

History of Discovery Oxidized Low-Density Lipoprotein and Atherosclerosis

Daniel Steinberg, Joseph L. Witztum

Chance and serendipity play major roles in the history of science. Too often, though, their contributions do not show up in formal publications. We all tend to shape history according to the styles of the times and according to our own biases. Acknowledged or unacknowledged, there is a tendency to want the scientific “story” to be neat and more or less linear. The genesis of the oxidized low-density lipoprotein (OxLDL) hypothesis owed a great deal to happenstance, but that may not be readily apparent from the formal articles. We take this opportunity to tell the whole story, at least as we remember it.

Several apparently unrelated events occurring at about the same time in Oslo, Norway; in Cleveland, Ohio; in La Jolla, California; in New York, New York; and in Dallas, Texas converged to lay the groundwork for the hypothesis that oxidative modification of LDL might be important in atherogenesis. Later developments regarding the relationship between OxLDL and the immune system in atherogenesis, again, were often smiled on by chance and serendipity.

Oslo, Cleveland, and La Jolla

In 1979, a young Norwegian researcher, Tore Henriksen, got in touch with our laboratory asking whether he could come to La Jolla to learn more about lipoproteins. He and his colleagues in Oslo had observed that under certain conditions, LDL was highly toxic for endothelial cells in culture, leading to cell death in just 24 hours.¹ Almost simultaneously and independently, Chisolm and coworkers in Cleveland made very similar observations.² Both groups noted that addition of whole serum or purified high-density lipoprotein to the medium completely prevented the toxic effects of the LDL, but the mechanism(s) involved remained unclear. Henriksen arrived in La Jolla in the summer of 1980 and demonstrated to us how drastically LDL affected the cultured cells. We agreed that the phenomenon was worth studying but urged him to first try to determine what, if anything, was happening to the LDL during the course of these incubations.

La Jolla and New York

In 1979, about the same time that Henriksen applied to come to our laboratory, one of us (D.S.) had applied to Zanvil Cohn at the Rockefeller University for a Visiting Scientist appointment to come to New York and learn something about macrophage biology. Concurrently, one of Cohn’s fellows, Eileen M. Mahoney, had applied to come to our laboratory in

La Jolla to learn more about lipoproteins. In short, there was a felicitous game of musical chairs in which no one was left without a seat! Mahoney went to work with Henriksen, and they quickly showed that the LDL isolated at the end of the incubation with endothelial cells was very different from the LDL that went into the incubation. It was much denser and had acquired a much greater negative charge.³ The extent of the changes depended on the concentration of LDL added and the duration of the incubation. The increase in density was quite dramatic, increasing to more than 1.063 in some studies, ie, to a density like that of high-density lipoprotein.^{4,5} The agarose gel electrophoretic mobility of the modified LDL almost doubled, and notably, the whole peak shifted without much broadening. Later studies would show that the increased net negative charge reflected mainly the masking of lysine ϵ amino groups on apolipoprotein B (apoB). The drastic increase in hydrated density was due to loss of cholesteryl esters and hydrolysis of (oxidized) phospholipids.

Dallas and La Jolla

In that same year, 1979, Goldstein et al⁶ published their surprising finding that peritoneal macrophages in culture take up and degrade native LDL but that they do so very slowly, too slowly to account for foam cell generation under the conditions tested. Because LDL is the primary source of the lipids in foam cells, Goldstein et al postulated that LDL must undergo some structural modification in vivo and that this modified form was what the macrophage took up. They found that chemically acetylated LDL was taken up avidly enough to make foam cells. However, acetyl-LDL itself had not (and still has not) been demonstrated in vivo, so the hunt was on for a biologically generated form of LDL that might behave biologically like acetyl-LDL.

Endothelial Cells Convert LDL to a Form Recognized by a Macrophage Scavenger Receptor(s)

This was the background that led Henriksen et al³ to test LDL previously incubated overnight with endothelial cells (endothelial cell [EC]-modified LDL) to see whether it might resemble acetyl-LDL in terms of macrophage binding and uptake. It did, and it competed with acetyl-LDL for binding and uptake. The competition was incomplete, implying the

From the Department of Medicine, University of California, San Diego, La Jolla, Calif.

Correspondence to Daniel Steinberg, MD, PhD, University of California, San Diego, Department of Medicine-MC 0682, 9500 Gilman Dr, La Jolla, CA 92093 (E-mail dsteinberg@ucsd.edu); or Joseph L. Witztum, MD, University of California, San Diego, Department of Medicine-MC 0682, 9500 Gilman Dr, La Jolla, CA 92093 (E-mail jwitztum@ucsd.edu).

(*Arterioscler Thromb Vasc Biol.* 2010;30:2311-2316.)

