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Review

How do macrophages sense modified low-density lipoproteins?

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ABSTRACT

In atherosclerosis, serum lipoproteins undergo various chemical modifications that impair their normal function. Modification of low density lipoprotein (LDL) such as oxidation, glycation, carbamylation, glucooxidation, *etc.* makes LDL particles more proatherogenic. Macrophages are responsible for clearance of modified LDL to prevent cytotoxicity, tissue injury, inflammation, and metabolic disturbances. They develop an advanced sensing arsenal composed of various pattern recognition receptors (PRRs) capable of recognizing and binding foreign or altered-self targets for further inactivation and degradation. Modified LDL can be sensed and taken up by macrophages with a battery of scavenger receptors (SRs), of which SR-*A*1, CD36, and LOX1 play a major role. However, in atherosclerosis, lipid balance is deregulated that induces inability of macrophages to completely recycle modified LDL and leads to lipid deposition and transformation of macrophages to foam cells. SRs also mediate various pathogenic effects of modified LDL on macrophages through activation of the intracellular signaling network. Other PRRs such Toll-like receptors can also interact with modified LDL and mediate their effects independently or in cooperation with SRs.

1. Introduction

Modification of circulating low-density lipoproteins (LDLs) play an essential role in atherogenesis, especially in initial stages associated with induction of inflammation, intimal accumulation of lipids, and foam cell formation. In atherosclerosis, LDL particles undergone various chemical modifications especially oxidation [1], acetylation [2], carbamylation [3], and glycation [4]. The formation of glycated LDL usually occurs at higher blood glucose presented in diabetic patients. LDL-affecting modifications (for example, oxidation and glycation) frequently coexist and can happen concomitantly leading to the formation of glycooxidized LDL [5]. Modified LDLs are cytotoxic and contribute to the damage of endothelial cells (ECs) that promotes endothelial dysfunction and atherogenesis. Modified LDLs enhance oxidative stress and reduce regenerative potential of the endothelium through promoting senescence of endothelial progenitor cells by modification and DNA damage [6].

Both lipids and protein moiety of LDL are subjected to modifications. LDL modification affects lipoprotein properties towards the enhancement of proatherogenic effects. For example, changes in apolipoprotein B (apoB), a main protein component of LDL, abolish the capacity of LDL to bind to LDL receptor [7]. Modified LDL can be recognized by scavenger receptors (SRs), which are expressed on the surface of macrophages and ECs [8].

2. LDL modifications related to atherosclerosis

LDL oxidation was first found by Henriksen et al. [9] who observed that overnight incubation of natural LDL with cultured ECs leads to the formation of modified LDL that are rapidly recognized and taken up by macrophages. The authors hypothesized that this modified LDL fraction promotes the intracellular lipid accumulation with subsequent transformation of lipid-laden macrophages to foam cells. Further investigations showed that ECs release molecular factors, which perform LDL conversion to oxidized LDL (oxLDL) [10].

2.1. Oxidized LDL

There are a variety of molecules that are able to mediate LDL oxidation. Reactive oxygen species (ROS) such as free radicals and hydroperoxides produced by ECs, monocytes, macrophages, neutrophils, and mast cells oxidize predominantly the lipid portion of LDL [11]. Metals such as Fe^{3+} and Cu^{2+} are also involved preferentially in oxidative modification of LDL phospholipids. Red blood cells, which extravasate

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from the intralaque neovessels within the lesion, undergo rapid destruction due to the lysis, serve as a constitutive source of iron, a potent prooxidant factor [12]. Lypoxygenases catalyze oxidation of fatty acids to hydroperoxide adducts that can mediate LDL oxidation [13].

Myeloperoxidase, a neutrophil-specific oxidase, produces hypochlorous acid (HOCL) and hypothiocyanous acids (HOSCN), by-products, able to oxidize apoB molecule at numerous sites [14]. ApoB is modified by myeloperoxidase at lysine residues with formation of chloramines, which are stable enough and remain a residual oxidative capacity [15]. Chloramine apoB derivatives are subjected to intramolecular rearrangements with formation of a Schiff base causing cross-linking and aggregation of proteins [16]. HOSCN is generated by myeloperoxidase *via* oxidation of serum thiocyanate and selectively modifies Cys residues on apoB. Both HOCL and HOSCN also contribute to the formation of foam cells from macrophages [17].

While oxidized, oxLDL become more prone to further oxidation since oxLDL particles contain less amounts of antioxidants compared with non-oxidized LDL [18]. LDL oxidation greatly enhances proatherogenic properties of LDL [19]. For example, LDL concentration required for initiation of foam cell formation (2 mg/ml) exceeds by 40-fold that of oxLDL (50 μg/ml) [20].

2.2. Carbamylated LDL

In LDL, apoB can be carbamylated in a non-enzymatic manner by cyanate originate from urea or thiocyanate. Cyanate reacts with NH_2-containing residues of a protein. Spontaneous dissociation of urea in an aquatic phase results in formation of ammonia and cyanate (OCN^-). Cyanate can isomerize to isocyanate, which irreversibly modifies NH2-containing groups in proteins and amino acids [21]. Carbamylated proteins usually lack a part of activity but commonly distributed in normal tissues at low levels without affecting normal metabolism [22]. Carbamylation is greatly enhanced in uremia (*i.e.* in subjects with chronic kidney disease, a well-established risk factor in atherosclerosis) and in smokers [23]. Levels of carbamylated LDL are especially increased in hemodyalisis patients with atherosclerosis [24]. Carbamylated LDL exhibit proatherogenic effects in vessels inducing EC damage and adhesiveness, proliferation of vascular smooth muscle cells (VSMCs), generation of oxidants, and stimulating endonuclease G that contributes to cell damage [3,25]. Although the role of carbamylated LDL in atherogenesis should be further studied, it is clear that this type of modified LDL has altered function and supports atherosclerosis progression in individuals with chronic kidney failure and heavy smokers.

