Lipoproteins, Macrophages and Atherosclerosis

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INTRODUCTION

The fatty streak, the earliest recognized gross lesion in the atherogenic process, is characterized by an accumulation of lipid-laden "foam cells". Recent studies have established that this lesion can -- and usually does -- develop beneath an intact endothelium. Furthermore, it has been shown that most of these cells are derived from circulating monocytes that enter the arterial wall and take up residence there as tissue macrophages. Some of these cells are derived from medial smooth muscle cells, particularly in later lesions, but the majority are macrophages. Early attempts to convert macrophages to foam cells by incubation in the presence of high concentrations of LDL were unsuccessful and this was traced to the rather low number of receptors for native LDL they express. Studies by Goldstein, Brown and coworkers established that the macrophage expresses a unique receptor that can take up chemically acetylated LDL at a sufficiently high rate to generate foam cells in culture. However, there was no evidence that chemical acetylation occurs to any significant extent in vivo. Thus, there remained an unresolved paradox regarding the mechanism of foam cell formation and thus the initiation of the fatty streak.

FOAM CELL GENERATION BY UPTAKE OF OXIDATIVELY MODIFIED LDL

In 1982 Henriksen, Mahoney and Steinberg demonstrated that incubation of native LDL in the presence of endothelial cells led to the generation of a modified form taken up four to ten times faster than native LDL -- but not by way of the native LDL receptor. This "endothelial cell-modified" LDL was taken up by the same receptor that recognizes acetylated LDL. Via and coworkers have partially purified this acetyl LDL receptor and shown that it also recognizes endothelial cell-modified LDL. The same modification can also be induced by incubation with arterial smooth muscle cells or with macrophages themselves.

We have established that the cell-induced modification depends on oxidation of the LDL by oxygen free radicals. It will not take place in the absence of transition metals (copper or iron) and is totally inhibited by the addition of antioxidants (e.g., alpha tocopherol or butylated hydroxytoluene). There is extensive oxidation of the unsaturated fatty acids in lecithin and probably those in cholesterol esters. The cholesterol nucleus itself undergoes oxidative modification but this has not yet been well characterized. During oxidative modification there is extensive hydrolysis of lecithin to lysolecithin by a phospholipase A2 activity associated closely with the LDL itself (see below). Fong and coworkers have shown that there is also direct oxidative attack on the apoprotein B-100, converting it to lower molecular weight fragments, 7 to 10 in number but none smaller than 70,000 daltons. Recent studies by Parthasarathy and coworkers show that these peptide fragments can be resolubilized in detergent and that they compete for the acetyl LDL receptor, establishing for the first time with certainty that the modification of apoprotein B is the crucial step accounting for conversion of LDL to a form that can generate foam cells.

Incubation of LDL in the presence of 5 μM copper ion even in the absence of any cells can mimic most of the changes induced by incubating LDL with cells. Thus it appears that the only essential contribution of the cells is...
the generation of oxygen free radicals. Since lecithin undergoes hydrolysis during copper-induced modification, and takes place in the absence of any cells, we conclude that the phospholipase $A_2$ activity resides in the LDL rather than being contributed by the cells. Oxidized lecithin has previously been shown to be a preferred substrate for phospholipase $A_2$ and this may link accelerated lecithin breakdown to lipid peroxidation. Conversely, the release of the two-position fatty acid from lecithin may facilitate the propagation reactions that lead to extensive lipid peroxidation in the LDL.

To summarize, we propose that one way in which elevated LDL levels can play a role in the initiation process is through its conversion to the oxidatively modified form which is then taken up more readily by macrophages than native LDL and converts the macrophages to foam cells.

Endothelial Cell Damage Induced by Oxidatively Modified LDL

Earlier studies by Henriksen et al. and by Hessler et al. established that LDL is toxic to cultured endothelial cells when they are incubated together in the absence of added serum. Later studies by Chisholm and coworkers showed that the toxicity was only evident if the LDL had undergone oxidative modification during the culture. It was prevented entirely by addition of antioxidants. Thus, a second way in which elevation of LDL levels might favor atherogenesis is by damage to the endothelium, functional damage at least and possibly, later, structural damage as well.

Recruitment and Retention of Macrophages Induced by Oxidatively Modified LDL

Quinn and coworkers have shown that oxidatively modified LDL acts as a chemoattractant for circulating monocytes. Consequently, high levels of LDL penetrating the artery wall might lead to the presence of oxidatively modified LDL in sufficient concentrations to help recruit monocytes into sites destined to become fatty streaks. On the other hand, the motility of resident peritoneal macrophages is inhibited by oxidatively modified LDL. Thus, the same molecule -- oxidatively modified LDL -- can first lure monocytes into the site of a developing lesion and then inhibit their exit after the monocytes have modified their phenotypic expression and acquired the characteristics of resident macrophages.

SUMMARY

We have presented an hypothesis that could account for the necessary steps by which an elevated level of LDL in the plasma could initiate the fatty streak lesion: 1) by helping to recruit circulating monocytes and causing them to be retained in the subendothelial space; 2) by favoring the generation of foam cells because of the more rapid uptake of oxidatively modified LDL via the acetyl LDL receptor; 3) by leading to endothelial damage and loss of endothelial function necessary to exclude LDL from entering and, later, by favoring aggregation of platelets at sites of endothelial injury.

REFERENCES

Goldstein JL, Ho YK, Basu SK, Brown MS (1979) Binding site on macrophages that mediates uptake and degradation of acetylated low density