© 2010 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.108.179697

presence of some receptors specific for EC-modified LDL and not acetyl-LDL. In what ways was LDL being modified?

One plausible hypothesis was that proteins or other compounds released from the damaged and dying endothelial cells were attaching to the LDL, thus increasing its density and making it a ligand for the macrophage receptors that recognized EC-modified LDL. However, no such conjugation could be demonstrated.

Another possibility was that enzymes released from the dying endothelial cells were chewing up the LDL. We tried incubating LDL with various enzymes and combinations of enzymes but could not replicate the changes that led to macrophage recognition.⁷

A possibility we seriously considered early on was oxidative modification. In 1983, the Cleveland Clinic group presented convincing evidence that oxidative changes were essential for converting LDL to the cytotoxic form.⁸ However, Henriksen had carried out incubations in nitrogen-washed flasks, and the EC modification appeared to be just as extensive as it was under oxygen. That same year, one of our new postdoctoral fellows came to us puzzled because his incubation of LDL with endothelial cells had yielded no modification. Another postdoc, Urs Steinbrecher, pointed out that the new postdoc had used Dulbecco's modified Eagle's medium rather than the Ham's F-10 medium we had been using routinely. He noted that Dulbecco's modified Eagle's medium contains lower concentrations of copper and other transition metals than Ham's F-10 and that supplementing Dulbecco's modified Eagle's medium with copper restored the EC modification, indicating that copper catalyzed oxidation was involved. We showed that EC modification was blocked by addition of EDTA, vitamin E, or a low concentration of whole serum.⁹ In retrospect, Henriksen's flasks washed with nitrogen must have still contained enough oxygen to support the oxidative conversion of LDL to EC-modified LDL. Thus, "EC-modified LDL" became "oxidatively modified LDL" (OxLDL). OxLDL was the first biologically plausible modification that could resolve the paradox originally pointed out by the Brown and Goldstein laboratory.

Inhibition of Atherogenesis in LDL Receptor-Deficient Rabbits by Probucol, an Antioxidant

Chance again played a key role in pointing us to probucol as an ideal agent for testing our hypothesis in animal models of atherosclerosis. At the time, probucol was in fairly broad use in the clinic for lowering plasma LDL levels, but its mechanism of action remained unknown. A visiting scientist, Marek Narusewicz, had undertaken a series of studies aimed at clarifying probucol's mechanism of action, starting with measurements of LDL flux in LDL receptor (LDLR)-deficient rabbits. Meanwhile, Sampath Parthasarathy was continuing his studies on the mechanisms of oxidative modification of LDL. One day he was given some labeled LDL by Narusewicz and got almost no *in vitro* modification! Yes, he had obtained LDL that had been harvested from a probucol-treated rabbit. We quickly were able to show that probucol was indeed a potent inhibitor of LDL oxidation.¹⁰ (Note that

the LDL-lowering effect of probucol does not depend on its antioxidant properties.)

In any case, here was a drug already approved for clinical use and already reported to have some effectiveness in human atherosclerosis, even in patients who lacked LDLRs.¹¹ We decided to plunge right ahead to see whether it worked in our LDL-receptor deficient rabbit model. Meanwhile, Toru Kita and his colleagues in Japan, impressed by the efficacy of probucol in patients with homozygous familial hypercholesterolemia and by our 1986 article on probucol as an antioxidant, started their independent studies at almost the same time. Both laboratories showed that probucol strongly inhibited the atherogenic process in WHHL rabbits.^{12,13} These findings were confirmed in a number of laboratories using various animal models and using several different antioxidants (reviewed in Steinberg¹⁴). Over the next decade, a wealth of cell culture and animal model evidence was published that supported involvement of LDL oxidation in the pathogenesis of atherosclerosis (reviewed in Steinberg et al¹⁵). By 1991, the strength of that evidence was such that a panel of experts convened by the National Heart, Lung and Blood Institute unanimously recommended initiation of clinical trials.¹⁶ Currently, PubMed lists 5877 manuscripts indexed under "oxidized LDL" and 2639 under "oxidized LDL and atherosclerosis." Space considerations preclude any detailed consideration of the extensive literature documenting the effectiveness of antioxidants in inhibiting atherogenesis in animal models, as well as all the new developments in the field that describes the impact of oxidation on other lipoproteins, such as high-density lipoprotein, but a number of more recent reviews are available.^{14,17-22}

What Is the Physiological Role of the Scavenger Receptors?

