Oxidized LDL Activates Fas-mediated Endothelial Cell Apoptosis

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Abstract

Oxidized low density lipoproteins (OxLDL) promote chronic inflammatory responses in the vasculature that give rise to atherosclerotic plaques. Fas ligand (FasL) is naturally expressed on the vascular endothelium where it can induce apoptosis in Fas-expressing immune cells as they enter the vessel wall. Although vascular endothelial cells are normally resistant to Fas-mediated cell death, OxLDL were shown to induce apoptosis in cultured endothelial cells and endothelium of arterial explants by a process that could be inhibited with Fas L neutralizing antibodies. OxLDL-induced cell death was also reduced in the aortic endothelium cultured from gld (FasL−/−) and lpr (Fas−/−) mice as compared with wild-type mice. OxLDL acted by sensitizing endothelial cells to death signals from the Fas receptor. Thus, the ability of OxLDL to promote Fas-mediated endothelial cell suicide may be a feature that contributes to their atherogenicity. (J. Clin. Invest. 1998. 102:1682–1689.) Key words: endothelium • oxidized LDL • apoptosis • Fas • Fas ligand

Introduction

It is firmly established that elevated plasma concentrations of LDL are associated with accelerated atherogenesis, and clinical trials demonstrate that LDL-lowering treatments reduce the risk of death in patients with coronary artery disease (1). Recent evidence suggests that oxidative modification of LDL renders it more atherogenic (2) and oxidized LDL (OxLDL) has been identified in atherosclerotic lesions (3, 4). OxLDL and its lipid constituents have numerous detrimental effects on endothelial cell function (5–7) including the induction of apoptosis (8–11).

Fas is a type I membrane protein that initiates an apoptotic signal when bound to its ligand, FasL (12). Fas is widely expressed, but few cell types express FasL. FasL is expressed by immune-privileged tissues of eye and testis (13, 14), as well as by some tumors, where it may contribute to their ability to evade immune detection (15–17). FasL is also expressed on vascular endothelial cells, where it appears to have an anti-inflammatory function (18). Under normal conditions, endothelial cells are resistant to Fas-mediated apoptosis, although they express detectable Fas on their cell surface (18). In contrast, Fas ligand can readily induce apoptosis in Fas-bearing vascular smooth muscle cells (19). Because recent evidence suggests that atherosclerosis is initiated by an aberrant immune response triggered by elevated lipid levels (20–22), we investigated whether oxidized lipid-induced endothelial cell apoptosis is mediated by the Fas cell death pathway. Here, we show that OxLDL induces endothelial apoptosis through Fas–FasL interaction, and that endothelial cells are markedly sensitized to Fas-mediated apoptosis by OxLDL. The results may provide insights about the pathogenesis of the accelerated atherosclerosis in patients with hyperlipidemia.

Methods

Cells and reagents. Human aortic endothelial cells (HAECs) were obtained from Clonetics (San Diego, CA). Human umbilical vein endothelial cells (HUVECs) were isolated as described (18). Both HAECs and HUVECs were cultured in EGM medium (Clonetics) containing 2% FBS, 10 ng/ml human EGF, 1.0 µg/ml hydrocortisone, and 12 µg/ml bovine brain extract. LDL was isolated by sequential ultracentrifugation (d = 1.019–1.063) from freshly drawn, citrated normolipidemic human plasma to which EDTA was added. LDL was oxidized in the presence of 5 µM CuSO4 for 24 h at 25°C, and the degree of oxidation was assessed by the increase of mobility on 1% agarose gel (1.3–1.5 versus native LDL). Different preparations of OxLDL displayed similar electrophoretic mobilities. For comparison, commercially available preparations of native and copper-oxidized LDLs (Sigma Chemical Co., St. Louis, MO, and Biomedical Technologies, Inc., Stoughton, MA, respectively) were used in selected experiments. The formation of thiobarbituric acid–reactive substances was 17.8 nanomoles of malondialdehyde/mg protein using an OxLDL preparation with relative electrophoretic mobility of 1.4. Minimally modified LDL (MM-LDL) was prepared by dialyzing native LDL against 9 µM FeSO4 in PBS for 72 h at 4°C as described (23). The electrophoretic mobility increased 1.1–1.2 versus native LDL. Acetylation of LDL was performed with excess acetic anhydride. Endotoxin contamination in OxLDL was measured with the coagulation Limulus amebocyte lysate assay using a commercially available kit (E-TOXATE, Sigma Chemical Co.).

