrophages that might derive from anti-inflammatory TREM2^{high} foam cells[114]. The local inflammatory milieu in plaques could contribute to the transition of TREM2^{high} lipid-associated macrophages to inflammatory PLIN2^{high}TREM1^{high} macrophages.

To what extent other macrophage subsets, such as pro-inflammatory MHCII^{+} cytokine-expressing macrophages, cavity macrophages or interferon-inducible macrophages that have a characteristic type I interferon signature, respond to (oxidized) lipids is unknown[115]. Cellular lipid accumulation and an insufficient capacity to handle cholesterol lead to an increased rate of foam cell apoptosis, which results in the accumulation of apoptotic and necrotic cells due to defective efferocytosis[116,117]. Indeed, atherogenic lipids have been shown to trigger CD36–TLR2-dependent apoptosis in endoplasmic reticulum-stressed macrophages in vitro[118]. The higher rate of cell death by several mechanisms leads to the release of intracellularly stored cholesterol and the formation of extracellular lipid deposits and acellular necrotic areas, which are characteristic of unstable, rupture-prone atherosclerotic plaques[119].

Progression of atherosclerosis: cholesterol crystal formation, DAMPs and inflammatory response

Formation of extracellular lipid deposits and their role in atherogenesis

The extracellular lipid deposits in atherosclerotic plaques contain oxidized lipids associated with oxLDL, as well as cholesterol crystals, which can also be derived from lipoprotein particles that were not scavenged by leukocytes[120] (Fig. 2). Atherosclerotic plaque acidification has been shown to increase extracellular lipid accumulation[121]. Although the presence of large cholesterol crystals was documented in the earliest microscopic examinations of atherosclerotic plaques[75], the functional role of microcrystals of cholesterol in the inflammatory response was identified only much later. Indeed, cholesterol crystals are now considered key damage-associated molecular patterns (DAMPs) that drive the inflammatory processes in atherosclerosis.

Cholesterol crystals are potent activators of all three complement pathways, which modulate atherosclerotic lesion formation[122]. When taken up by macrophages or generated in lysosomes, cholesterol crystals also trigger the activation of the NLRP3 inflammasome, an intracellular pattern recognition receptor complex that promotes the generation and release of active IL-1β and IL-18 (refs. 123,124). Deficiencies in several components of the NLRP3 signalling complex have mostly been shown to limit atherosclerotic lesion formation in mice[125], and inhibition of IL-1β with a monoclonal antibody has been shown to significantly reduce the occurrence of ASCVD events in patients with previous myocardial infarction compared with placebo[126]. The FDA approved the use of a low dose of the broad anti-inflammatory agent colchicine for patients with established ASCVD, because this approach has been shown to reduce cardiovascular events compared with placebo in clinical trials[127]. Moreover, the ongoing ZEUS trial[128] is testing the efficacy of ziltivekimab, an antibody targeting the pro-inflammatory cytokine IL-6, which is downstream of IL-1β, for the prevention of cardiovascular events in patients with cardiovascular disease, chronic kidney disease and inflammation.

Adducts formed by phospholipid peroxidation products, which have been termed oxidation-specific epitopes (OSEs), also show robust immunoreactivity and are another class of DAMPs[79]. Phosphocholine-containing oxPLs present in oxLDL are recognized by CD36, which cooperates with TLR4 and TLR6 in transmitting pro-inflammatory signals in macrophages, resulting in nuclear factor-κB (NF-κB) activation and the transcription of various chemokines, such as CXCL1, CXCL2, CCL9 and CCL5, as well as components of the NLRP3 inflammasome[129,130]. Although studies using in vitro models of oxLDL need to be interpreted with care, specific active moieties of oxLDL, such as phosphocholine-containing oxPLs, have been clearly documented in vivo[82]. Indeed, the importance of phosphocholine-containing oxPLs in driving the inflammatory response during atherogenesis is underscored by experimental data by Witztum and colleagues demonstrating that Western diet-fed *Ldlr*$^{-/-}$ mice overexpressing a single-chain variable fragment of the oxPL-neutralizing phosphocholine-specific antibody E06 have reduced levels of systemic inflammation and decreased atherosclerosis[82,131]. In addition, malondialdehyde adducts also trigger chemokine secretion by macrophages, which is dependent on scavenger receptor-mediated uptake[132].

