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Oxidative Modification of LDL: Its Pathological Role in Atherosclerosis

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Abstract Oxidized low-density lipoprotein (OxLDL) is a well-known risk marker for cardiovascular diseases. OxLDL has shown a variety of proatherogenic properties in experiments performed in vitro. In addition, immunological studies using monoclonal antibodies have revealed the occurrence of OxLDL in vivo in atherosclerotic lesions and patients' plasma specimens. Resent clinical studies have indicated the prospective significance of plasma OxLDL measurements; however, the behavior and metabolism of OxLDL in vivo is poorly understood. The mechanism by which LDL is oxidized is not clear, and the modified structures of OxLDL are not yet fully understood, partly because OxLDL is a mixture of heterogeneously modified particles. Here, I discuss the recent studies on oxidative modifications in OxLDL and its clinical and pathological features.

Keywords Oxidized low-density lipoprotein · LC-MS/MS · Myeloperoxidase · ELISA · Monoclonal antibody · Oxidized phospholipids

Oxidation of LDL

Low-density lipoprotein (LDL) is a major cholesterol carrier in human plasma. Very low-density lipoprotein (VLDL) is formed in the liver through the assembly of apolipoprotein B (apoB) and lipid molecules in the endoplasmic reticulum. VLDL particles are then secreted into the circulation. In the blood flow, lipoprotein lipase hydrolyzes triacylglycerol (TG) in VLDL, and the resulting fatty acids are delivered to peripheral tissues. By hydrolyzing TG, VLDL is reduced in size and increased in density, and finally, LDL is generated. The major constituent of LDL is cholesterol (in both free and ester forms).

Each LDL particle contains one molecule of apoB protein. Other proteins such as apolipoprotein E (apoE), paraoxonase, and PAF-acetylhydrolase (PAF-AH; also called Lp-PLA_2) are also present in LDL particles. Phospholipids and free cholesterol align on the surface of a particle, while TG and cholesteryl ester are packed in the central core of the particle. Judging from the amino acid sequence, apoB protein does not contain putative transmembrane domains, but there exist amphipathic α-helices where hydrophobic amino acid side chains align on one side of the α-helix cylinder and hydrophilic amino acid side chains gather on the other side. Thus, apoB is thought to cover the surface of the particle, rather than penetrating its core [1, 2].

Under conditions of oxidative stress, lipid molecules containing polyunsaturated fatty acids (PUFA) in LDL are easily oxidized. A variety of lipid oxidation products are formed, and subsequently, apoB protein is covalently modified by these oxidized lipids [3, 4]. Radical chain reactions on PUFA lead to formation of lipid hydroperoxides, and secondary cleavage reactions produce a variety of aldehyde compounds such as malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and acrolein. These aldehyde compounds modify amino acid residues in the lipoproteins to form various adducts (Fig. 1). For example, the ε-amino group of lysine residues reacts with aldehyde compounds such as acrolein to form a Schiff's base. 4-HNE, a highly reactive compound generated during lipid peroxidation reactions, readily reacts with histidine and cysteine residues [5].

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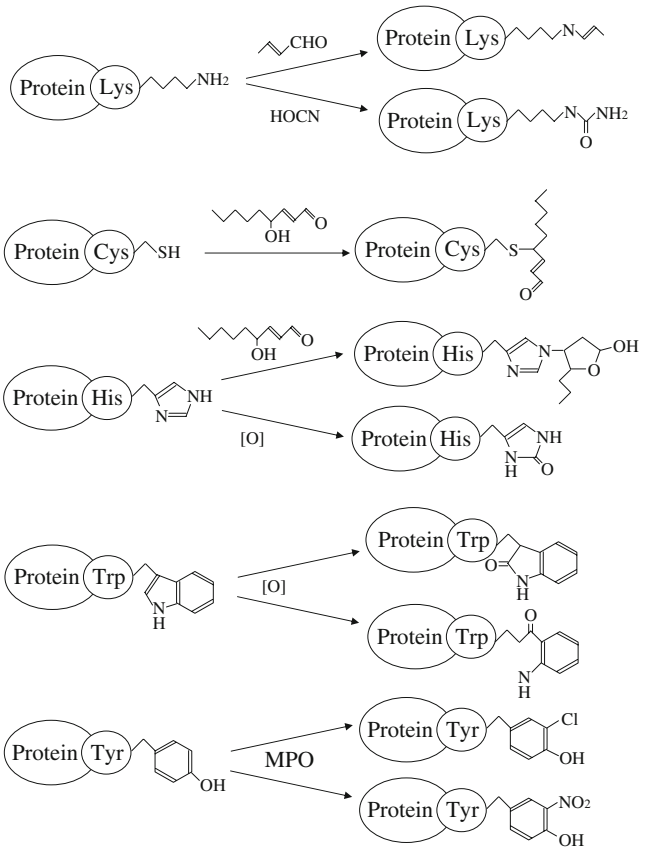

Fig. 1 Typical amino acid modifications generated during oxidative modification of LDL. Amino acid side chains of lysine, cysteine, histidine, and tryptophan residues are depicted and modified with various lipid oxidation products and oxidants such as cyanate (*HOCN*). Tyrosine residues are modified by the action of myeloperoxidase (*MPO*)

Modified tyrosine residues such as 3-chlorotyrosine or 3-nitrotyrosine are formed through myeloperoxidase (MPO)-dependent reactions [6–8]. MPO, released mainly from activated neutrophils, is an enzyme that generates hypochlorous acid, which kills bacteria. MPO is also involved in another type of apoB modification. It generates cyanate from isothocyanate and hydrogen peroxide; lysine residues in proteins are easily carbamylated by cyanate [9]. In addition to chemical modifications of amino acid side chains, cleavage of peptide chains [10] and cross-linking of polypeptides [11] occur during radical chain reactions. Thus, it is hard to define OxLDL structurally, since it is a mixture of heterogeneously modified lipoprotein particles.