Detection of Fas and FasL expression on endothelial cells. 90% confluent HAECs and HUVECs were incubated with OxLDL (150 µg protein/ml) or L-α-palmitoyl lysophosphatidylcholine (LPC, 45 µM, Sigma Chemical Co.) at 37°C, 5% CO2 for 13 h, and detached from the culture plate with 0.5% EDTA. To determine FasL expression, endothelial cells were incubated with an anti-FasL antibody (C-20, Santa Cruz Biotechnology, Santa Cruz, CA) or with rabbit IgG followed by a FITC-conjugated antibody against rabbit Ig (Biosource, Camarillo, CA). To determine Fas expression, endothelial cells were incubated with an FITC-conjugated anti-Fas monoclonal antibody.
Detection of DNA fragmentation by agarose gel electrophoresis. HUVECs (10^6) were incubated in the presence or absence of native LDL (300 μg protein/ml), OxLDL (300 μg protein/ml), LPS (100 endotoxin U/ml), or a neutralizing anti-FasL antibody (24) (10 μg/ml, 4H9, MBL, Nagoya, Japan) for 36 h. Attached cells and floating cells were combined and lysed in 0.33 ml of lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.2% Triton X-100) followed by incubation with 0.1 mg/ml RNAase A for 1 h at 37°C. Ethanol-precipitated DNA was resuspended in TE buffer, fractionated on 1.5% agarose gel in 1× TBE buffer, and stained with ethidium bromide.

Detection of DNA fragmentation by TdT-mediated dUTP nick-end labeling (TUNEL). 70% confluent HUVECs were incubated in the presence or absence of OxLDL (300 μg protein/ml), a neutralizing anti-FasL antibody (24) (10 μg/ml, 4H9), or an agonistic anti-Fas antibody (0.5 μg/ml, CH11, MBL) for 16 h at 37°C. Cell viability was measured by means of MTT (dimethylthiazol-diphenyltetrazolium bromide) assay (24) and percentage of cell death was calculated as 100 - viability of treated endothelial cells/viability of untreated endothelial cells.

Results

To examine cell surface Fas and FasL, FACS analysis was performed on HUVECs treated with OxLDL or Lα-palmitoyl lysophosphatidylcholine (LPC-C16:0), a component of OxLDL. Incubation with OxLDL (150 μg protein/ml) or LPC-C16:0 (45 μM) upregulated FasL expression on HUVECs (Fig. 1). In contrast, treatments with OxLDL or LPC did not detectably alter HUVEC cell surface Fas expression. Treatment of HAECs with OxLDL or LPC also led to an increase in cell surface FasL expression, whereas Fas expression remained unchanged (M. Sata and K. Walsh, unpublished data). Higher doses of oxidized lipid, that more effectively induce cytotoxicity, also up-regulated FasL expression as determined by Western immunoblotting (M. Sata and K. Walsh, unpublished data).

HAECs treated with OxLDL displayed characteristics of apoptosis including cell shrinkage and nuclear condensation (Fig. 2). The expression of the apoptotic phenotype was...
markedly attenuated when cultures were coincubated with 4H9 anti-FasL antibody that neutralizes the cytotoxicity of FasL (24). Interestingly, OxLDL–treated endothelial cells protected from death by anti-FasL antibody still attained a distinctive elongated cell morphology (27) (Fig. 2 A), indicating that other actions of OxLDL are not blocked by the neutralizing anti-FasL antibody. Similar observations were also made with HUVECs treated with 4H9 and HAECs treated with C-20 anti-FasL antibody that also has a neutralizing activity (26) (M. Sata and K. Walsh, unpublished data). In the presence of neutralizing anti-FasL antibody, HUVEC apoptosis could be induced with 10 μM staurosporine, indicating that other apoptosis-inducing signals were not inhibited by the antibody (data not shown).