The innate immune activation by lipid DAMPs not only promotes the secretion of downstream effector cytokines, such as IL-6, but also propagates vascular inflammation and results in the activation of adaptive immune responses and the subsequent recruitment of T cells, which have been shown to modulate atherosclerosis by participating in a maladaptive immune response[133] (Fig. 2).

Dendritic cells, T cells and B cells in the atherosclerotic plaque

In the adventitia of normal arteries, lymphocytes and dendritic cells (DCs) are an immune–vascular unit that mediates vascular homeostasis. Importantly, conventional DC subsets take up cholesterol-containing lipoproteins in the setting of dyslipidaemia, which results in their activation in an oxysterols receptor LXRβ-dependent manner[134]. Plasmacytoid and conventional DC subsets can sense vascular antigens and promote both tolerogenic and inflammatory responses, depending on the type of T cell they instruct[133,135]. Whereas early homeostatic responses typically promote the expansion of regulatory T (T_{reg}) cells, the sustained pro-inflammatory and dyslipidaemic environment of atherosclerotic plaques alters the DC activation state, favouring the generation of CD4^{+} T helper (T_{H}) cells and effector T (T_{eff}) cells. Interestingly, oxPLs (as they are found in oxLDL) have been shown to induce a hyperactivated DC state[136]. Both CD4^{+} T_{eff} and T_{reg} cells are likely to be primed in secondary lymphoid organs and recruited to the artery wall by specific chemokine–chemokine receptor pairs[137]. Experimental studies in atherosclerosis-prone mice have documented the contribution of different DC and T cell subsets in atherosclerotic lesion formation, but insights into the antigen specificity of these responses is

still limited[135,137]. Of note, Ley and colleagues have shown that many $CD4^+$ T cells that show specificity for apoB peptides, which are presented by antigen-presenting cells, might initially have a T_{reg} cell identity[138,139]. Only later, triggered by the local milieu of inflammatory cytokines, continuous antigen exposure and cellular metabolic alterations associated with progressing atherosclerotic lesions, do other T cell subsets with greater cytotoxic and inflammatory functions dominate, including those with an exT_{reg} cell phenotype[140]. Indeed, T_{reg} cells protect mice from atherosclerotic lesion formation, whereas pro-inflammatory T_H1 cells, including T_H1-like exT_{reg} cells, promote atherogenesis by propagating inflammation[137]. The pivotal role of T cells in atherogenesis is also underscored by accumulating evidence that patients with cancer who are treated with immune checkpoint inhibitors, which unleash T cell activity, have an increased risk of vascular inflammation, atheroprogression and ASCVD events[141–144]. In humans, T cells have been reported to be a prominent cellular component of atherosclerotic lesions, showing autoimmune-like reactivity[145,146], and atherosclerotic plaques from symptomatic compared with asymptomatic patients have a higher prevalence of chronically activated effector memory T cells with IFNγ signatures[145,146]. $CD8^+$ T cells are also found particularly in advanced atherosclerotic plaques, where they show various phenotypes and exert their function by mediating target cell lysis[147]. Some $CD8^+$ T cells have specificity for apoB[148].

In contrast to T cells, B cells are usually absent from intimal atherosclerotic lesions, but they are present in the so-called artery tertiary lymphoid organs, which are found in the adventitia surrounding advanced atherosclerotic plaques and are sites for germinal centre (GC) reactions[149] (Fig. 2). GC responses, which involve the help of T follicular helper cells, lead to the generation and clonal expansion of B cells that undergo affinity maturation and class switching and can further differentiate into memory B cells and long-lived plasma cells. Hyperlipidaemia has been found to promote GC responses in mice[150]. Atherosclerosis is generally reduced in mice with B cells that are unable to class switch or to generate GC-dependent IgG antibodies, and was increased in antibody-deficient *Ldlr*$^{-/-}$ mice that received IgG preparations from hyperlipidaemic *Apoe*$^{-/-}$ mice compared with IgG from normolipidaemic wild-type donor mice[149]. The specificity of these pathogenic IgG antibodies is unknown, but they might include anti-oxLDL antibodies as well as autoantibodies to classic self-antigens such as nucleic acids[151]. Indeed, in response to excessive lipid accumulation, DCs can promote the formation of autoantibodies by producing key survival factors, such as BAFF and APRIL[152], for antibody-producing cells[134]. Of note, both IgM and IgG are present in atherosclerotic plaques of humans and many of these immunoglobulins bind to epitopes of oxLDL[153].