Copper treatment has been widely used to produce in vitro models of OxLDL. Incubation of isolated LDL fraction with micromolar concentrations of copper sulfate at 37°C is a very simple way of oxidizing these fractions. Lipid peroxidation reactions proceed after a lag period of 60–90 min. Then, marked increases in lipid peroxidation parameters are observed, such as absorbance at 233 nm indicating conjugated diene formation, presence of thiobarbituric reactive substance indicating formation of small aldehyde compounds, and agarose gel electromobility indicating an increase in negative charges. Although copper-induced oxidation of LDL does not proceed in the presence of plasma, Cu-OxLDL has provided much information on oxidative modifications of biological molecules. Monoclonal antibodies (mAbs) recognizing Cu-OxLDL have been raised in several studies (Table 1). By using these anti-OxLDL mAbs, progress has been made in the characterization of the oxidized materials in atherosclerotic lesions, and the bioactive molecules formed in atherogenic lipoproteins.

Table 1 Three classes of monoclonal antibodies used for detection of OxLDL or related compounds

Names of mAbs	Recognizing structures	References
Class 1: Recognizes oxidative modifications		
DLH3	Oxidized PC	[12, 13]
E06	Phosphorylcholine (oxidized PC)	[19]
NA59	4-HNE	[21]
5F6	Acrolein-Lys adduct (N^{ϵ}-(3-formyl-3, 4-dehydropiperidino)lysine: FDP-lysine)	[23]
82D3	2-alkenal-Lys adducts (N^{ϵ}-(5-ethyl-2-methylpyridinium)lysine: EMP-lysine)	[27]
DLH2	Cross-linked protein (MDA, glutaraldehyde)	[21]
MDA-lys	MDA-lysine adducts	[25]
MDA-2	MDA	[21]
ML25	MDA	[26]
Class 2: Recognizing an apoB fragment		
4E6	Part of apoB that conformationally appears when at least 60 lysine residues of apoB-100 are substituted with aldehydes	[28, 29]
Class 3: Recognizing OxLDL-binding proteins		
WB-CAL-1	β2-GPI	[30, 31]

Several antibodies found in the literature are listed in this table. Note that this table is not a comprehensive list of anti-OxLDL mAbs, there exist numerous other antibodies raised by a variety of methods

Anti-OxLDL antibodies

A number of immunological studies have been conducted on OxLDL, and mAbs against OxLDL have been raised and utilized. These mAbs are divided into three categories. One group of mAbs recognizes chemically modified structures originating from oxidized lipids, the second recognizes an apoB fragment that changes its conformation during oxidation of LDL, and the third recognizes non-LDL proteins forming tight complexes with OxLDL in plasma.

An anti-OxLDL mAb, DLH3, was produced by immunizing mice with a homogenate of human atheroma [12]. DLH3 recognizes oxidized phosphatidylcholine (OxPC), including 1-palmitoyl-2-(9-oxononanoyl) PC (also called POVPC; Fig. 2) [13]. OxPC containing aldehyde group can bind to proteins to form adducts, and both free OxPC and conjugated forms are antigenic to DLH3 mAb. Immunohistochemical examinations using DLH3 demonstrated that OxPC-modified proteins, very likely OxLDL, are abundant in human atherosclerotic lesions [12, 14]. In addition, accumulation of OxPC in various diseased tissues were observed using DLH3. OxPC has been shown to present in hepatocytes in the livers of patients with nonalcoholic steatohepatitis [15], retinas from those with age-related macular degeneration [16], chondrocytes from the cartilages with osteoarthritis [17], and injured spinal cords in CD36 knockout mice [18].

Palinski et al. raised an antiphosphorylcholine mAb, E06, as one of the natural antibody clones from an apoE knockout (apoE-KO) mouse, which is an animal model widely used for hypercholesterolemia and spontaneous atherosclerosis [19]. E06 readily binds to OxPC and OxLDL; however, it recognizes the phosphorylcholine moiety of OxPC. Interestingly, they found that the amino acid sequence of the variable region of the E06 antibody is identical to that of a well-characterized antibody for tuberculosis, T15 [20]. This observation raised the possibility that the biological responses related to OxLDL, such as scavenger receptor-mediated endocytosis and autoantibody-dependent clearance of OxLDL, could be a part of the defense system against infectious diseases and xenobiotics.