Eight preparations of OxLDL were tested and all produced similar extents of apoptosis in HAECs and HUVECs. Though preparations varied in their dose-response characteristics, cell death typically occurred at concentration of OxLDL above 250 μg/ml in medium containing 2% FBS. In comparison, others have reported that 16% apoptosis when HUVECs were treated with 25 μg protein/ml OxLDL for 48 h in serum-free medium (11) or 54% apoptosis when the endothelial cell line CRL-1998 was treated with 200 μg protein/ml OxLDL for 24 h in serum-free medium (8). In our studies, neutralizing anti-FasL antibody was most effective in inhibiting apoptosis at OxLDL concentrations below 400 μg/ml, and less effective at higher OxLDL concentrations.

Genomic DNA prepared from OxLDL-treated HUVECs showed a DNA ladder upon electrophoresis, a well established marker of apoptosis (Fig. 2 B). OxLDL-induced DNA frag-
mentation was markedly attenuated by coincubation with neutralizing anti-FasL antibody. Native LDL did not induce DNA fragmentation in HUVECs, nor did endotoxin, a common contaminant of lipoprotein preparations, when incubated at greater than a 300-fold excess of the levels detected in the OxLDL preparation (M. Sata and K. Walsh, unpublished data).

HUVECs treated with OxLDL also showed decreased mitochondrial function in the MTT assay (Fig. 2 C), another indicator of apoptotic cell death (24). Consistent with the data presented above, incubation with neutralizing anti-FasL antibody inhibited the decrease in cell viability that was induced by incubation with OxLDL. LPC-C18:0, a component of OxLDL, also induced endothelial cell death as assessed by decreased mitochondrial function (Fig. 2 C). Neutralizing anti-FasL antibody inhibited apoptosis induced by 45 μM LPC-C18:0. At higher LPC-C18:0 concentrations (> 60 μM) the neutralizing antibody was ineffective (M. Sata and K. Walsh, unpublished data). MM-LDL also promoted endothelial cell apoptosis and coincubation with the neutralizing anti-FasL antibody reduced cell death induced by this agent (Fig. 2 C). Unlike OxLDL that is recognized by the endothelium cell scavenger receptor (28, 29), MM-LDL is recognized by the LDL receptor, yet it still promotes atherogenesis by acting as an inflammatory agent (23, 30). Acetylated LDL, which is internalized by endothelial cells but lacks biological effects (31, 32), did not affect endothelial cell viability. LPC-C12:0, which does not inhibit endothelial cell migration (6), also did not affect endothelial cell viability. Cholesterol and oxysterols (7-ketocholesterol and 25-hydroxycholesterol), as well as the phosphatidylcholine PC-C18:2/16:0, did not kill endothelial cells.

Arterial organ culture was used to study endothelial cell viability in a system with intact morphology and minimal cell division (25). Apoptotic endothelial cell death was detected in rings prepared from rabbit carotid artery that were incubated with OxLDL (100 μg protein/ml in 0.5% FBS) for 24 h as assessed by fluorescent detection of TUNEL-positive DNA (Fig. 3). OxLDL induces endothelial cell apoptosis through Fas–FasL interaction in an organ culture of rabbit carotid artery. Rings from rabbit carotid arteries were treated with OxLDL in the absence or presence of the neutralizing anti-FasL antibodies (αFasL, 4H9, 10 μg/ml; and C-20, 10 μg/ml) and harvested at 24 h and 64 h. (A) Rings harvested at 24 h were stained with TUNEL (green) to detect fragmented chromatin and propidium iodide (red) to detect total chromatin and examined by laser confocal microscopy. Apoptotic nuclei are shown as yellow with a filter for rhodamine and fluorescein. The internal elastic lamina containing autofluorescent elastin is visible with a filter specific for fluorescein. Bar, 25 μM. (B) Arteries harvested at 64 h were stained for CD31 (clone JC/70A, DAKO), an endothelial cell marker, using FastRed chromagen (Biogenex, San Ramon, CA). Endothelium is indicated by arrows. Bar, 50 μM.
Endothelial cells from gld or lpr mice are resistant to OxLDL-induced apoptosis. Aortic rings from male C57BL/6J mice, B6Smn-