While we were focused on the role of scavenger receptors in atherosclerosis, we had to stop and ask why they were there at all. After all, atherosclerosis exerts no selective genetic pressure one way or the other, because it becomes a significant disorder only after the reproductive years are over. Besides, the macrophages of rabbits and mice, species that never spontaneously develop atherosclerosis, also express these receptors. So the question was what are the physiological ligands they recognize and what is their physiological function? We asked what looks like OxLDL and whether it might bind like OxLDL. Well, LDL looks a little like the outer layer of a cell membrane—a phospholipid/cholesterol monolayer with protein embedded in it. Maybe an oxidatively damaged cell would look enough like OxLDL to be recognized by the receptors that bind OxLDL. One of our graduate students, Gilbert Sambrano, took on that problem and quickly showed that red blood cells oxidatively damaged by previous incubation with Cu⁺⁺ and ascorbic acid adhered very tightly to cultured mouse peritoneal macrophages; undamaged red blood cells did not. No serum was present, so the binding of the oxidized red blood cells was not dependent on opsonizing antibodies (Abs), ie, not due to binding to the Fc receptor. Moreover, the binding was competitively reversed almost completely by OxLDL but not by native LDL or acetyl LDL.²³ Sambrano went on to show that apoptotic thymocytes, like oxidatively damaged red blood cells, bound tightly to

macrophages and that this binding was inhibited almost 50% by OxLDL.²⁴ We postulated that the scavenger receptors on the macrophage are there to recognize and phagocytose apoptotic and necrotic cells and that these receptors recognize the same or similar ligands on OxLDL (discussed in detail below).

Which of the Candidate Scavenger Receptors Play Important Roles in Atherogenesis?

The acetyl LDLR discovered by Brown et al²⁵ was cloned by Kodama in the laboratory of Monty Krieger²⁶ and renamed scavenger receptor A-1. That same year, Carl P. Sparrow, a postdoctoral fellow in our laboratory, compared the binding and uptake of acetyl LDL and OxLDL by mouse peritoneal macrophages.²⁷ He showed that OxLDL binding and uptake could not be accounted for by the acetyl LDLR alone. In fact, most of the binding of OxLDL was not competed for by acetyl LDL, and he concluded that “there is more than one macrophage ‘scavenger receptor’ for modified lipoproteins.”²⁷

At about this time we made an observation that seemed highly promising but turned out to be a red herring. Elke Ottnad, one of our postdoctoral fellows, was on the hunt for the putative second macrophage scavenger receptor and identified a 94- to 97-kDa membrane protein that bound OxLDL with very high affinity on a ligand blot.²⁸ Acetyl LDL, on the other hand, did not bind tightly. We went on to identify this binding protein as macrophage scavenger receptor 1 (MSR1), analogous to human CD68.^{29,30} However, try as we might, we never could get clear-cut evidence for functionality. CD68 bound OxLDL tightly on ligand blots, but it was simply not the “missing” OxLDL receptor. That had already been discovered by Endemann et al in 1993 and identified as CD36.³¹ Subsequent studies leave little doubt that CD36 accounts for most of the binding and degradation of OxLDL by macrophages in culture.^{32,33}

An Unexpected Turn in the Road Leads Us to Investigate the Role of the Immune System and Inflammation in Atherogenesis

In 1982 and 1983, we were trying to assess how much of LDL degradation *in vivo* was occurring via the specific LDLR pathway and how much via alternative pathways. The landmark studies of Brown and Goldstein led to the prediction that in humans, 66% or more of the clearance of LDL from plasma occurred via the LDLR pathway. To test this hypothesis, Shepherd et al had injected cyclohexanedione-modified LDL into patients along with native LDL.³⁴ Because lysine residues of apoB were required for the interaction of apoB with the LDLR, it was reasoned that if one blocked enough lysine residues chemically, the clearance of the modified LDL would represent LDLR-independent clearance. In comparison with the clearance of native LDL, which is a sum of LDLR-specific and -nonspecific clearance, one could calculate that portion due to the LDLR pathway. This earlier study suggested that only 33% of clearance was due to the LDLR pathway.³⁴ However, it was subsequently shown that *in vivo* the cyclohexanedione modification of lysines was reversible. In our laboratory, we had shown that nonenzymatic glucosylation of apoB, in the presence of the reducing agent cyanoborohydride, covalently and irreversibly blocked many

lysine residues and drastically reduced the affinity of glu-LDL for the LDLR, making it a useful “reagent” for quantifying LDLR-independent uptake *in vivo*.^{35,36} Antero Kesäniemi injected native and glu-LDL into normal subjects, which allowed us to calculate that up to 75% of LDL clearance was accounted for by the LDLR pathway, in agreement with the Brown and Goldstein predictions.³⁷ Because it was believed that the LDLR was dysregulated in diabetes, we next injected glu-LDL into diabetic subjects. Whereas the glu-LDL tracer was cleared very slowly in normal subjects, remarkably, in 3 of the 4 diabetic subjects we studied, there was a very rapid clearance of the glu-LDL tracer, which was subsequently shown to be due to autoantibodies (autoAbs) to their own glu-LDL.³⁸

