

Atherosclerosis: cell biology and lipoproteins Alan Daugherty

Cardiovascular Division, Washington University School of Medicine, St. Louis, MO, USA

Current Opinion in Lipidology 1997, 8:U11-U12

© Rapid Science Publishers
ISSN 0957-9672

Oxidative damage continues to attract interest as a possible cause of atherosclerotic lesion development. The explosion of research in this arena may be traced to early cell culture studies that demonstrated that endothelium could modify LDL through oxidative mechanisms. Cell-mediated oxidation of LDL yielded a material that exhibited a wide spectrum of biological activities that are considered proatherogenic. The potential role of oxidation in the disease process garnered more interest when oxidatively modified lipoproteins were detected in evolving lesions. In addition, a role for oxidation was implied by the demonstration that several structurally diverse antioxidants decreased the extent of lesion formation in animal models of atherosclerosis (reviewed in [1] and [2]). However, a question critical to validating the basic tenet of the oxidation hypothesis remains unanswered: how do lipoproteins become oxidized?

Cell culture studies have implicated free copper and iron in lipoprotein oxidation. However, the extrapolation of these in-vitro findings to the pathophysiology of the disease may not be valid because neither copper nor iron are known to exist in an unbound state *in vivo*, especially in the concentrations that are required to promote LDL oxidation. Several other oxidant mechanisms have been proposed; however, none have been proven [2]. Although caeruloplasmin, the major copper-carrying protein in plasma, has previously been shown to have antioxidant properties, Fox and colleagues [2] have made observations suggesting a converse effect in which caeruloplasmin oxidizes LDL. Ehrenwald *et al.* [3] attempted to resolve this contradiction by comparing the intact 132 kDa native protein and degraded material. They demonstrated that the intact protein oxidized LDL, while proteolytic cleavage of intact caeruloplasmin yielded a 115 kDa fragment that inhibited copper-induced LDL oxidation. Additional studies [4] demonstrated that zymosan-activated human U937 monocytic cells released caeruloplasmin and facilitated LDL oxidation. A striking result was the demonstration of cell-mediated LDL oxidation in copper-free RPMI 1640 medium.

In addition to macrophages, two other vascular cell types present in atherosclerotic lesions, endothelial and smooth muscle cells, have been shown to oxidize LDL, although this cell-mediated oxidation required coincubation with relatively high concentrations of transition metal ions. Mukhopadhyay *et al.* [5**] reported the promotion of LDL oxidation by exogenously applied caeruloplasmin during incubation with cultured endothelial and smooth muscle cells in copper-free medium. As in the previous report [3], in a cell-free system proteolytic cleavage of intact protein abolished oxidant properties. Treatment of the protein with Chelex-100 also abolished oxidant activity by removing one of the seven coppers present in intact caeruloplasmin. The oxidation of LDL required the secretion of superoxide by these two vascular cell types, as determined by the correlation of the rate of superoxide production with LDL oxidation; superoxide production quantitatively accounted for LDL oxidation, and the inhibitory effect of exogenous superoxide dismutase. Overall, these studies demonstrate that any role of caeruloplasmin in atherogenesis may result from multiple actions that vary depending on the structure of the protein within lesions.

The role of iron metabolism in the development of atherosclerosis is also a subject of considerable debate. In a recent study Pang *et al.* [6**] observed that ferritin levels increase dramatically in arterial tissue during atherogenesis. Differential screening was performed by use of cDNA libraries created with poly (A)+ mRNA from normal and atherosclerotic human aortas and two genes were found to be highly expressed in atherosclerotic lesions: L- and H-ferritin. L-chain-rich ferritin is most abundant in iron storage organs such as spleen and liver, while the H-chain-rich form predominates in most other organs. Not only have L- and H-ferritin been identified in human atherosclerotic lesions, but an increase in mRNA for these proteins was found in atherosclerotic tissues of rabbits fed a cholesterol-enriched diet, coincident with the initial appearance of atherosclerotic lesions. Expression of ferritin mRNA was stimulated in cultured macrophages by interleukin-1 and tumor necrosis factor. The latter cytokine may be of relevance to the increases noted in lesion ferritin mRNA, since there have been many reports that tumor necrosis factor is secreted in atherosclerotic lesions. Although ferritin is present in atherosclerotic lesions, its role is still uncertain. One major function of ferritin is the chelation of intracellular iron, which may be considered to be an antioxidant property. Alternatively, it has been suggested

that ferritin is a source of iron that therefore promotes oxidative damage.

These recent articles serve to show the continued interest in the oxidation theory of atherosclerosis. They also illustrate the many complexities that will be faced in determining the validity of this hypothesis.

References

- 1 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.
- 2 Daugherty A, Roselaar SE: **Lipoprotein oxidation as a mediator of atherogenesis: insights from pharmacological studies.** *Cardiovasc Res* 1995, **29**:297-311.
- 3 Ehrenwald E, Chisolm GM, Fox PL: **Intact human ceruloplasmin oxidatively modifies low density lipoprotein.** *J Clin Invest* 1994, **93**:1493-1501.
- 4 Ehrenwald E, Fox PL: **Role of endogenous ceruloplasmin in low density lipoprotein oxidation by human U937 monocytic cells.** *J Clin Invest* 1996, **97**:884-890.
- 5 Mukhopadhyay CK, Ehrenwald E, Fox PL: **Ceruloplasmin enhances**
 - **smooth muscle cell and endothelial cell-mediated low density lipoprotein oxidation by a superoxide-dependent mechanism.** *J Biol Chem* 1996, **271**:14773-14778.

Demonstration of the ability of vascular cells to facilitate LDL oxidation by ceruloplasmin.

- 6 Pang JHS, Jiang MJ, Chen YL, Wang FW, Wang DL, Chu SH, Chau LY:
 - **Increased ferritin gene expression in atherosclerotic lesions.** *J Clin Invest* 1996, **97**:2204-2212.

One of the first applications of differential display to identify genes that are expressed in atherosclerotic tissue.

Recommended reading

- Bowen MA, Bajorath J, Siadak AW, Modrell B, Malack AR, Marquardt H, Nadler SG, Aruffo A: **The amino-terminal immunoglobulin-like domain of activated leukocyte cell adhesion molecule binds specifically to the membrane-proximal scavenger receptor cysteine-rich domain of CD6 with a 1:1 stoichiometry.** *J Biol Chem* 1996, **271**:17390-17396.

Demonstration of a function of the scavenger receptor cysteine-rich domain in CD6.

- Caulinglaser T, Watson CA, Pardi R, Bender JR: **Effects of 17 beta-estradiol on cytokine-induced endothelial cell adhesion molecule expression.** *J Clin Invest* 1996, **98**:36-42.

A novel mechanism by which estradiol may influence atherosclerosis.

- Lindstedt L, Lee M, Castro GR, Fruchart JC, Kovanen PT: **Chymase in exocytosed rat mast cell granules effectively proteolyzes apolipoprotein AI-containing lipoproteins, so reducing the cholesterol efflux-inducing ability of serum and aortic intimal fluid.** *J Clin Invest* 1996, **97**:2174-2182.

Degranulated mast cells have been found in human atherosclerosis. This manuscript describes a mechanism that may be responsible for a detrimental effect on this cell type.

- Murugesan G, Fox PL: **Role of lysophosphatidylcholine in the inhibition of endothelial cell motility by oxidized low density lipoprotein.** *J Clin Invest* 1996, **97**:2736-2744.

Identification of the factor in oxidized LDL that inhibits wound healing of endothelium.