In contrast to IgG antibodies, anti-oxLDL IgM antibodies are thought to mediate protective effects. Several epidemiological studies have shown that high plasma levels of IgM against epitopes of oxLDL are associated with a lower risk of cardiovascular events[154]. Similarly, mice deficient in or with increased levels of natural IgM antibodies, which mostly bind to OSEs, show increased or reduced atherosclerosis, respectively[155–157]. The atheroprotective mechanisms include neutralization of the pro-inflammatory effects of oxLDL and inhibition of oxLDL-induced foam cell formation. However, the same OSEs found on oxLDL are also present on dying cells and a subset of extracellular vesicles[79]. Therefore, OSE-specific IgM can mediate additional protective functions by promoting the clearance and inhibiting the pro-inflammatory and pro-coagulatory properties of dying cells and extracellular vesicles, respectively[158]. Innate-like B1 cells secreting IgM are present in the perivascular adipose tissue of healthy arteries, where they might mediate important homeostatic functions in this regard[159].

Several strategies are also being investigated to exploit adaptive immune responses for anti-inflammatory therapies, including the expansion of T_{reg} cells and neutralizing anti-OSE-based antibody therapies targeting crucial moieties of oxLDL[133].

Beyond LDL: relative atherogenicity of apoB-containing lipoproteins

Are all apoB-containing lipoproteins equally atherogenic?

Conceptually, in atherogenesis, apoB can be considered to be an 'anchor' — the common structural protein that, because of its properties (as described above), facilitates the retention of apoB-containing lipoproteins at susceptible sites in artery walls. ApoB itself is not inherently atherogenic; instead, it is the 'cargo' of the lipoprotein constituents that is first anchored and then released that has pro-atherogenic effects. Given the marked compositional variation in apoB-containing lipoproteins, from large VLDL and chylomicron remnants to small LDL and Lp(a), it should not be surprising that particles have different potentials to cause pathogenic changes. Even for relatively uniform LDL particles, variation in the proteome and the lipidome, and possibly in the glycosylation pattern, modulates their affinity for artery wall proteoglycans, supporting the proposition that not all apoB-containing lipoproteins are equally atherogenic[160,161]. We have introduced the concept of 'relative per-particle atherogenicity', defined as the increase in ASCVD risk per unit change in lipoprotein concentration, relative to the benchmark of LDL[160,161]. We now develop this concept further by categorizing pro-atherogenic properties as being 'intrinsic', 'acquired' or 'permissive', as illustrated in Fig. 3.

The major apoB-containing lipoprotein classes — LDL; triglyceride-rich lipoproteins (TRLs), comprising chylomicrons and VLDL, and their remnants (TRL/remnants); and Lp(a) — vary considerably in size, content of major and minor lipid components, and the ancillary apolipoproteins present. TRLs and their remnants contain apoC-I, apoC-II, apoC-III and apoE[162], whereas Lp(a) has the large, highly polymorphic apo(a) attached to apoB[163] (Fig. 3). All these features can alter the atherogenic potential of a lipoprotein.

LDL atherogenicity

LDL particles are fairly homogeneous in size and composition, and variation in particle atherogenicity is likely to be dependent mainly on the conformation of apoB, as detailed above[3]. LDL subclasses exist, and the small, dense subfraction that accumulates in individuals with elevated plasma triglyceride levels has been reported to be more atherogenic than normal-sized LDL[164]. This higher atherogenicity despite a lower cholesterol content per particle[3,165] is potentially explained by the greater ability of small, dense LDL to bind to artery wall proteoglycans[3,166,167].

LDL can be classified as having primarily intrinsic pro-atherogenic properties, based on the ability of its apoB to bind to artery wall proteoglycans and on its cholesterol 'load' (Fig. 3). The relative homogeneity of LDL in the general population allows the use of LDL metrics as a benchmark when investigating the quantitative association of other lipoproteins with ASCVD[3,160,161].