Indeed, these Abs reacted not only with the glu-LDL but a variety of other similarly glucosylated proteins; that is, the Abs were hapten specific. Together with Linda Curtiss, we injected murine glu-LDL into mice to generate glucitolysine-specific monoclonal antibodies (mAbs), which were very useful for identifying glucosylated proteins in general.³⁹ Moreover, Steinbrecher went on to show that other subtle modifications of autologous LDL, as opposed to modifications of other autologous proteins, led to an equally robust, hapten-specific immune response.⁴⁰ Thus, even simple methylation of autologous guinea pig LDL led to methyllysine-specific Abs when injected into a guinea pig. This set of observations proved of great value in the development of mAbs directed against oxidation-specific epitopes that were subsequently used to prove the presence of OxLDL *in vivo* in animals and humans.

Looking back, it is remarkable again how lucky we were that the first set of subjects in whom we injected the glu-LDL tracer did not have significant titers of autoAbs to glucosylated epitopes. Indeed, we subsequently showed that not only diabetic subjects but many nondiabetic subjects had such titers, and we became aware of other investigators who had observed rapid clearance of glu-LDL in nondiabetic subjects as well! If in our initial studies we had observed the accelerated clearance of the glu-LDL, we might have assumed that it was due to denaturation of the LDL and never pursued the remarkable immunogenicity of modified forms of LDL, a property that potentially explains many of the features of atherogenesis accompanied by LDL oxidation.

Later, to demonstrate the presence of OxLDL *in vivo*, we took advantage of the hapten-directed immunogenicity of modifications of LDL. We immunized guinea pigs and mice with autologous OxLDL and malondialdehyde-modified LDL (MDA-1), generating antisera and mAbs to “oxidation-specific” epitopes present on OxLDL.^{41,42} These were used by Wulf Palinski, Michael Rosenfeld and Seppo Ylä-Herttuala, to immunostain atherosclerotic lesions and to show that LDL eluted from the arteries of rabbits and humans contained such oxidation-specific epitopes, as well as all the other physical and biological properties of OxLDL generated *in vitro*.^{43–46} Furthermore, autoAbs to OxLDL were present on the LDL in lesions as part of immune complexes.⁴⁷ These experiments left no doubt that OxLDL was indeed generated *in vivo* in the artery wall. We further showed that autoAbs to such epitopes were present in humans and were related to the extent of atherosclerosis in animal models and in some

epidemiological studies.^{43,48–52} To further understand the potential importance of such immune responses, Palinski immunized rabbits and later mice with a model OxLDL, homologous MDA-LDL, actually expecting this to exacerbate lesion formation. In fact, we found the opposite! The immunization was atheroprotective,^{53,54} and these results were soon confirmed by others (reviewed in other articles^{55,56}). Indeed, the observation that OxLDL was immunogenic and that the ensuing immune responses were sufficiently important to modulate atherogenesis were of great heuristic value and contributed significantly to then growing awareness of the importance of immune responses in the pathogenesis of atherosclerosis.

Discovery of Concerted Innate Immune Response to Oxidation-Specific Epitopes

The observation that autoAbs to OxLDL existed in murine models of atherosclerosis prompted us to generate hybridomas to OxLDL using splenic cells isolated from cholesterol-fed apoE mice.⁴² Together with Linda Curtiss, we generated more than 1,500 viable hybridomas from a single splenic fusion, and a minimum of 33% of these generated IgM mAbs to one or more models of OxLDL, attesting to the profound immunologic response to the abundant oxidation-specific epitopes generated during atherogenesis.

A panel of IgM mAbs was cloned from these hybridomas, designated the “E0” series, that bound to a variety of oxidation-epitopes. Each of these E0 Abs bound to *both* the lipid and the protein moieties of OxLDL, a surprising finding that forced us to conclude that they were binding to oxidized lipids and that some of those oxidized lipids had become covalently bound to the apoB. To better understand the nature of these unusual mAbs, Peter Shaw sequenced the genes responsible for the heavy and light chain variable regions responsible for antigen binding. Surprisingly, the genes of all the OxLDL-specific mAbs were “germline” rearrangements typical of true natural antibodies (NABs). Furthermore, 7 separately cloned NABs were identical to each other in both the heavy and light chains and identical to the T15 idiotype, an IgA NAb first described 30 years earlier.⁵⁷ T15 had been well studied, as it provides optimal protection to mice against lethal infection with *Streptococcus pneumoniae*. Indeed, T15 had been crystallized with its antigen, phosphocholine (PC), which is present on the bacteria not as a phospholipid but covalently linked to a carbohydrate present on the cell wall polysaccharide. Thus, both T15 and our IgM NABs—typified by E06—bound the same PC epitope, present as either the PC headgroup of oxidized phospholipid (PC) or as PC linked to the cell wall of pathogens—a true case of molecular mimicry between OxLDL and pathogens.

