Low Density Lipoprotein Oxidation and Its Pathobiological Significance*

Daniel Steinberg‡

From the Department of Medicine, University of California, San Diego, La Jolla, California 92093-0682

The fact that low density lipoprotein (LDL) is extremely susceptible to oxidative damage has been known for some time (1, 2), but until quite recently this was primarily a nuisance for the student of lipoprotein metabolism. It now appears that oxidation of LDL plays a significant role in atherogenesis.

Beginning in the 1980s evidence began to accumulate that cholesterol accumulation in the developing atherosclerotic lesion was probably not due to the uptake of native LDL by way of the Brown/Goldstein LDL receptor but instead due to the uptake of some modified form of LDL (then still unidentified) by way of one or more alternative receptors (also then unidentified). This conclusion grew from two well accepted observations. First, patients and animals totally lacking the LDL receptor nevertheless accumulate cholesterol in foam cells much the same way as do patients and animals with normal LDL receptors; second, the two cell types in lesions that give rise to cholesterol-laden foam cells (the monocyte/macrophage and the smooth muscle cell) do not accumulate cholesterol in vitro even in the presence of very high concentrations of native LDL (3, 4). This paradox could be resolved if circulating LDL underwent some form of modification and if the modified form, rather than native LDL itself, then served as the ligand for delivery of cholesterol to developing foam cells. Acetylation of LDL in vitro generated a modified LDL that could induce cholesterol accumulation in macrophages (3). The uptake of this acetylated LDL was by way of a new receptor designated the acetyl LDL receptor (later cloned and renamed scavenger receptor A (SRA)) (5). SRA, unlike the LDL receptor, is not down-regulated when the cholesterol content of the cell increases. Thus, acetyl LDL could, in principle, account for foam cell formation. However, there was (and still is) no evidence that acetylation of LDL occurs to any extent in vivo. Another modified form of LDL emerged as a candidate when it was shown that simply incubating LDL overnight with a monolayer of arterial endothelial cells converted it to a form that was taken up much more rapidly by macrophages and capable of increasing their cellular cholesterol content (6–8). The uptake was specific and saturable, and it occurred in part by way of the acetyl LDL receptor. Incubation with smooth muscle cells could also modify LDL in much the same way (7, 8). This cell-mediated modification turned out to be, very simply, oxidative modification (9, 10). The addition of antioxidants to the culture medium completely blocked cell-induced modification, and the changes induced by the cells could be duplicated by incubating LDL in the presence of transition metals in the absence of cells. Thus, oxidative modification induced by cells appeared to be a biologically plausible modification of LDL that could account for foam cell formation and the initiation, or at least acceleration, of the atherosclerotic process.

Between 1985 and 1989, 62 papers were published about “oxidized LDL”; between 1992 and January 1997, 727 papers were published about “oxidized LDL.” This intense interest springs largely from the increasing evidence that oxidative modification of LDL plays a significant role in experimental atherosclerosis and thus may represent a target for interventions to slow the progress of the disease (11, 12).

This review is limited to oxidative modification of LDL, but it should be noted that other modified forms of LDL (e.g., aggregated LDL or LDL-containing immune complexes) can also induce foam cell formation and could contribute to atherogenesis (13–15).

**Early Events in Atherogenesis**

To put the following discussion of oxidized LDL and its pathobiological effects into a context, we begin with a brief summary of current views on the initiation of the atherosclerotic lesion. More detailed discussions are available elsewhere (16).

An increase in plasma LDL levels leads to an increase in the adherence of circulating monocytes to arterial endothelial cells and at the same time to an increased rate of entry of LDL into the intima, resulting in a higher steady state concentration of LDL in the intima. There the LDL can undergo oxidative modification catalyzed by any of the major cell types found in arterial lesions, i.e., endothelial cells, smooth muscle cells, or macrophages. Even minimally oxidized LDL (MM-LDL) can increase adherence and penetration of monocytes, in part by stimulating release of MCP-1 from endothelial cells (17). MM-LDL can also stimulate release of MCSF, which can induce differentiation of the monocyte into a cell with the phenotypic pattern of the tissue macrophage, including an increase in expression of SRA (18). More fully oxidized LDL (Ox-LDL) is itself directly chemotactic for monocytes, and it is also, of course, one of the major ligands for SRA and other receptors on the arterial macrophage that contribute to foam cell formation. Soon after a lesion is initiated there is fragmentation of the internal elastic membrane and migration of smooth muscle cells from the media up into the intima. These smooth muscle cells do not normally express SRA but can be induced to do so (19). This may be the basis for the contribution that smooth muscle cells make to the foam cell population. A centrally important point is that the fatty streak lesion, while being clinically silent itself, is the precursor of the more complex lesions that cause stenosis and limited blood flow. These complex lesions ultimately represent the sites of thrombosis leading to myocardial infarction.