Relative atherogenicity of TRL/remnants

Most studies elucidating how apoB-containing lipoproteins induce atherogenesis have focused on LDL, because it is the predominant carrier of cholesterol in the blood, and the principal means of delivering

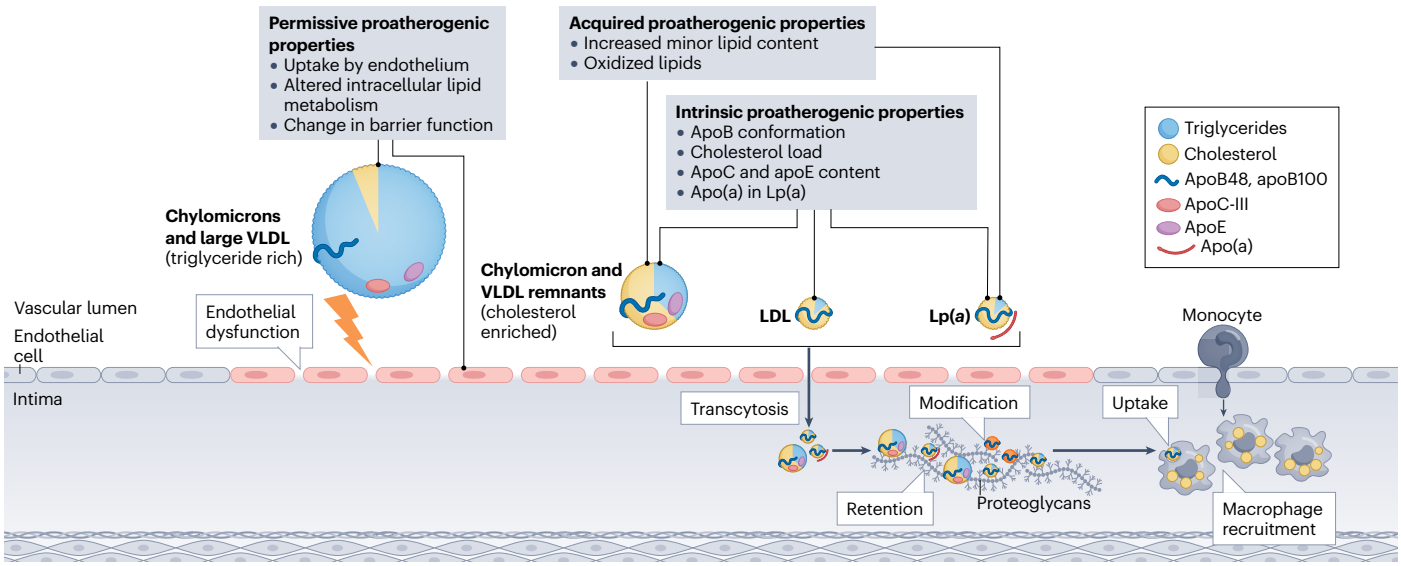

Fig. 3 | Concepts in atherogenicity of apoB-containing lipoproteins. Apolipoprotein B (apoB)-containing lipoproteins are complex structures that vary substantially in size and composition. In the circulation, they undergo metabolic transformations, either gaining or losing constituents. This figure conceptualizes the notion that the atherogenic properties of a lipoprotein can be usefully categorized as being either intrinsic or acquired. Intrinsic properties are inherent to the core structure of a lipoprotein, such as the presence of apoB and major lipids. Acquired properties result from the lipoprotein gaining potentially atherogenic components (such as minor apoproteins or oxidized lipids) by transfer or by metabolic modification occurring in the circulation or in the artery wall. In addition, permissive atherogenicity describes circumstances in which a particle itself does not enter the artery wall but induces pro-atherogenic changes, such as by increasing the atherogenic potential of other lipoprotein species. Lp(a), lipoprotein(a).

cholesterol into the artery wall. However, with the rising prevalence of obesity and type 2 diabetes, attention has widened to consider in greater detail the contribution of TRLs and their remnants (TRL/remnants) to atherogenesis, because these particles accumulate in individuals with these conditions. Furthermore, worldwide epidemiological studies have shown that TRL/remnants are major risk factors for cardiovascular disease in the general population, and Mendelian randomization studies have clearly demonstrated that TRL/remnants are causally associated with an increased risk of ASCVD events[168]. The availability of large cohorts with genetic, phenotypic and outcome data has made it possible to investigate and quantify the relative atherogenicity of lipoproteins. Theoretically, by selecting allelic variants (single-nucleotide polymorphisms (SNPs)) that affect mainly a single lipoprotein class, it is possible to compare the increase in ASCVD risk associated with a change in the concentration of different types of lipoprotein. Furthermore, by focusing on changes in apoB, the atherogenicity of lipoproteins can be compared on a per-particle basis.