Sohvi Hörkkö subsequently showed that E06 (or T15) could inhibit the binding and uptake of OxLDL by macrophage scavenger receptors CD36 and SR-B1, implying that the PC headgroup was a ligand for such receptors.⁵⁸ NABs are one arm of innate immunity, and as such they are thought to arise as a result of natural selection. Just as we had reasoned that the scavenger receptors evolved to mediate uptake of apoptotic cells, we reasoned that E06 would bind to such

epitopes on apoptotic cells as well. Indeed, Mi-Kyung Chang and Christoph Binder showed that E06 could bind to and inhibit the uptake of apoptotic cells by macrophages *in vitro*, and together with Judy Berliner and others, showed that the content of OxPL on the surface of apoptotic cells was greatly increased.^{59,60} Thus, *in vivo*, the PC of oxidized phospholipids serves as a ligand mediating uptake and removal of apoptotic cells, and E06 facilitates this uptake by binding, fixing complement, and mediating removal via complement-dependent processes. Chang and Binder subsequently showed that the innate protein C-reactive protein binds to the same PC of OxLDL and apoptotic cells,⁶¹ similar to its known ability to bind to the PC present on the cell wall of *S pneumoniae*. Thus, PC is both a pathogen-associated molecular pattern and a danger-associated molecular pattern, recognized by multiple pattern recognition receptors of innate immunity, eg, scavenger receptors, NABs, and pentraxins. Binder showed the potential importance of such interactions by showing that immunization of LDLR^{-/-} mice with heat-killed *S pneumoniae* led to dramatic increases in E06 titers and atheroprotection.⁶² Similarly, other oxidation-specific epitopes found on OxLDL, such as malondialdehyde modified-type adducts, for example, are also danger-associated molecular patterns recognized by their own set of NABs and other innate proteins and have their analogues on microbes as well.⁶³

Understanding these relationships helps to explain the involvement of innate immune responses in inflammatory diseases in general and atherosclerosis in particular. It is remarkable that the serendipitous observation of the rapid clearance of glu-LDL not only led to the development of oxidation-specific mAbs that provided crucial evidence of the existence of OxLDL *in vivo* but helped develop the now widely appreciated importance of both adaptive and innate immune mechanisms in atherogenesis and inflammation, and their potential interactions with the microbial world as well.

Is the Oxidative Modification Hypothesis Relevant to the Human Disease?

The large clinical trials of antioxidants, most of them using vitamin E or beta carotene, have been resoundingly negative. Meta-analysis of the data from these studies (n≈80,000) shows no benefit at all with regard to cardiovascular outcomes.¹⁹ Do these disappointingly negative results mean that the oxidative modification hypothesis is irrelevant in the human disease? Not necessarily. As discussed recently (see Steinberg¹⁴ and references cited above), there are many reasons these trials may have failed to show an effect even though oxidative modification may be an important element in the pathogenesis of the human disease as it appears to be in many animal models. For example, vitamin E may be the wrong antioxidant in humans. The dosage may have been too low. Importantly, treatment may have been started too late in life. Antioxidant treatment may be beneficial only in some subset of patients, eg, patients subject to unusual oxidative stress. After all, the hypothesis is not that *any* antioxidant, at *any* dosage, in *any* individual will necessarily be effective.

The original hypothesis considered that oxidative modification played a quantitatively significant role in the patho-

genesis of atherosclerosis in most individuals, but an updated hypothesis might suggest it to be most relevant in those individuals or groups who are under the greatest oxidative stress, such as those with renal failure on hemodialysis or special subsets of diabetic subjects.^{64–66} Of course, the implication is that one day an effective antioxidant intervention will be found that will slow the progress of the disease. Learning how to identify subjects who are under highest oxidative stress and therefore most likely to benefit from effective antioxidant intervention is of major importance if we hope to appropriately test this hypothesis and develop effective therapies. It would be a mistake to ignore the strong scientific basis of the hypothesis and assume prematurely that the human disease is categorically different from that in experimental animals, including the nonhuman primate.⁶⁷

Need for More Basic Research

Despite intensive research on the oxidative modification hypothesis, many fundamental questions remain unanswered to this day. Which of the several candidate enzyme systems for LDL oxidation are the most important *in vivo*? Does nonenzymatic oxidation play a role? What are the physiological systems that help protect LDL from oxidative damage, and what are the optimal ways to inhibit this oxidation *in vivo*? What is the ultimate fate of OxLDL? Why does immunization to boost titers of Abs against OxLDL appear to ameliorate atherogenesis, whereas animals and patients with more severe atherosclerosis tend to have higher antibody titers? Is the beneficial impact of immunization due to the humoral immunity itself or to the accompanying cellular responses?