As described above, various aldehyde compounds generated during lipid peroxidation reactions are capable of forming adducts with proteins. mAbs recognizing other oxidized lipids, such as 4-HNE or acrolein, have been used to detect OxLDL and other oxidized materials accumulating in atherosclerotic lesions [21–27]. Immunohistochemical examinations on human atheromatous lesions using antibodies against various lipid peroxidation products have reported accumulation of lipid peroxidation epitopes in foam cells in the lesions, but not in the extracellular spaces, suggesting that the antigens were removed from the tissues

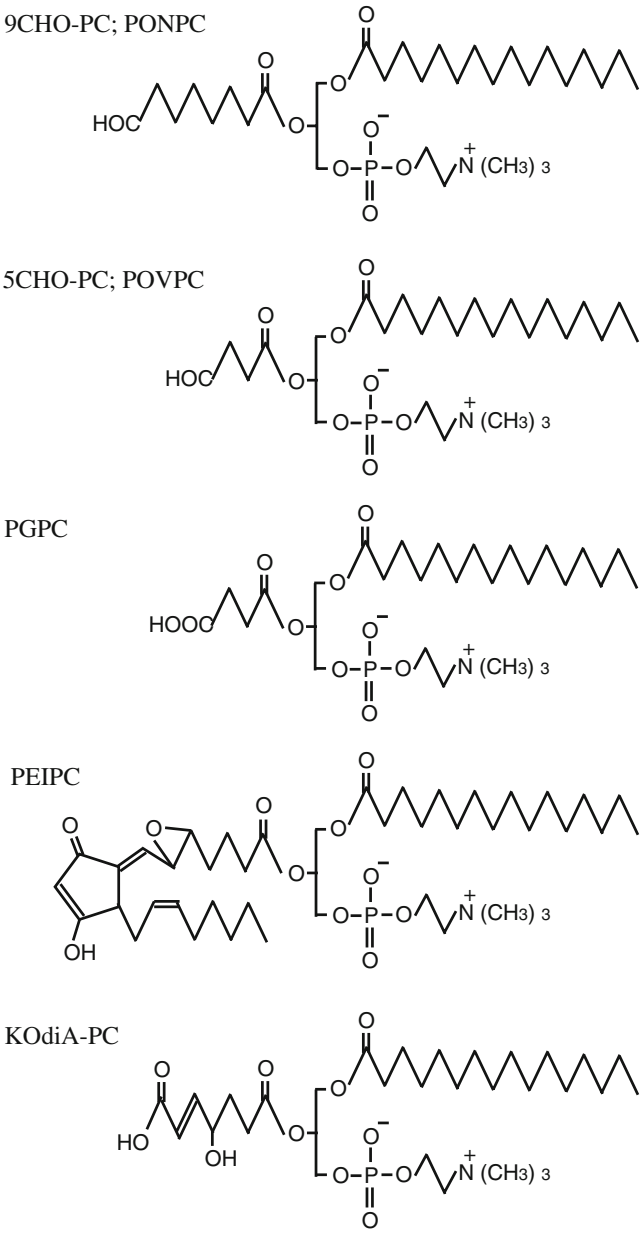

Fig. 2 Typical OxPC products generated in OxLDL. *PONPC* 1-palmitoyl-2-(9-oxononanayl) PC, *POVPC* 1-palmitoyl-2-(5-oxovaleroyl) PC, *PGPC* 1-palmitoyl-2-glutaroyl PC, *PEIPC* 1-palmitoyl-2 eicosaisoprostane PC, *KOdiAPC* keto-hydroxy dicarboxylic acid PC

by macrophages through scavenger receptor-mediated uptake of OxLDL and the antigens deposited in the cells [12, 21].

Some antibodies recognize specific fragments of apoB protein [28, 29]. Because these antibodies do not bind to native LDL, the epitope regions of apoB normally face the inside of the particle but become exposed to the outside when LDL is modified. Such conformational changes could occur after either chemical or oxidative modifications. Actually, the antibodies bind to OxLDL, MDA-LDL, and acetylated LDL, suggesting that this type of mAb may not be specific to oxidative modifications.

Antiphospholipid antibody syndrome (APS) is known as an autoimmune disease with thromboembolic complications. It was thought that patients with APS have antibodies that bind to phospholipids such as cardiolipin, but it turned out that most of the antiphospholipid antibodies recognize several phospholipid-binding proteins. Kobayashi et al. clearly demonstrated that β2-glycoprotein I (β2-GPI) bound to negatively charged phospholipids and OxLDL and that antiphospholipid mAbs raised from APS model mice recognize such complexes containing OxLDL [30–32]. C-reactive protein (CRP), a major acute phase protein in the circulation, binds to OxLDL through recognition of the phosphorylcholine moiety formed during oxidative modifications [31–33]. They showed that mAbs against β2-GPI or CRP, in combination with anti-apoB mAbs, are useful in detecting plasma OxLDL. Thus, antibodies that recognize some proteins forming complexes with OxLDL in the plasma can behave like anti-OxLDL mAbs.

OxPC

OxPCs have been studied extensively to date, partly because they are known bioactive compounds. The phospholipid fraction separated from the minimally modified LDL induces MCP-1 expression in endothelial cells, induction of heme oxygenase-1 in human aortic endothelial cells, enhances interleukin 6-induced hepatic paraoxonase expression, and modulates Toll-like receptor responses [34–37]. Three active compounds were isolated and identified as 1-palmitoyl-2-(5-oxovaleroyl)-PC (or POVPC), 1-palmitoyl-2-glutaroyl-PC (PGPC), and 1-palmitoyl-2-epoxyisoprostane-PC (PEIPC; Fig. 2) [38, 39]. Podrez et al. extensively studied OxPC products which can act as ligands for CD36 scavenger receptor [40]. They found that a series of OxPC products containing α,β-unsaturated carbonyl groups bind strongly to CD36. Recently, it was proposed that some OxPCs could form conjugated products from two molecules of OxPC through aldol condensation reactions [41].