Early studies selected a small number of SNPs that seemed to affect only TRLs (such as variants in *LPL*) and compared them with SNPs that seemed to affect only LDL (such as variants in *LDLR*). Each set of SNPs altered plasma levels of apoB, and when the ASCVD risk per standardized change in apoB (particle number) was calculated, it was found to be similar for TRL and LDL. This result suggested that both lipoprotein classes were equally atherogenic[169]. However, in a subsequent investigation[160], we used an arguably more objective approach by including all 1,125 SNPs affecting TRL/remnants and LDL in the Mendelian randomization analysis and found that no variant affected only one lipoprotein class[11,170]. We divided the SNPs into subsets based on their effect on TRL/remnants relative to LDL. For the set of SNPs that more affected TRL/remnants, the ASCVD risk per standardized change in apoB was increased by an odds ratio of 1.76 (95% CI 1.58–1.96), compared with an odds ratio of 1.33 (95% CI 1.26–1.40) for the set of SNPs that more affected LDL[160]. Further investigation refined this observation and revealed that TRL/remnant particles have an approximately fourfold greater atherogenicity than LDL[171], a finding with implications for our understanding of atherogenesis, cardiovascular risk assessment and the interpretation of clinical trials.

Mechanisms of TRL atherogenicity

Various mechanisms might explain the high atherogenicity of TRL/remnants. First, the increased atherogenic potential might be due to the high cholesterol content per particle or to preferential binding of TRL/remnants to artery wall proteoglycans due to an altered apoB conformation or to the presence of apoE or apoC-III[162,172–175]. These features can all be considered to increase the intrinsic atherogenicity of the particle (Fig. 3). The fact that TRL/remnants contain about two times more cholesterol per apoB than LDL means that the cholesterol load delivered per particle is higher, and evidence also indicates that remnant particles can be ingested by macrophages without the requirement for modification (unlike LDL)[176]. Furthermore, whereas LDL contains a single molecule of apoB per particle, TRLs contain several molecules of apoE in addition to apoB, allowing TRL and their remnants to bind more strongly to proteoglycans than LDL. Therefore, an augmented LDL-like mechanism of cholesterol deposition at atherosclerotic lesion sites might be at least partly responsible for the higher atherogenicity of TRL/remnants than of LDL. In support of this contention, we observed in genetic studies that TRL/remnant cholesterol content seemed to explain a substantial portion of the increased per-particle atherogenicity[171]. However, we and others have also observed that cholesterol from TRL/remnants is associated with a

higher ASCVD risk per standardized increase (per millimole per litre) than cholesterol from LDL, suggesting that factors other than just cholesterol load contribute to the higher atherogenicity of TRL/remnant particles[160,171,177,178].

A second plausible explanation lies in the observed relationship between plasma triglyceride levels and inflammation. A strong, positive association exists between plasma triglyceride levels and biomarkers of chronic systemic inflammation[162,179,180]. The mechanism underlying this link is unclear, but it seems to be causal given that SNPs affecting TRL concentration influence plasma C-reactive protein levels and blood cell indices of inflammation[171]. This finding might reflect states of metabolic inflammation associated with lipid accumulation in the liver and adipose tissue rather than vascular inflammation. Conversely, TRLs carry high levels of apoC-III, which has been shown to trigger activation of the NLRP3 inflammasome in human monocytes, a process associated with the production of reactive oxygen species and pro-inflammatory cytokines[181]. An additional potential mechanism linked to inflammation might be that, during intravascular lipolysis, TRL/remnants accumulate minor lipids (such as ceramide) and partial lipolysis products (such as diglycerides and lysophospholipids), which when released from TRL/remnants that have been retained in the subendothelial space can trigger pathogenic pathways[11,170]. Alternatively, similar bioactive lipids can be generated by in situ lipolysis of proteoglycan-bound TRL/remnants, given that lipoprotein lipase is secreted by cells present in the arterial wall[88]. Free fatty acids released during the lipolysis of TRL triglyceride have been linked to pro-inflammatory effects on endothelial cells and monocyte-derived macrophages[182,183]. This action is more pronounced when triglycerides are rich in saturated fatty acids rather than polyunsaturated fatty acids[182,184]. Therefore, although the major lipid in TRL — triglyceride — might not itself contribute to atherogenesis, metabolic changes induced in TRL constituents with the generation of partial lipolysis products and also oxidized lipid species (due to the presence of reactive oxygen species in the artery wall) can be considered to be examples of acquired atherogenicity[185–189] (Fig. 3).