The discovery of the role of oxidation of LDL in atherogenesis and the important immune responses to the oxidation-specific epitopes generated has been a long journey for us, and serendipity has played a major role. For us, it has been an exciting journey, but much remains to be learned. Importantly, it remains to be seen whether these observations on the role of OxLDL in animal models will lead to clinical interventions that can supplement lipid-lowering regimens and add significantly to our ability to control atherosclerosis and its complications.

Disclosures

None.

References

- Henriksen T, Evensen SA, Carlander B. Injury to human endothelial cells in culture induced by low density lipoproteins. *Scand J Clin Lab Invest.* 1979;39:361–368.
- Hessler JR, Robertson AL Jr, Chisolm GM III. LDL-induced cytotoxicity and its inhibition by HDL in human vascular smooth muscle and endothelial cells in culture. *Atherosclerosis.* 1979;32:213–229.
- Henriksen T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated low density lipoproteins. *Proc Natl Acad Sci U S A.* 1981;78:6499–6503.
- Henriksen T, Mahoney EM, Steinberg D. Interactions of plasma lipoproteins with endothelial cells. *Ann N Y Acad Sci.* 1982;401:102–116.
- Henriksen T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of biologically modified low density lipoprotein. *Arteriosclerosis.* 1983;3:149–159.
- Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci U S A.* 1979;76:333–337.
- Fong LG, Parthasarathy S, Witztum JL, Steinberg D. Nonenzymatic oxidative cleavage of peptide bonds in apoprotein B-100. *J Lipid Res.* 1987;28:1466–1477.
- Morel DW, Hessler JR, Chisolm GM. Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipid. *J Lipid Res.* 1983;24:1070–1076.
- Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci U S A.* 1984;81:3883–3887.
- Parthasarathy S, Young SG, Witztum JL, Pittman RC, Steinberg D. Probucol inhibits oxidative modification of low density lipoprotein. *J Clin Invest.* 1986;77:641–644.
- Yamamoto A, Matsuzawa Y, Yokoyama S, Funahashi T, Yamamura T, Kishino B. Effects of probucol on xanthomata regression in familial hypercholesterolemia. *Am J Cardiol.* 1986;57:29H–35H.
- Carew TE, Schwenke DC, Steinberg D. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants *in vivo* can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc Natl Acad Sci U S A.* 1987;84:7725–7729.
- Kita T, Nagano Y, Yokode M, Ishii K, Kume N, Ooshima A, Yoshida H, Kawai C. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc Natl Acad Sci U S A.* 1987;84:5928–5931.
- Steinberg D. The LDL modification hypothesis of atherogenesis: an update. *J Lipid Res.* 2009;50(suppl):S376–S381.
- Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med.* 1989;320:915–924.
- Steinberg D. Antioxidants in the prevention of human atherosclerosis: summary of the proceedings of a National Heart, Lung, and Blood Institute Workshop: September 5–6, 1991, Bethesda, Maryland. *Circulation.* 1992;85:2337–2344.
- Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free Radic Biol Med.* 2000;28:1815–1826.
- Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? *Circulation.* 2002;105:2107–2111.
- Kris-Etherton PM, Lichtenstein AH, Howard BV, Steinberg D, Witztum JL. Antioxidant vitamin supplements and cardiovascular disease. *Circulation.* 2004;110:637–641.
- Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. *Physiol Rev.* 2004;84:1381–1478.
- Shao B, Heinecke JW. HDL, lipid peroxidation, and atherosclerosis. *J Lipid Res.* 2009;50:599–601.
- Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, Edwards PA, Fogelman AM. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. *Arterioscler Thromb Vasc Biol.* 1996;16:831–842.
- Sambrano GR, Parthasarathy S, Steinberg D. Recognition of oxidatively damaged erythrocytes by a macrophage receptor with specificity for oxidized low density lipoprotein. *Proc Natl Acad Sci U S A.* 1994;91:3265–3269.
- Sambrano GR, Steinberg D. Recognition of oxidatively damaged and apoptotic cells by an oxidized low density lipoprotein receptor on mouse peritoneal macrophages: role of membrane phosphatidylserine. *Proc Natl Acad Sci U S A.* 1995;92:1396–1400.
- Brown MS, Basu SK, Falck JR, Ho YK, Goldstein JL. The scavenger cell pathway for lipoprotein degradation: specificity of the binding site that mediates the uptake of negatively-charged LDL by macrophages. *J Supramol Struct.* 1980;13:67–81.
- Kodama T, Reddy P, Kishimoto C, Krieger M. Purification and characterization of a bovine acetyl low density lipoprotein receptor. *Proc Natl Acad Sci U S A.* 1988;85:9238–9242.
- Sparrow CP, Parthasarathy S, Steinberg D. A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. *J Biol Chem.* 1989;264:2599–2604.
- Ottand E, Parthasarathy S, Sambrano GR, Ramprasad MP, Quehenberger O, Kondratenko N, Green S, Steinberg D. A macrophage receptor for oxidized low density lipoprotein distinct from the receptor for acetyl low density lipoprotein: partial purification and role in recognition of oxidatively damaged cells. *Proc Natl Acad Sci U S A.* 1995;92:1391–1395.