**Additional Potentially Proatherogenic Properties of Oxidized LDL**

As already mentioned, the first property of oxidized LDL to be discovered that makes it more atherogenic than native LDL is that it is recognized by the scavenger receptors and can therefore give rise to foam cells (7). Additional potentially proatherogenic properties became apparent soon thereafter, including the fact that Ox-LDL is itself a chemotactic agent for monocytes (20) and that it inhibits the motility of tissue macrophages (21). Oxidized LDL is cytotoxic for endothelial cells in culture (22); it inhibits the vasodilatation that is normally induced by NO (23); it is mitogenic for macrophages and smooth muscle cells (24, 25); it can stimulate the release of MCP-1 and MCSF from endothelial cells (17, 18). Ox-LDL is immunogenic, and autoantibodies are commonly found both in animals and patients (26–28). Titers tend to be higher in patients with more rapidly progressive disease (27), but, paradoxically, immunization of rabbits with OxLDL to raise antibody titer actually inhibits lesion progression (29).

There are as many as 20 additional biological effects that have been described, but almost none of these has been evaluated in vivo. In any case, these examples will suffice to demonstrate that the oxidative modification of LDL leads to a possibly very large array of consequences above and beyond the generation of foam cells that could be important in atherogenesis. It is important also
to stress that some of these biological effects can be exercised by minimally modified LDL. Many of these appear to be attributable to partially oxidized phospholipids that may mimic the effects of platelet-activating factor or of autacoids (30, 31). The immunogenicity of OxLDL appears also to be attributable in part to oxidized phospholipids, possibly complexed with protein or other lipids (32, 33). Before leaving this topic, it is worth noting, as discussed further below, that the oxidation of the lipoprotein matrix of a plasma membrane is somewhat analogous to the oxidation of an LDL particle. Just as oxidized LDL can exert a number of effects, including regulation of gene expression, oxidation of the plasma membrane of a cell may give rise to analogous biological effects that could be relevant during apoptosis or under conditions of high oxidative stress.

The Nature of “Oxidized LDL”

Originally, oxidized LDL was defined primarily in terms of its biological properties, notably the fact that it was no longer a ligand for the native LDL receptor but was a ligand for the acetyl LDL receptor and that its uptake by macrophages was therefore much more rapid, sufficient to cause cholesterol accumulation. This degree of oxidation could be effected by incubation overnight with cultured cells in the appropriate medium or by incubation with 5–10 μM Cu²⁺ for 8–16 h. Later studies showed that after much gentler oxidative modification (too little to alter its binding by the LDL receptor and yet not enough to make it a ligand for the acetyl LDL receptor) the oxidized LDL had different and potentially very important biological properties of other kinds, including the ability to stimulate the release of endothelial cells of MCP-1 and M-CSF (17, 18). This minimally oxidized LDL, designated “MM-LDL,” is very different from LDL incubated overnight with copper ions, which may deserve to be redesignated as “maximally oxidized LDL.” Obviously there is potentially a continuous spectrum of degrees of oxidation and a great deal of molecular heterogeneity in what we call “oxidized LDL” (34). Even if oxidative conditions are controlled as precisely as possible, the product will still vary from experiment to experiment depending on the composition of the starting LDL. LDL particles rich in polyunsaturated fatty acids are more readily oxidized than are LDL particles enriched in saturated fatty acids or monounsaturated fatty acids (35). The content of vitamin E and other naturally occurring indigenous antioxidants will influence the susceptibility of LDL preparations to oxidation under any given set of conditions. The enormous complexity of the problem is evident when one considers that the average LDL particle contains about 700 molecules of phospholipids, 600 of free cholesterol, 1600 of cholesterol esters, 185 of triglycerides, and 1 of apolipoprotein B (apoB) containing 4536 amino acid residues! Both the lipids and the protein are subject to oxidation and both are indeed oxidized. Direct oxidative damage to proteins is discussed by Berlett and Stadtman the previous Minireview in this series (74), and almost certainly there is some direct apoB oxidation. Cholesterol is converted to oxysterols, especially at the 7-position. The polyunsaturated fatty acids in cholesterol esters, phospholipids, and triglycerides are subject to free radical-initiated oxidation and can participate in chain reactions that amplify the extent of damage. A key feature of LDL oxidation is the breakdown of these polyunsaturated fatty acids to yield a broad array of smaller fragments, 3–9 carbons in length, including aldehydes and ketones that can become conjugated to other lipids (especially amino lipids) or to the apoB (36). For example, malondialdehyde (or other aldehydes) generated during oxidation can form Schiff bases with the ε-amino groups of lysine residues and can go on to generate cross-links generated during oxidation can form Schiff bases with the apoB (36). For example, malondialdehyde (or other aldehydes) can become conjugated to other lipids (especially amino lipids) or to polyunsaturated fatty acids to yield a broad array of smaller fragments, 3–9 carbons in length, including aldehydes and ketones that can become conjugated to other lipids (especially amino lipids) or to the apoB (36). For example, malondialdehyde (or other aldehydes) generated during oxidation can form Schiff bases with the ε-amino groups of lysine residues and can go on to generate cross-links.
macrophages, and smooth muscle cells, are involved in its oxidation. However, there is no convincing in vivo evidence to implicate one or another of these as more important than the others.