A third potential mechanism linking TRL to pathogenic changes in artery walls lies in the cellular changes that occur in the endothelium when it is exposed to TRL. The observation that large TRL particles (chylomicrons and large VLDL) are too big to cross the endothelium by transcytosis led to the belief that these particles did not contribute to atherogenesis[190]. However, studies now indicate that endothelial cells can internalize circulating large TRLs via the SR-B1 receptor[185]. This uptake promotes changes in intracellular lipid metabolism and deleterious effects on the normal quiescent state of endothelial cells, impairing their barrier function[186,191,192]. Two studies have uncovered a novel mechanistic link between large TRLs and vascular disease, mediated by the induction of lipid droplet formation in the endothelium[191,193]. The accumulation of these droplets in endothelial cells promoted atherosclerosis and hypertension. More specifically, when mice were exposed to a high-fat diet or subjected to the loss of adipose triglyceride lipase (one of the major intracellular lipases), triglyceride-rich droplets formed in the endothelial layer of artery walls. Moreover, the presence of these droplets was associated with inhibition of nitric oxide production, thereby reducing endothelium-mediated relaxation and promoting vasoconstriction, which in turn contributed to the development of hypertension. In addition, there was activation of the NF-κB pathway, leading to upregulation of VCAM1, a factor that facilitates leukocyte adhesion to endothelial cells and promotes atherosclerotic plaque inflammation[191–193]. These changes in the state of the endothelium can in turn induce increased influx of other apoB-containing lipoproteins (remnants and LDL) into the artery wall, and can be classed as permissive atherogenic effects[186] (Fig. 3).

Finally, when considering the role of TRL in atherosclerosis, the observation that the plasma levels of chylomicrons and large VLDL increase markedly after a meal must be taken into account. An acute elevation in the plasma levels of TRL and their remnants during the postprandial phase in humans has been shown to result in impaired vasodilatation, increased production of pro-inflammatory cytokines, heightened endothelial inflammatory response, increased expression of VCAM1 and monocyte activation[182,183]. These effects are mediated through both direct and indirect mechanisms[182,183]. In addition, chylomicrons and VLDL bind to and transport coagulation factor VII and factor X[194,195]. Consequently, throughout the day as meals are absorbed, the appearance of TRLs and their remnants can create conditions that promote atherosclerotic plaque rupture and thrombus formation.

Relative atherogenicity of Lp(a)

Lp(a) is formed in the liver by the addition of apo(a) to an LDL particle. Apo(a), the product of the *LPA* gene, is a large, highly polymorphic protein of unknown function that is found in a few animal species, including humans. Apo(a) shares substantial homology with plasminogen and, on this basis, was initially thought to be a regulator of thrombosis[163]. The plasma concentration of Lp(a) is highly variable and largely under genetic control. Epidemiological studies have consistently shown that Lp(a) is one of the strongest inherited causal risk factors for ASCVD and, accordingly, Lp(a) is considered to be a prime target for novel drug-based intervention[163,196,197]. Attempts have been made to quantify the relationship between Lp(a) and ASCVD, and a range of results were obtained, suggesting that large reductions — approximately 50–100 mg/dl in total Lp(a) mass — might be required to see the same benefit as a 1 mmol/l reduction in plasma LDL cholesterol level[163,198,199]. However, Mendelian randomization studies focusing on the apoB present in Lp(a) and LDL reported that Lp(a) particles were substantially (approximately sixfold) more atherogenic than LDL particles[161], a result that was confirmed using measured Lp(a) and apoB levels[200]. This finding suggests that lowering plasma Lp(a) levels might be a useful intervention in a wider group of people than previously thought.