29. Ramprasad MP, Fischer W, Witztum JL, Sambrano GR, Quehenberger O, Steinberg D. The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosialin, the mouse homologue of human CD68. *Proc Natl Acad Sci U S A*. 1995;92:9580–9584.
30. Ramprasad MP, Terpstra V, Kondratenko N, Quehenberger O, Steinberg D. Cell surface expression of mouse macrosialin and human CD68 and their role as macrophage receptors for oxidized low density lipoprotein. *Proc Natl Acad Sci U S A*. 1996;93:14833–14838.
31. Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoprotein. *J Biol Chem*. 1993;268:11811–11816.
32. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW. Scavenger receptors class A-III and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. *J Biol Chem*. 2002;277:49982–49988.
33. Silverstein RL, Febbraio M, CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. *Sci Signal*. 2009;2:re3.
34. Shepherd J, Bicker S, Lorimer AR, Packard CJ. Receptor-mediated low density lipoprotein catabolism in man. *J Lipid Res*. 1979;20:999–1006.
35. Witztum JL, Mahoney EM, Branks MJ, Fisher M, Elam R, Steinberg D. Nonenzymatic glucosylation of low-density lipoprotein alters its biologic activity. *Diabetes*. 1982;31:283–291.
36. Steinbrecher UP, Witztum JL, Kesaniemi YA, Elam RL. Comparison of glucosylated low density lipoprotein with methylated or cyclohexanedione-treated low density lipoprotein in the measurement of receptor-independent low density lipoprotein catabolism. *J Clin Invest*. 1983;71:960–964.
37. Kesaniemi YA, Witztum JL, Steinbrecher UP. Receptor-mediated catabolism of low density lipoprotein in man. Quantitation using glucosylated low density lipoprotein. *J Clin Invest*. 1983;71:950–959.
38. Witztum JL, Steinbrecher UP, Kesaniemi YA, Fisher M. Autoantibodies to glucosylated proteins in the plasma of patients with diabetes mellitus. *Proc Natl Acad Sci U S A*. 1984;81:3204–3208.
39. Curtiss LK, Witztum JL. A novel method for generating region-specific monoclonal antibodies to modified proteins. Application to the identification of human glucosylated low density lipoproteins. *J Clin Invest*. 1983;72:1427–1438.
40. Steinbrecher UP, Fisher M, Witztum JL, Curtiss LK. Immunogenicity of homologous low density lipoprotein after methylation, ethylation, acetylation, or carbamylation: generation of antibodies specific for derivatized lysine. *J Lipid Res*. 1984;25:1109–1116.
41. Palinski W, Ylä-Herttuala S, Rosenfeld ME, Butler SW, Socher SA, Parthasarathy S, Curtiss LK, Witztum JL. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis*. 1990;10:325–335.
42. Palinski W, Hökkö S, Miller E, Steinbrecher UP, Powell HC, Curtiss LK, Witztum JL. 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*. 1996;98:800–814.
43. Palinski W, Rosenfeld ME, Ylä-Herttuala S, Gurtner GC, Socher SS, Butler SW, Parthasarathy S, Carew TE, Steinberg D, Witztum JL. Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci U S A*. 1989;86:1372–1376.
44. Ylä-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest*. 1989;84:1086–1095.
45. Ylä-Herttuala S, Palinski W, Rosenfeld ME, Steinberg D, Witztum JL. Lipoproteins in normal and atherosclerotic aorta. *Eur Heart J*. 1990;11(suppl E):88–99.
46. Rosenfeld ME, Palinski W, Ylä-Herttuala S, Butler S, Witztum JL. Distribution of oxidation specific lipid-protein adducts and apolipoprotein B in atherosclerotic lesions of varying severity from WHHL rabbits. *Arteriosclerosis*. 1990;10:336–349.
47. Ylä-Herttuala S, Palinski W, Butler SW, Picard S, Steinberg D, Witztum JL. Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. *Arterioscler Thromb*. 1994;14:32–40.
48. Salonen JT, Ylä-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssonen K, Palinski W, Witztum JL. Autoantibody against oxidized LDL and progression of carotid atherosclerosis. *Lancet*. 1992;339:883–887.
49. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler Thromb*. 1994;14:605–616.
50. Palinski W, Tangirala RK, Miller E, Young SG, Witztum JL. Increased autoantibody titers against epitopes of oxidized LDL in LDL receptor-deficient mice with increased atherosclerosis. *Arterioscler Thromb Vasc Biol*. 1995;15:1569–1576.