Another important property of OxPC in lipoproteins is that it can be hydrolyzed by some enzymes. PAF-AH hydrolyzes not only PAF but also PC analogs containing a hydrophilic short chain moiety at the sn-2 position [42]. POVPC, which has a five-carbon aldehyde-containing acyl group, is a good substrate for this enzyme. There is some controversy as to whether PAF-AH plays a protective role in atherogenesis by hydrolyzing OxPC or rather acts as a proatherogenic factor by releasing lysoPC and oxidized acyl chains [43]. Recent studies reported that PAF-AH could be a good marker for vulnerable atherosclerotic plaques, since PAF-AH is released from inflammatory cells and produces lysoPC and oxidized fatty acid derivatives [44]. More studies including epidemiological observations are needed to elucidate the pathological roles of PAF-AH.

Plasma OxLDL

The anti-OxPC mAb DLH3 has also been utilized to measure OxLDL in human plasma. OxLDL present in the human circulation was detected with high sensitivity by sandwich enzyme-linked immunosorbent assay (ELISA). In this system, LDL fractions were separated by ultracentrifugation from each plasma sample. DLH3 mAb precoated in the microtiter wells recognizes OxPC adducts in OxLDL, and OxLDL particles captured in the wells are detected by anti-apoB antibodies and alkaline phosphatase-conjugated second antibodies [45]. Plasma OxLDL levels increased in patients with cardiovascular diseases such as acute myocardial infarction (AMI), and the increases correlate with the severity of the disease symptoms [46]. Increased plasma OxLDL levels were also observed in patients with acute phase cerebral infarction [47]. In addition to vascular diseases, increased OxLDL levels were found in patients with carotid arteriosclerosis and those receiving hemodialysis, patients with lecithin–cholesterol acyltransferase deficiency [45, 48–50].

Other mAbs also have been utilized for ELISA measurement of plasma OxLDL. Witztum et al. used the antiphosphorylcholine mAb E06 and a chemiluminescence detector to develop a dual sandwich ELISA system [51, 52]. In this procedure, two sandwich assays are performed for each plasma sample to determine relative concentrations of OxLDL and LDL separately, because LDL concentration in plasma varies between individuals. Finally, they calculated the OxLDL/apoB ratio, i.e., number of oxidized modifications per an apoB-containing particle. This value obtained with E06 procedure is, in part, similar to that obtained with DLH3 procedure, as both procedures detect the ratio of oxidized products in LDL. They reported a significant increase in the OxLDL/apoB ratio in patients with AMI, those with receiving percutaneous intervention treatment and those receiving hemodialysis [53–55].

Tanaga et al. [56] reported that plasma levels of modified LDL in patients with cardiovascular diseases were higher than those in nonpatient subjects measured using a competitive ELISA kit using an anti-MDA mAb, ML25 [26]. MDA is a lipid peroxidation product generated from PUFA during oxidative modification of LDL. MDA easily binds to the ε-amino group of lysine residues to form adducts such as Schiff's base.

Measurement of modified LDL in human plasma samples using a second class of anti-OxLDL mAb, those recognizing an apoB fragment, was also reported [57]. This procedure is based on a simple competition ELISA, in which a known

amount of 4E6 mAb is pre-incubated with plasma samples, and then, the remaining mAb are allowed to bind to precoated OxLDL in microtiter wells. In this assay, the values obtained are the concentrations of plasma OxLDL. Increased concentration of OxLDL in patients with AMI was observed using this measuring system [58]; however, recent reports pointed out that the OxLDL/LDL ratio rather than OxLDL concentration was a potentially useful predictor for cardiovascular diseases [59]. An OxLDL-measuring kit based on 4E6 mAb is commercially available from Mercodia Inc. (Sweden). Since only one mAb is used, it should be noted that the antigen detected may not necessarily be an oxidatively modified apoB.

Plasma OxLDL as a predictive marker

A subject of much discussion is whether OxLDL formed in vivo can act as a cause of atherosclerosis or whether it is generated as a result of lesion formation. It is still difficult to answer this question, but recent progress with in vivo OxLDL studies has provided new evidence suggesting that OxLDL can be used to predict future atherosclerotic events. AMI patients had increased OxLDL levels at the acute phase, which was three times higher than the level in control subjects, and these levels had decreased almost to the basal level at discharge from the hospital. Naruko et al. followed more than 100 patients with AMI after their discharge from the hospital and found that the patients who suffered from restenosis during the following 6 months had higher OxLDL levels at the time of discharge than the patients without restenosis (1.03±0.65 ng/5 μg LDL vs. 0.61±0.34 ng/5 μg LDL) [60]. Plasma OxLDL levels could reflect the balance between oxidative stresses facilitating LDL modifications and the clearance rate of OxLDL from the circulation [61, 62]. It can be speculated that high OxLDL levels at the acute phase of AMI could be caused by massive release of OxLDL from the ruptured plaques, whereas high OxLDL levels during the stable phase may indicate that the patients have strong sources of OxLDL production. These sources include exposure to strong oxidative stresses and presence of unstable plaques somewhere in the circulatory system that releases OxLDL from the lesions.