2.3. Glycated and glycooxidized LDLs

Elevated generation of advanced glycosylation end products (AGEs) including those on LDL is a hallmark of diabetic hyperglycemia [26,27]. AGEs intensively accumulate in the wall of diabetic vessels. The rate of non-enzymatic glycation depends on glucose levels and the duration of exposure [28]. LDL glycation involves phospholipids [29] and apoB [30]. ApoB is mainly glycated on lysine residues located in the LDL receptor-binding domain [30], which in turn affects the binding capacity of LDL to the receptor [19]. The levels of AGE-apoB were increased by 4-fold in diabetic subjects [29]. Since glycation significantly impairs the interaction between LDL and the LDL receptor, glycated LDL begin to be preferentially recognized by SRs on macrophages and indeed could stimulate conversion to foam cells [31].

Except for increased formation of AGEs, hyperglycemia also contributes to oxidative stress [32]. Glycated LDL are sensitive to oxidative modification [33,34]. In glycated LDL, oxidation leads to hydrolysis of glycerolipids such as triglyceride and choline-phospholipid and increase in lysophosphatidylcholine (LPC). Oxidation can induce apoB fragmentation of apoB although all apoB fragments retain in the LDL particle [35]. Formation of glycooxidized LDL increases their atherogenicity at least through generation of highly proatherogenic LPC. LPC is a main phospholipid constituent of OxLDL that mediates the atherogenic activity of OxLDL and glycooxidized LDL. LPC alters function of a range of immune (monocytes, macrophages, T cells, dendritic cells) and vascular (EC, VSMC) cells by inducing proinflammatory properties [36]. LPC also activates several proatherogenic signaling pathways serving as a ligand for specific G protein-coupled receptors [37].

3. Subendothelial retention and accumulation of modified LDL

Quantitative analysis of endocytotic vesicles in luminal ECs in experimental hyperholesterinemia in rabbits showed a significant increase in the numbers of vesicles in the endothelium in the aortic areas where the development of early atherosclerotic lesions occurred [38]. Along the luminal surface, endocytotic vesicles can join each other, thus forming transendothelial channels (Fig. 1A–C) [38]. Both LDL and modified LDL can pass the endothelium across ECs by vesicular transport or through the endothelial barrier [39]. The injection of [^{125}I]-labeled LDL into the blood circulation in the Anichkov's experimental model showed that LDL penetrated the endothelial monolayer by means of vesicular transport as well as *via* spaces between endothelial cell-to-cell contacts which become expanded from very earlier stages of atherosclerosis in atheroprone areas of the aorta (Fig. 2A–E) [38]. Autoradiographic analysis demonstrated the accumulation and retention of LDL in the arterial intima (Fig. 2A) [38].

In the subendothelial layer, lipoproteins interact with extracellular matrix (ECM) proteins such as proteoglycans. Positively charged amino acid residues of apoB are involved in the interaction with negatively charged sulphate groupd in proteoglycans [40]. In apoB, there are two proteoglycan-binding sequences enriched with basic amino acids and located in the N- and C-terminal regions [41]. The interaction of lipoproteins with subendothelial matrix proteins may result in lipoprotein retention. Modified LDL are retained by the intimal ECM more strongly than native LDL. The atherogenicity of LDL and modified LDL is primarily depends on their affinity to artery wall proteoglycans. Indeed, mice that have LDL with impaired proteoglycan-binding function develop less advanced atherosclerosis [42].

Retention of modified LDL in the subendothelial layer could induce inflammation. For example, oxLDL retained in the intimal ECM can

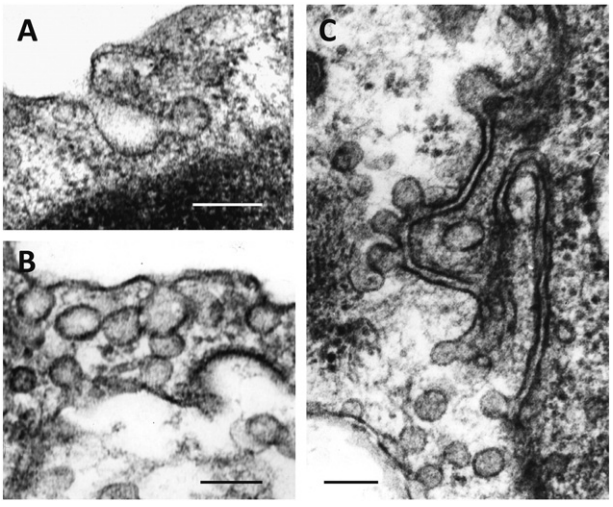

Fig. 1. Edocytotic vesicles in the cytoplasm of luminal endothelial cells (A-C), observed in the aorta of rabbits with experimental hypercholesterolemia (Anitchkov's model of atherosclerosis). (A, B): Formation and aggregation of edocytotic vesicles (A), accompanied by the formation of transendothelial channels (B) in the cytoplasm of luminal endothelial cells (ECs) (B). (C): Attachment of edocytotic vesicles to plasmalemma in zone of endothelial cell-to-cell contact. Transmission Electron Microscopy (TEM). Scale bars = 150 nm (A–C).
Images adapted from [38].