51. Tsimikas S, Palinski W, Witztum JL. Circulating autoantibodies to oxidized LDL correlate with arterial accumulation and depletion of oxidized LDL in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2001;21:95–100.
52. Tsimikas S, Brilakis ES, Lennon RJ, Miller ER, Witztum JL, McConnell JP, Kornman KS, Berger PB. Relationship of IgG and IgM autoantibodies to oxidized low density lipoprotein with coronary artery disease and cardiovascular events. *J Lipid Res*. 2007;48:425–433.
53. Palinski W, Miller E, Witztum JL. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc Natl Acad Sci U S A*. 1995;92:821–825.
54. Freigang S, Hökkö S, Miller E, Witztum JL, Palinski W. Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes. *Arterioscler Thromb Vasc Biol*. 1998;18:1972–1982.
55. Binder CJ, Hartvigsen K, Witztum JL. Promise of immune modulation to inhibit atherogenesis. *J Am Coll Cardiol*. 2007;50:547–550.
56. Hansson GK, Nilsson J. Vaccination against atherosclerosis? Induction of atheroprotective immunity. *Semin Immunopathol*. 2009;31:95–101.
57. Shaw PX, Hökkö S, Chang MK, Curtiss LK, Palinski W, Silverman GJ, Witztum JL. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. *J Clin Invest*. 2000;105:1731–1740.
58. Hökkö S, Bird DA, Miller E, Itabe H, Leitinger N, Subbanagounder G, Berliner JA, Friedman P, Dennis EA, Curtiss LK, Palinski W, Witztum JL. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J Clin Invest*. 1999;103:117–128.
59. Chang MK, Bergmark C, Laurila A, Hökkö S, Han KH, Friedman P, Dennis EA, Witztum JL. Monoclonal antibodies against oxidized low-density lipoprotein bind to apoptotic cells and inhibit their phagocytosis by elicited macrophages: evidence that oxidation-specific epitopes mediate macrophage recognition. *Proc Natl Acad Sci U S A*. 1999;96:6353–6358.
60. Chang MK, Binder CJ, Miller YI, Subbanagounder G, Silverman GJ, Berliner JA, Witztum JL. Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. *J Exp Med*. 2004;200:1359–1370.
61. Chang MK, Binder CJ, Torzewski M, Witztum JL. 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*. 2002;99:13043–13048.
62. Binder CJ, Hökkö S, Dewan A, Chang MK, Kieu EP, Goodyear CS, Shaw PX, Palinski W, Witztum JL, Silverman GJ. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between *Streptococcus pneumoniae* and oxidized LDL. *Nat Med*. 2003;9:736–743.
63. Hartvigsen K, Chou MY, Hansen LF, Shaw PX, Tsimikas S, Binder CJ, Witztum JL. The role of innate immunity in atherogenesis. *J Lipid Res*. 2009;50(suppl):S388–S393.
64. Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, Knecht A, Weissgarten Y, Brunner D, Fainaru M, Green MS. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet*. 2000;356:1213–1218.
65. Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. *Circulation*. 2003;107:992–995.
66. Milman U, Blum S, Shapira C, Aronson D, Miller-Lotan R, Anbinder Y, Alshiek J, Bennett L, Kostenko M, Landau M, Keidar S, Levy Y, Khemlin A, Radan A, Levy AP. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2–2 genotype: a prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol*. 2008;28:341–347.
67. Sasahara M, Raines EW, Chait A, Carew TE, Steinberg D, Wahl PW, Ross R. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol: I: is the extent of atherosclerosis related to resistance of LDL to oxidation? *J Clin Invest*. 1994;94:155–164.

KEY WORDS: antioxidants ■ atherosclerosis ■ immune system ■ macrophages ■ oxidized low-density lipoprotein

Arteriosclerosis, Thrombosis, and Vascular Biology



JOURNAL OF THE AMERICAN HEART ASSOCIATION

Oxidized Low-Density Lipoprotein and Atherosclerosis Daniel Steinberg and Joseph L. Witztum

Arterioscler Thromb Vasc Biol. 2010;30:2311-2316

doi: 10.1161/ATVBAHA.108.179697

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231

Copyright © 2010 American Heart Association, Inc. All rights reserved.

Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:

<http://atvb.ahajournals.org/content/30/12/2311>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
<http://atvb.ahajournals.org/subscriptions/>