While oxidation of LDL in the artery wall has received the most attention, it seems very likely that oxidation of LDL takes place at many other sites, perhaps at all sites of inflammation. Because of increased vascular permeability at sites of inflammation, the concentration of LDL in the inflammatory fluid would be higher than it is in normal extracellular fluid. Because of the infiltration by neutrophils and monocyte/macrophages the conditions for LDL oxidation at inflammatory sites would be propitious. However, LDL oxidation at peripheral sites would not have the same significance as oxidation of LDL in the artery wall unless the LDL oxidized at peripheral sites reenters the bloodstream and is subsequently delivered to the artery. If LDL in the periphery were to undergo limited oxidation before reentering the blood it would have a prolonged half-life, as discussed above, and it could then be taken up into developing arterial lesions. Being already partially oxidized, this LDL might make an unusually large contribution to the further progression of the lesion. Immunochemical studies have provided evidence for the presence of oxidized LDL (or at least of antigens closely related to it) at sites of inflammation (47). The functional significance of this remains to be explored.

Antioxidant Inhibition of Atherogenesis in Experimental Models

If oxidative modification of LDL plays a significant role in atherosclerosis, its inhibition by an appropriate antioxidant should slow the progression of the disease. Indeed this has now been demonstrated in several different animal models (the LDL receptor-deficient rabbit, the cholesterol-fed New Zealand White rabbit, the cholesterol-fed hamster, the cholesterol-fed cynomolgus monkey, the LDL receptor-deficient mouse, and the apoprotein E-deficient mouse) and using one of several different antioxidants (probucol, butylated hydroxytoluene, diphenylphenylenediamine, and vitamin E (58)). A total of 23 studies has been reported of which 16 were strongly positive (more than 50% inhibition of the rate of progression), 2 were borderline, and 5 negative. An important question to be asked is whether the antioxidants exerted their inhibitory effect on lesion progression only because of their antioxidant properties or, possibly, because of additional biological properties. This is the same kind of problem that arises with the use of any inhibitor in biology. In fact the first antioxidant tested, probucol, does indeed have additional biological properties that might be relevant (48), including the ability to inhibit interleukin-1 release and to increase expression of cholesterol ester transfer protein. However, the fact that two antioxidants as structurally diverse as probucol and diphenylphenylenediamine share the ability to inhibit atherogenesis suggests that the effect is attributable primarily to their shared antioxidant properties. Further evidence that the effect depends upon antioxidant activity comes from the rough parallelism observed in some studies between the effectiveness of these compounds in protecting circulating LDL from oxidation in an ex vivo test system and their effectiveness in inhibiting atherogenesis (49).

At this time there is insufficient evidence, however, to allow a convincing in vivo evidence to implicate one or another of these as more important than the others.

Is OxLDL Relevant to the Human Disease?

The basic pathobiology of experimental atherosclerosis appears to be very much the same as that of the human disease, suggesting that antioxidants should work in humans. Furthermore, epidemiologic studies have repeatedly shown a negative correlation between levels of dietary intake or plasma levels of antioxidant vitamins, on the one hand, and, risk of coronary heart disease, on the other (65). On the other hand, the time scale over which lesions develop in animal models is very short (weeks or months) compared with the time scale over which human lesions evolve (decades). Also, the degree of antioxidant protection we can achieve in humans may be less than that achieved in animal studies.