Although the presence of OxLDL has been established in lesions and patients' plasma, it is not clear whether OxLDL causes atherosclerotic lesion formation or is a result of lesion development. Previous studies on immune systems and OxLDL suggested a proatherogenic property of OxLDL in vivo. It is reported that mice lacking humoral immunity, but not cellular immunity, develop larger atherosclerotic lesions than control mice [63]. It is well known that there are autoantibodies against OxLDL in plasma. Shoji et al. reported a reciprocal relationship between plasma OxLDL levels and plasma anti-OxLDL autoantibody levels in healthy people [64], suggesting that autoantibodies have a role in enhancing the clearance of OxLDL from the circulation. Splenectomy experiments provided further evidence on the role of anti-OxLDL antibodies and the proatherogenic property of OxLDL. Removal of the spleen from an apoE-KO mouse led to worsening of atherosclerotic lesions. Injection of B cells from apoE-KO mice, but not T-cells, reversed the effect of spleen removal on atherogenesis [65]. Very recently, it has been demonstrated that hepatic overexpression of LOX-1, a scavenger receptor, by adenovirus administration reduced plasma OxLDL levels and attenuated atherosclerotic lesion development [66]. These observations strongly suggest that changes in plasma OxLDL levels would affect development of atherosclerotic lesions.

OxLDL structures in vivo

As outlined above, OxLDL is composed of heterogeneously modified lipoprotein particles, which makes its structural analysis very difficult. Immunological methods have so far only successfully detected a specific part of all the modifications using specific mAbs. On the other hand, comprehensive structural analysis of modified lipoprotein particles by liquid chromatography–mass spectrometry (LC-MS)/MS analysis offers a novel approach of identifying OxLDL structures. We introduced an on-membrane procedure of tryptic digestion of proteins for the preparation of LC-MS/MS samples [67]. It is not easy to separate or recover apoB

Table 2 Modified amino acid residues in native LDL fraction and copper-induced OxLDL

Native LDL	
His (+16)	H2245, H2253, H3960
Kynurenine (Trp (+4))	W1114
MP-Lys (+76)	Lys293
Copper-induced oxLDL	
His (+16)	H375, H569, H1113, H1864, H2245, H2253, H3281, H3960
Trp (+16)	W556, W1114, W4087
Kynurenine (Trp (+4))	W556, W1114, W2659, W4087
HNE-His (+156)	His3281
MP-Lys (+76)	Lys293

The sites of modified amino acid residues and types of modifications were analyzed by the LC-MS/MS technique (cited with permission from Obama et al. [67], copyright Wiley-VCH Verlag GmbH & Co. KGaA). Kynurenine is a fluorescent amino acid formed from tryptophan through oxidative cleavage of the indole ring.
MP-Lys N^{ε} -(3-methylpyridinium)lysine (an acrolein-Lys adduct)

on polyacrylamide gels, because the apoB protein is enormous (over 500 kDa) and largely hydrophobic. Thus, apoB is not a suitable protein for processing via the conventional in-gel digestion technique used to prepare tryptic fragments of the samples. We separated the LDL fraction from human plasma by ultracentrifugation, and an aliquot of LDL was blotted onto polyvinylidene difluoride membranes. When delipidation and tryptic digestion were performed on the membranes, the efficiency of tryptic digestion and recovery of the resulting peptides were greatly improved, so that the sequence coverage of apoB protein on LC-MS/MS analysis was more than doubled. Therefore, it enabled determination of the site of oxidative modification in such a huge protein.

It is interesting to note that there are only a few highly susceptible sites of oxidation in apoB. Several modified peptides have been found in the native human LDL fraction. Since the estimated amount of OxLDL present in native LDL is approximately 1/5,000–1/10,000, these modified peptides can be attributed to minute amounts of OxLDL present in human plasma. There are some acrolein-modified lysine residues (MP-lys) in the native LDL fraction (Table 2). In addition, oxidized tryptophan residues are formed in OxLDL. More importantly, the sites of modification in the native LDL fraction do not overlap those in copper-oxidized LDL. This supports the idea that copper-mediated oxidation does not occur under in vivo conditions.

MPO is one plausible candidate for the in vivo oxidant responsible for OxLDL formation. Podrez et al. reported that MPO-dependent oxidative modification of LDL could occur even in the presence of 50% serum [68], whereas serum is known as a potent antioxidant under in vitro conditions. MPO is mainly released from activated neutrophils, and a recent report demonstrated that neutrophils accumulate in atherosclerotic lesions [69].

LDL modification with carbamylation of the ε-amino group of lysine residues through MPO action has been reported [9]. Amino groups can be easily carbamylated by cyanate, which is generated from isothiocyanate and hydrogen peroxide in the presence of MPO. Carbamylated LDL has been shown to induce foam cell formation of mouse peritoneal macrophages and apoptosis of endothelial cells. The carbamylated lysine residues increased in plasma specimens from patients with cardiovascular diseases. Since isothiocyanate is abundant in cigarette smoke, the proatherogenic effect of smoking can be explained, at least in part, by this type of LDL modification.

Elucidation of OxLDL structures is certainly important for understanding the mechanisms of OxLDL formation under in vivo conditions. Recent progress in LC-MS/MS approaches could provide more powerful tools to address this issue.

Conclusion

Immunological techniques, especially ELISA detection of OxLDL in the circulation, provide evidence for the presence of in vivo OxLDL and its relationship to cardiovascular diseases. Recent studies have demonstrated that plasma OxLDL has a predictive potential for secondary prevention. OxLDL could lead to atherosclerotic lesion formation. To address this issue, more studies on the time-course behavior of OxLDL in vivo, its structural analysis, and the molecular mechanisms of OxLDL generation are needed.

