Studies from several laboratories suggest that oxidized LDL may play an important role in atherogenesis. Our group previously showed that treatment of aortic endothelial cells with low levels of MM-LDL caused increased expression of MCP-1, M-CSF, tissue factor, and a monocyte-binding protein. In these studies MM-LDL was produced by storage of native LDL. We now show that cocultures of endothelial and smooth muscle cells can also produce MM-LDL from native LDL. This production of MM-LDL by cells is prevented by preincubating the LDL with probucol or vitamin E. However, addition of antioxidants to MM-LDL did not block its action. In past studies we also showed that endothelial cells exhibit differential sensitivity to the effects of MM-LDL. We report herein that in resistant cells there is no elevation of catalase, glutathione peroxidase, or copper-zinc-dependent SOD. However, manganese-dependent SOD is elevated in resistant cells. Ways in which MM-LDL production may be elevated in poorly controlled diabetes subjects are discussed. Diabetes 41 (Suppl. 2):74–76, 1992

Several studies showed that oxidized lipoproteins are present in the artery wall and that the amount of oxidized lipid is increased in the atherosclerotic plaque (1–4). LDL isolated from the vessel wall was shown to contain oxidized lipids and protein (3,4), and monoclonal antibodies to MDA lysine and hydroxynonenal show localization of this product in lesions of the artery wall (1,2). These oxidized lipoproteins may play an important role in atherogenesis from the pre–fatty streak stage through the definitive fibrous plaque. Evidence of this role comes from studies showing that probucol and other antioxidants can inhibit plaque formation in cholesterol-fed animals (5,6).

The mechanisms by which oxidized lipoproteins can accelerate the atherogenic process have been explored in vitro and in vivo studies by several groups. Highly oxidized lipoproteins are toxic for dividing smooth muscle cells and fibroblasts (7). At a high concentration, oxidized LDL chemotactically attracts monocytes and stimulates cholesterol loading of macrophages (8,9).

The cells of the vessel wall are capable of oxidizing lipoproteins in vitro (10–15), and both superoxides and lipoxygenase products have been implicated in the oxidative process.

Our group has been examining the role of MM-LDL in the early events of atherosclerosis. In our published studies, stored LDL or LDL oxidized by iron were used (16). These MM-LDL particles show very little apoB oxidation and are taken up by the LDL receptor. They contain approximately 2–5/nmol of TBARS/mg of cholesterol and low levels of peroxides and epoxides. We showed that treatment of endothelial cells with MM-LDL induces the binding of monocytes, but not neutrophils, to the endothelial monolayer. This increased binding lasts for at least 48 h. Treatment of human aortic endothelial cells or smooth muscle cells with MM-LDL stimulates the synthesis of MCP-1, a monocyte chemotactic factor (17). This increase in synthesis is induced at the mRNA level. The increased monocyte chemotactic activity induced by MM-LDL appears to result entirely from MCP-1. We also showed that treatment of rabbit aortic and human aortic endothelial cells with MM-LDL induces the production of the colony-stimulating factors M-CSF and GM-CSF (18), and that M-CSF and GM-CSF can be induced in vivo when mice are injected with MM-LDL (19). This induction occurs in both LPS-resistant and LPS-sensitive mice.
addition, we showed that treatment of aortic valve endothelial cells with MM-LDL increases the production of tissue factor by these cells (20). Both CSF induction and tissue factor synthesis are induced at mRNA level.

NEW WORK

Work by Navab et al. in our group (21) recently showed that MM-LDL can be made by cocultures of human aortic endothelial cells and smooth muscle cells. Native LDL was added to cocultures for 24 h. The LDL was then reisolated from the coculture. The isolated LDL induced the production of MCP-1 when incubated with previously unexposed cocultures. Conditioned medium from cocultures was also active in inducing unexposed endothelial cell cultures to bind monocytes. The process by which the cocultures convert native LDL to MM-LDL depend on oxidation because the conversion was blocked by pre-incubating LDL with probucol or vitamin E. Adding probucol or vitamin E to conditioned medium did not block its effects on monocyte binding or on the production of MCP-1.

In our earlier work, we reported that endothelial cells from different individuals exhibited different sensitivities to the effects of MM-LDL (16). We further reported that resistance to the effects of MM-LDL could be induced by exposure of sensitive endothelial cells for 24 h to low levels of MM-LDL. In addition, resistant cells became sensitive when cells were treated with cycloheximide before the addition of MM-LDL. These studies suggested that a protein might be involved in the protection of cells from the effects of MM-LDL and that this protein might be present at higher levels in resistant cells. Cushing et al. (17) recently surveyed a number of antioxidant proteins and cofactors to determine which were induced in rabbit aortic endothelial cells by exposure to low levels of MM-LDL. Neither cytoplasmic nor membrane glutathione peroxidase were induced by MM-LDL, and the levels of glutathione were not elevated by MM-LDL. The levels of Cu-Zn SOD protein and catalase were not increased by MM-LDL. However, the level of activity of Mn SOD was increased three- to fourfold; the level of mRNA for Mn SOD was increased approximately fivefold. Although these observations do not prove a protective role for Mn SOD, they suggest that the amount of this enzyme may be important in the handling of MM-LDL by exposed endothelial cells.

DISCUSSION

There are several possible factors related to oxidation of LDL that may account for the increased risk of athero-sclerosis and stroke seen in people with poorly controlled diabetes. First, LDL oxidation is elevated in the blood of diabetic animals (22,23). This increase may be due to the increase in triglyceride in all classes of particles in diabetic people with hypertriglyceridemia; fatty acids associated with triglycerides may be more susceptible to oxidation than those associated with cholesterol in the LDL particle. In addition, glycosylation of LDL in the vessel wall may accelerate oxidation because, as discussed by others at this meeting, the glycosylation reaction increases the production of free radicals. There are many antioxidant systems including enzymes and vitamins in animals and humans that resist the oxidation of lipids. In the person with poorly controlled diabetes, the balance between oxidation and antioxidation may be shifted toward increased oxidation, causing increased formation of oxidized lipoproteins.

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