Several clinical trials on effects of 

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Raison d’Etre

OxLDL is bound and internalized by at least two and possibly three different macrophage receptors. Because these receptors tend to have a much broader ligand specificity than previously studied receptors they have been designated “scavenger receptors” or “multiligand” receptors (51, 52). The best studied example is the original acetyl LDL receptor, now redesignated scavenger receptor A, which occurs in two differentially spliced forms (SRAI and SRAII) that have very similar ligand binding specificity. Unlabeled acetyl LDL can only inhibit some 40–60% of the binding of OxLDL to mouse peritoneal macrophages (7, 8), and macrophages from mice in which SRA has been targeted show only a partial defect in OxLDL binding, implying that additional receptors are involved (53). Recent evidence shows that SRA can also play a role in the adherence of macrophages to plastic surfaces (54) and to glycosylated collagen (55).

The pathogenetic role of SRA in atherosclerosis has now been demonstrated by crossing SRA-targeted mice with apoprotein E-targeted mice and finding a highly significant 58% reduction in lesion severity (53). Since OxLDL is one of the major naturally occurring ligands for SRA, these findings further support the oxidative modification hypothesis of atherosclerosis.

The B class of scavenger receptors includes CD36 (56) and SR-B1 (57). Finally, a receptor with scavenger receptor-like activity has been cloned from Drosophila (58), and it has been designated as the first member of a new class of scavenger receptors, SRC.

Recent studies have shown that macroscialin and its human homologue, CD68, can bind OxLDL in ligand blots and that antibodies against CD68 can partially inhibit the binding and uptake of OxLDL by a human monocyte-derived cell line, the THP-1 cell line (59, 60). However, only a very small fraction of macroscialin or of CD68 is expressed on the plasma membrane, and their importance in the uptake of OxLDL by normal monocytes/macrophages remains to be further evaluated.

As mentioned above, receptors with at least some of the properties of SRA and SRB can be found all the way back to Drosophila (58). Why have these receptors persisted in evolution? Surely it can have nothing to do with any role they play in atherosclerosis. Atherosclerosis is almost exclusively a human disease, and in any case, its clinical effects occur after the preclinical period is over so there cannot be any selective genetic pressure (positive or negative). We have suggested that oxidative damage to a cell membrane may generate lipid-protein products similar to those found in oxidatively damaged LDL (61). Indeed, the binding of oxidatively damaged red blood cells to macrophages is competitively inhibited by OxLDL but, interestingly, not by acetyl LDL. The binding of apoptotic thymocytes to macrophages is also inhibited by oxidized LDL (62), a finding compatible with the hypothesis. The two best studied receptors for oxidized LDL, SRA and CD36, also bind apoptotic cells. The role of CD36 in this respect has been extensively studied (63) and appears to involve cooperative interaction with αvβ3 and thrombospondin. Recent studies show that peritoneal macrophages from mice in which SRA has been “knocked out” show a deficit in the phagocytosis of apoptotic thymocytes (64). Thus it may be that as we search for receptors that bind oxidatively damaged LDL we are at the same time on the trail of receptors whose primary function is to recognize damaged (apoptotic) cells.

Receptors for OxLDL and Their Evolutionary Raison d’Etre

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Several clinical trials on effects of β-carotene have, unfortunately to test its possible efficacy in preventing cancer, have also recorded cardiovascular events (66–68). All of them have been negative with respect to effects on either cancer or cardiovascular disease. Unfortunately, it was not recognized until recently that β-carotene is actually relatively ineffective in protecting LDL (much less effective than vitamin E). Carefully conducted trials in human subjects show that supplementation even with very large doses of β-carotene (doses sufficient to increase the β-carotene concentration in the LDL fraction severalfold) fails to protect the circulating LDL against oxidation ex vivo (69, 70). β-Carotene is an effective antioxidant activity comes from the rough parallelism observed in some studies between the effectiveness of these compounds in protecting circulating LDL from oxidation in an ex vivo test system and their effectiveness in inhibiting atherogenesis (49). At this time there is insufficient evidence, however, to allow a convincing in vivo evidence to implicate one or another of these as more important than the others.
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Vitamin E, on the other hand, is very effective in protecting circulating LDL against oxidation ex vivo (69, 71, 72). The degree of protection in vivo is a function of the extent to which the vitamin E content of the circulating LDL is increased, and doses of 400–800 IU of vitamin E daily in a placebo-controlled, double-blind trial in patients with established coronary heart disease (70). Those randomized to vitamin E showed 47% fewer nonfatal myocardial infarctions and cardiovascular deaths (the primary end point) than the control group, and the result was significant at the p = 0.001 level. Additional trials are in progress. Decisions about the use of antioxidants in human atherosclerosis should be deferred until additional data become available.

REFERENCES