References

1. Stocker R (1994) Lipoprotein oxidation: mechanistic aspects, methodological approaches and clinical relevance. Curr Opin Lipidol 5:422–433
2. Hevonoja T, Pentikäinen MO, Hyvönen MT, Kovanen PT, Ala-Korpela M (2000) Structure of low density lipoprotein (LDL) particles: basis for understanding molecular changes in modified LDL. Biochim Biophys Acta 1488:189–210
3. Witztum JL, Steinberg D (1991) Role of oxidized low density lipoprotein in atherosclerosis. J Clin Invest 88:1785–1792
4. Itabe H (1998) Oxidized phospholipids as a new landmark in atherosclerosis. Prog Lipid Res 37:181–207
5. Uchida K, Stadtman ER (1992) Modification of histidine residues in proteins by reaction with 4-hydroxynonenal. Proc Natl Acad Sci U S A 89:4544–4548
6. Podrez EA, Abu-Soud HM, Hazen SL (2000) Myeloperoxidase-generated oxidants and atherosclerosis. Free Radic Biol Med 28:1717–1725
7. Hazen SL, Heinecke JW (1997) 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. J Clin Invest 99:2075–2081
8. Leeuwenburgh C, Hardy MM, Hazen SL, Wagner P, Oh-ishi S, Steinbrecher UP et al (1997) Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. J Biol Chem 272:1433–1436
9. Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Hörkkö S, Barnard J, Reynolds WF, Topol EJ, DiDonato JA, Hazen SL (2007) Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nat Med 13:1176–11874
10. Stadtman ER (1992) Protein oxidation and aging. Science 257:1220–1224
11. Itabe H, Jimi S, Kamimura S, Suzuki K, Uesugi N, Imanaka T et al (1998) Appearance of cross linked proteins in human atheroma and rat pre-fibrotic liver detected by a new monoclonal antibody. Biochim Biophys Acta 1406:28–39
12. Itabe H, Takeshima E, Iwasaki H, Kimura J, Yoshida Y, Imanaka T et al (1994) A monoclonal antibody against oxidized lipoprotein recognizes foam cells in atherosclerotic lesions. Complex formation of oxidized phosphatidylcholine and polypeptides. J Biol Chem 269:15274–15279
13. Itabe H, Yamamoto H, Imanaka T, Suzuki M, Kawai Y, Nakagawa Y, Suzuki A, Takano T (1996) Oxidized phosphatidylcholines that modify proteins. Analysis by anti-oxidized low density lipoprotein monoclonal antibody. J Biol Chem 271:33208–33217
14. Imanaga Y, Sakata N, Tekebayashi S, Matsunaga A, Sasaki J, Arakawa K et al (2000) In vivo and in vitro evidence for