Role of Lp(a) in atherogenesis

In statistical models, the ASCVD risk associated with LDL cholesterol can be fully accounted for by adjusting for apoB, but the risk associated with Lp(a) cannot be explained in the same way[161,201]. This finding suggests that the increased atherogenicity of Lp(a) is likely to be due to components that are unique to Lp(a) and which are not present on LDL. Leading candidates include the apo(a) protein and minor lipid constituents, such as oxPLs[82,202,203].

Apo(a) on Lp(a) might promote atherogenesis by binding to laminin, a component of the extracellular matrix in artery walls. As noted for TRL/remnants, augmented particle retention in the artery wall can conceivably facilitate cholesterol deposition and consequently the initiation and growth of atherosclerotic lesions[204]. However, most research on the apo(a) protein has focused on its structural similarity to the fibrinolytic zymogen plasminogen. Apo(a) contains multiple copies of sequences similar to plasminogen kringle IV, followed by sequences similar to kringle V and the protease domain of plasminogen[205,206]. Nevertheless, the role of Lp(a) in the regulation of thrombosis and in thrombotic disease remains unclear[207]. Although

apo(a) can inhibit plasmin-mediated fibrinolysis in vitro, ex vivo studies have shown no change in clot lysis times using plasma taken from humans before and after reducing Lp(a) levels with an apo(a) antisense oligonucleotide[208]. Earlier studies in humans did suggest a positive association between high plasma Lp(a) levels and venous thromboembolism[209,210], but subsequent reports are inconclusive[211,212], and genetic evidence does not support this association except at extremely high plasma Lp(a) levels[197].

Another consideration about the role of apo(a) in cardiovascular disease is the marked size polymorphism of the protein. Most people carry *LPA* alleles coding for apo(a) protein of different lengths, and genetic studies suggest that ASCVD risk is influenced not only by the total plasma Lp(a) concentration but also by the size of the apo(a) protein, which is determined by the number of kringle IV repeats present[163,197,213]. The specific effect of this polymorphism on the overall atherogenic potential of Lp(a) remains unclear.

OxPLs formed by enzymatic and non-enzymatic lipid peroxidation are found in tissues throughout the body. These minor lipid species have a crucial role as endogenous DAMPs, which are recognized by the innate immune system and trigger a sterile inflammatory response[81]. For example, oxPLs can activate the NF-κB pathway and engage the NLRP3 inflammasome, leading to the production of pro-inflammatory cytokines[79]. Although oxPLs are present in all lipoproteins in the circulation, about 85% of lipoprotein-associated oxPLs in humans are carried by Lp(a)[214], either bound to the apo(a) protein or located on the surface of the particle[215].

OxPLs can promote atherogenesis by increasing the expression of adhesion molecules and cytokines in endothelial cells, thereby facilitating the infiltration of inflammatory cells into the vessel wall, and by stimulating the proliferation of VSMCs and macrophages[216]. Studies in animal models have shown that reducing the cellular uptake of oxPLs substantially decreases atherosclerosis[131]. Likewise, female double transgenic mice expressing human apoB100 and human apo(a) have increased atherosclerosis with a vulnerable plaque phenotype compared with those expressing human apoB100 only[217]. Translating these findings to humans, plasma oxPL levels have been identified as novel risk factors for ASCVD in a number of epidemiological studies[82].

The results of the genetic association studies described above are encouraging but a note of caution is warranted in using these findings to predict the outcome of Lp(a)-lowering clinical trials, because we do not yet understand the basis of the greater atherogenicity of Lp(a). If the presence of apo(a) alters the conformation of apoB and this change increases particle atherogenicity, or if apo(a) itself has important pro-atherogenic properties, then Lp(a) can be considered to have high intrinsic atherogenicity[163] (Fig. 3). Conversely, the greater atherogenicity of Lp(a) might be an acquired characteristic, due primarily to the presence of minor lipids (such as oxPLs) and other potentially bioactive molecules (such as diglycerides and lysophosphatidic acid) that provoke pro-inflammatory pathways[82,214,218]. In an informative investigation in humans, these minor lipid species were shown to be enriched in Lp(a) and could activate monocytes, increasing the capacity of these monocytes to transit across an endothelial layer[218]. However, profound lowering of plasma Lp(a) levels did not lead to a concomitant decrease in total plasma diacylglycerol levels, and the decrease in oxPLs was much less than that of Lp(a)[218]. These intriguing results might be explained by redistribution of these minor lipid species to other lipoproteins that were not reduced by the treatment.