glycoxidation of low density lipoprotein in atherosclerotic plaques. Atherosclerosis 150:343–355
15. Ikura Y, Ohsawa M, Suekane T, Fukushima H, Itabe H, Jomura H et al (2006) Localization of oxidized phosphatidylcholine in nonalcoholic fatty liver disease: impact on disease progression. Hepatology 43:506–514
16. Suzuki M, Kamei M, Itabe H, Yoneda K, Bando H, Kume N et al (2007) Oxidized phospholipids in the macula increase with age and in eyes with age-related macular degeneration. Mol Vis 13:772–778
17. Akagi M, Kanata S, Mori S, Itabe H, Sawamura T, Hamanish C (2007) Possible involvement of the oxidized low-density lipoprotein/lectin-like oxidized low-density lipoprotein receptor-1 system in pathogenesis and progression of human osteoarthritis. Osteoarthr Cartil 15:271–290
18. Naba I, Yoshikawa H, Sakoda S, Itabe H, Suzuki H, Kodama T, Yanagihara T (2000) Onion-bulb formation after a single compression injury in the macrophage scavenger receptor knockout mice. Exp Neurol 166:83–89
19. Palinski W, Hörkkö S, Miller E, Steinbrecher UP, Powell HC, Curtiss LK, Witztum JL (1996) Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma. J Clin Invest 98:800–814
20. Binder CJ, Hörkkö S, Dewan A, Chang M-K, Kieu EP, Goodyear CS, Shaw PX, Palinski W, Witztum JL, Silverman GJ (2003) Pneumococcal vaccination decrease atheroscleostic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. Nat Med 9:736–743
21. Rosenfeld ME, Khoo JC, Miller E, Parthasarathy S, Parinski W, Witztum JL (1991) Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid–protein adducts. J Clin Invest 87:90–99
22. Toyokuni S, Miyake N, Hiai H, Hagaiwara M, Kawakishi S, Osawa T, Uchida K (1995) The monoclonal antibody specific for the 4-hydroxy-2-nonenal histidine adduct. FEBS Lett 359:189–191
23. Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y et al (1998) Protein-bound acrolein: potential markers for oxidative stress. Proc Natl Acad Sci U S A 95:4882–4887
24. Kawai Y, Saito A, Shibata N, Kobayashi M, Yamada S, Osawa T et al (2003) Covalent binding of oxidized cholesteryl esters to protein: implications for oxidative modification of low density lipoprotein and atherosclerosis. J Biol Chem 278:21040–21049
25. Haberland ME, Fong D, Cheng L (1988) Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. Science 241:215–218
26. Kotani K, Maekawa M, Kanno T, Kondo A, Toda N, Manabe M (1994) Distribution of immunoreactive malondialdehyde-modified low-density lipoprotein in human serum. Biochim Biophys Acta 1215:121–125
27. Ichihashi K, Osawa T, Toyokuni S, Uchida K (2001) Endogenous formation of protein adducts with carcinogenic aldehydes: implications for oxidative stress. J Biol Chem 276:23903–23913
28. Holvoet P, Perez G, Zhao Z, Brouwers E, Bernar H, Collen D (1995) Malondialdehyde-modified low density lipoproteins in patients with atherosclerotic disease. J Clin Invest 95:2611–2619
29. Holvoet P, Macy E, Landeloos M, Jones D, Jenny NS, Van de Werf F, Tracy RP (2006) Analytical performance and diagnostic accuracy of immunometric assays for the measurement of circulating oxidized LDL. Clin Chem 52:760–764
30. Kobayashi K, Kishi M, Atsumi T, Bertolaccini ML, Makino H, Sakairi N, Yamamoto I, Yasuda T, Khamashta MA, Hughes GRV, Koike T, Voelker DR, Matsuura E (2003) Circulating oxidized LDL forms complexes with β2-glycoprotein I: implication as an atherogenic autoantigen. J Lipid Res 44:716–726
31. Kobayashi K, Tada K, Itabe H, Ueno T, Liu PH, Tsutsumi A, Kuwana M, Yasuda T, Shoenfeld Y, de Groot PG, Matsuura E (2007) Distinguished effects of antiphospholipid antibodies and anti-oxidized LDL antibodies on oxidized LDL uptake by macrophages. Lupus 16:929–938
32. Matsuura E, Kobayashi K, Tabuchi M, Lopez LR (2006) Oxidative modification of low-density lipoprotein and immune regulation of atherosclerosis. Prog Lipid Res 45:466–486
33. Chang MK, Binder CJ, Torzewski M, Witztum JL (2002) C-reactive protein binds to both oxidized LDL and apoptotic cells through recognition of a common ligand: phosphorylcholine of oxidized phospholipids. Proc Natl Acad Sci U S A 99:13043–13048
34. Berliner JA, Territo MC, Sevanian A, Ramin S, Kim JA, Bamshad B, Esterson M, Fogelman AM (1990) Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. J Clin Invest 85:1260–1266
35. Ishikawa K, Navab M, Leitinger N, Fogelman AM, Lusis AL (1997) Induction of heme oxygenase-1 inhibits monocyte transmigration induced mildly oxidized LDL. J Clin Invest 100:1209–1216
36. Van Lenten BJ, Wagner AC, Navab M, Fogelman AM (2001) Oxidized phospholipids induce changes in hepatic paraoxonase and apoJ but monocyte chemoattractant protein-1 via interleukin-6. J Biol Chem 276:1923–1929
37. Walton KA, Cole AL, Yeh M, Subbanagounder G, Krutzik SR, Modlin RL et al (2003) Specific phospholipid oxidation products inhibit ligand activation of toll-like receptors 4 and 2. Arterioscler Thromb Vasc Biol 23:1197–1203
38. Watson AD, Leitinger N, Navab M, Faull KF, Hörkkö S, Witztum JL et al (1997) Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interaction and evidence for their presence in vivo. J Biol Chem 272:13597–13607
39. Watson AD, Subbanagounder G, Welsbie DS, Faull KF, Navab M, Jung ME et al (1999) Structural identification of novel pro-inflammatory epoxyisoprostane phospholipid in mildly oxidized low density lipoprotein. J Biol Chem. 274:24787–24798
40. Podrez EA, Poliakov E, Zhongzhou S, Zhang R, Deng Y, Sun M et al (2002) Identification of a novel family of oxidized phospholipids that serve as ligands for the macrophage scavenger receptor CD36. J Biol Chem 277:38503–38516
41. Friedman P, Hörkkö S, Steinberg D, Witztum JL, Dennis EA (2002) Correlation of antiphospholipid antibody recognition with the structure of synthetic oxidized phospholipids. Importance of Schiff base formation and aldol condensation. J Biol Chem 277:7010–7020
42. Stremler KE, Stafforini DM, Prescott SM, Zimmerman GA, McIntyre TM (1989) An oxidized derivative of phosphatidylcholine is a substrate for the platelet-activating factor acetylhydrolase from human plasma. J Biol Chem 264:5331–5334
43. Nabav M, Berliner JA, Sabbanagounder G, Hama S, Lusis AJ, Castellani LW, Reddy S, Shih D, Shi W, Watson AD, Van Lenten BJ, Vora D, Fogelman AM (2001) HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. Arterioscler Thromb Vasc Biol 21:481–488
44. Zalewski A, Macphee C (2005) Role of lipoprotein-associated phospholipase A_2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol 25:923–931
45. Itabe H, Yamamoto H, Imanaka T, Shimamura K, Uchiyama H et al (1996) Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. J Lipid Res 37:45–53

46. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otuska M et al (2001) Oxidized low density lipoprotein relates to plaque destabilization in human coronary atherosclerotic lesions. Circulation 103:1955–1960
47. Miyazaki H, Matsuoka H, Itabe H, Usui M, Ueda S, Okuda S et al (2000) Hemodialysis impairs endothelial function via oxidative stress. Effects of vitamin E-coated dialyzer. Circulation 101:1002–1006
48. Nishi K, Itabe H, Uno M, Kitazato KT, Horiguchi H, Shinno K, Nagahiro S (2002) Oxidized LDL in carotid plaques and plasma associates with plaque instability. Arterioscler Thromb Vasc Biol 22:1649–1654
49. Uno M, Kitasato K, Nishi K, Itabe H, Nagahiro S (2003) Elevation of plasma oxidized LDL in acute cerebral infarction. J Neurol Neurosurg Psychiatry 74:312–316
50. Itabe H, Hosoya R, Karasawa K, Jimi S, Saku K, Takebayashi S et al (1999) Metabolism of oxidized phosphatidylcholines formed in oxidized low density lipoprotein by lecithin-cholesterol acyltransferase. J Biochem 126:153–161
51. Penny WF, Ben-Yehuda O, Kuroe K, Long J, Bond A, Bhargava V, Peterson JF, McDaniel M, Julicano J, Witztum JL, Ross J Jr, Peterson KL (2001) Improvement of coronary artery endothelial dysfunction with lipid-lowering therapy: heterogeneity of segmental response and correlation with plasma-oxidized low density lipoprotein. J Am Coll Cardiol 37:766–774
52. Fang JC, Kinlay S, Bhargava V, Hikita H, Witztum JL, Selwyn AP, Ganz P (2006) Circulating autoantibodies to oxidized LDL correlate with impaired coronary endothelial function after cardiac transplantation. Arterioscler Thromb Vasc Biol 26:2044–2048
53. Tsimikas S, Witzutum JL, Miller ER, Sasiela WJ, Szarek M, Olsson AG, Schwartz GG (2004) High-dose atorvastatin reduces total plasma levels of oxidized phospholipids and immune complexes present on apolipoprotein B-100 in patients with acute coronary syndromes in the MIRACL trial. Circulation 110:1406–1412
54. Tsimikas S, Lau HK, Han K-R, Shortal B, Miller ER, Segev A, Curtiss LK, Witztum JL, Strauss BH (2004) Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a). Short-term and long-term immunologic responses to oxidized low-density lipoprotein. Circulation 109:3164–3170
55. Tsimikas S, Bergmark C, Beyer RW, Patel R, Pattison J, Miller E, Juliano J, Witztum JL (2003) Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. J Am Coll Cardiol 41:360–370
56. Tanaga K, Bujo H, Inoue M, Mikami K, Kotani K, Takahashi K, Kanno T, Saito Y (2002) Increased circulating malondialdehyde-modified LDL levels in patients with coronary artery diseases and their association with peak sizes of LDL particles. Arterioscler Thromb Vasc Biol 22:662–666
57. Holvoet P, Donck J, Landeloos M, Brouwers E, Luijtens K, Arnout J, Lasaffre E, Vanrenterghem Y, Collen D (1996) Correlation between oxidized low density lipoproteins and von Willebrand factor in chronic renal failure. Thromb Haemost 76:663–669
58. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, Collen D, Muls E, Van de Werf F (2001) Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol 21:844–1848
59. Girona J, Manzanares JM, Marinón F, Cabré A, Heras M, Guardiola M, Ribalta J, Masana L (2008) Oxidized to non-oxidized lipoprotein rations are associated with aeteriosclerosis and the metabolic syndrome in diabetic patients. Nutr Metab Cardiovasc Dis 18:380–387
60. Naruko T, Ueda M, Ehara S, Itoh A, Haze K, Shirai N, Ikura Y, Ohsawa M, Itabe H, Kobayashi Y, Yamagishi H, Yoshiyama M, Yoshikawa J, Becker AE (2006) Persistent high levels of plasma oxidized low-density lipoprotein after acute myocardial infarction predict stent restenosis. Arterioscler Thromb Vasc Biol 26:877–883
61. Itabe H (2003) Oxidized low-density lipoproteins: what is understood and what remains to be clarified. Biol Pharm Bull 26:1–9
62. Itabe H, Ueda M (2007) Measurement of plasma oxidized low-density lipoprotein and its clinical implications. J Atheroscler Thromb 14:1–11
63. Fyfe AI, Qiao J-H, Lusis AJ (1994) Immune-deficient mice develop typical atherosclerotic fatty streaks when fed an atherogenic diet. J Clin Invest 94:2516–2520
64. Shoji T, Nishizawa Y, Fukumoto M, Shimamura K, Kimura J, Kanda H, Emoto M, Kawagishi T, Morii H (2000) Inverse relationship between circulating oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibody levels in healthy subjects. Atheroscler 148:171–178
65. Caligiuri G, Nicoletti A, Poirer B, Hansson GK (2002) Protestive immunity against atherosclerosis carried by B cells of hypercholesterolemic mice. J Clin Invest 109:745–753
66. Ishigaki Y, Katagiri H, Gao J, Yamada T, Imai J, Uni K, Hasegawa Y, Kaneko K, Ogihara T, Ishihara H, Sato Y, Takikawa K, Nishimichi N, Matsuda H, Sawamura T, Oka Y (2008) Impact of plasma oxidized low-density lipoprotein removal on atherosclerosis. Circulation 118:75–83
67. Obama T, Kato R, Masuda Y, Takahashi K, Aiuchi T, Itabe H (2007) Analysis of modified apolipoprotein B-100 structures formed in oxidized low-density lipoprotein using LC-MS/MS. Proteomics 7:2132–2141
68. Podrez EA, Schmitt D, Hoff HF, Hazen SL (1999) Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro. J Clin Invest 103:1547–1560
69. van Leeuwen M, Gijbels MJJ, Duijvestijn A, Smook M, van de Gaar MJ, Heeringa P, de Winther MPJ, Tervaert LWC (2008) Accumulation of myeloperoxidase-positive neutrophils in atherosclerotic lesion in LDLR$^{-/-}$ mice. Arterioscler Thromb Vasc Biol 28:84–89