Of note, Lp(a) has been identified as a causal risk factor for aortic valve stenosis through mechanisms related to promotion of calcification of valve tissue[163]. The role of Lp(a) in this disease is beyond the scope of this Review, but this association points to the possibility that Lp(a) might be involved in pathological mechanisms beyond atherogenesis.

An integrated view of apoB-related atherogenesis

Any assessment of the atherogenicity of apoB-containing lipoproteins must consider their relative abundance in the blood circulation. TRL/remnants and Lp(a) might be more atherogenic than LDL on a per-particle basis, but LDL is much more abundant. Typically, the concentration of apoB contained in LDL is about 80 mg/dl and that contained in TRL/remnants is about 10 mg/dl, whereas the concentration of apoB contained in Lp(a) varies from almost zero to about 20 mg/dl[163]. Lowering plasma LDL levels is, therefore, still the first-line intervention strategy. However, in people who are receiving LDL-lowering therapy and who achieve the new guideline-recommended targets of a plasma LDL cholesterol level of <1.4 mmol/l (55 mg/dl)[219], the concentration of apoB contained in LDL would be <35 mg/dl, and other apoB-containing lipoproteins, if their plasma levels are elevated, would contribute substantially to the residual risk of ASCVD.

Identifying the causes of the increased particle atherogenicity of different apoB-containing lipoproteins might be important because it is conceivable that only the intrinsic atherogenic effects are decreased in direct proportion to reducing the concentration of the apoB-containing lipoprotein in the bloodstream. Indeed, for LDL, which has mainly intrinsic atherogenicity, a meta-analysis of trials has shown a linear relationship between LDL cholesterol reduction and ASCVD risk reduction[220]. If the cause of increased TRL/remnant and Lp(a) atherogenicity is partly acquired, then reductions in the plasma levels of these lipoprotein classes by specific lipid-lowering treatments might or might not lead to a proportional reduction in ASCVD event rates. Acquired bioactive components might partly or wholly redistribute to other lipoproteins and continue to exert a pro-atherogenic influence.

When clinical trial experience of novel therapies such as specific Lp(a)-lowering and TRL/remnant-lowering agents is available, the outcome can be compared with quantitative predictions, especially those from Mendelian randomization studies, which have many of the features of a randomized clinical trial. Information on therapies that can reduce plasma Lp(a) levels by >80% will be published in the next few years[221]. Trials on lowering of plasma TRL/remnant levels have produced mixed results. Early investigations of fibrates, which lower plasma TRL/remnant levels by 25–40%, demonstrated reductions in the risk of ASCVD[222]. However, in the PROMINENT trial[223], pemafibrate lowered plasma triglyceride and TRL cholesterol levels by 25–30% but showed no reduction in the risk of ASCVD. This outcome might be attributable to the concomitant rise in plasma LDL cholesterol and apoB levels in those receiving pemafibrate[224,225], even allowing for the difference in atherogenicity between TRL/remnants and LDL particles[171].

Conclusions

A large body of evidence supports the predominant role of LDL as a causative agent in initiating and progressing the formation of atherosclerotic plaques. We now have a detailed understanding of how LDL penetrates, is retained by and elicits a maladaptive response in the artery wall at sites of susceptibility. The other apoB-containing lipoproteins — TRL and Lp(a) — seem to be even more atherogenic than archetypal LDL but are usually present at lower concentrations in the

plasma. Multiple targets for intervention can be identified, including lowering the plasma levels of apoB-containing lipoproteins and modulating the cellular responses to the presence of lipoproteins in artery walls. These strategies remain to be tested in clinical outcome trials.

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Competing interests

The authors declare no competing interests.

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