**Figure 4.**

Endothelial cells from gld or lpr mice are resistant to OxLDL-induced apoptosis. Aortic rings from male C57BL/6J mice, B6Smn-

gldFasL−/− mice, and B6-MRL-lprFas−/− mice were incubated with OxLDL (150 μg protein/ml) for 72 h. Sections were stained for CD31 (clone MEC 13.3, PharMingen) with FastRed chromagen, counterstained with Mayer’s hematoxylin, and observed in lower (top) or higher magnification (bottom).

3 A). Treatment with OxLDL also led to notable losses of endothelium by 64 h (Fig. 3 B). Consistent with observations with cultured cells, apoptosis and loss of endothelium was inhibited by coinoculating the OxLDL with neutralizing anti-FasL antibodies. OxLDL did not induce apoptosis in medial smooth muscle cells under the conditions of these assays.

OxLDL-induced endothelial cell disruption was also examined in cultured arterial rings from gld or lpr mice that lack functional FasL or Fas, respectively (n = 5 for each group). Endothelium from C57BL/6J wild-type mice was largely destroyed after a 3-d incubation with OxLDL (Fig. 4). However, aortic endothelia of gld or lpr mice were relatively resistant to OxLDL-induced cell death. 49.7±6.6% of the endothelium was disrupted in aortic rings from wild-type mice after treatment with 150 μg protein OxLDL, but only 10.4±1.1% or 9.9±1.7% of the luminal surface lost endothelium in rings from gld or lpr mice, respectively (P < 0.05, P < 0.05). These data provide genetic evidence that the Fas/FasL interaction is essential for OxLDL-induced endothelial cell apoptosis.

To elucidate how oxidized lipids activate the Fas death pathway, cultured HAECs or HUVECs were incubated with combinations of neutralizing anti-FasL antibody and agonistic anti-Fas antibody in the presence or absence of OxLDL or LPC. Typically endothelial cells are resistant to Fas-mediated cell death in response to Fas ligand overexpression (18) or ligation of cell surface Fas with agonistic anti-Fas antibodies (Fig. 5). Surprisingly, incubation with OxLDL permitted the agonistic anti-Fas antibody to trigger apoptosis, as detected by FACS analysis after TUNEL staining, when endogenous FasL was blocked by the neutralizing antibody (Fig. 5 B). These findings indicate that oxidized lipids kill endothelial cells by increasing the responsiveness of cells to Fas ligation.

**Discussion**

Previously, we showed that vascular endothelial cells express both Fas and FasL, and that these cells are normally resistant to Fas-mediated apoptosis (18). Here, we demonstrate that oxidized lipids induce apoptosis in endothelial cells by activating the Fas death pathway. Oxidized lipid-induced endothelial cell apoptosis, identified by changes in cell shape, nuclear condensation, DNA fragmentation, and reduced mitochondrial function, was inhibited by incubation with a neutralizing antibody to FasL. Neutralizing anti-FasL antibodies also inhibited oxidized lipid-induced endothelial cell death in cultured arterial rings, and aortic endothelia prepared from FasL− or Fas-deficient mice were resistant to OxLDL-induced death. Collectively, these data indicate that the Fas/FasL pathway is a key feature of apoptosis induced by acute exposure to oxidized lipid.

Endothelial cell apoptosis induced by moderate levels of copper-oxidized LDL, ferrous-oxidized (minimally modified) LDL, LPC-C16:0, or LPC-C18:0 was blocked by treatment with the neutralizing anti-FasL antibody. At higher levels of oxidized lipids (OxLDL > 400 μg protein/ml, LPC-C16:0 > 80 μM, or LPC-C18:0 > 60 μM), cell death was unaffected by inhibition of the Fas pathway (data not shown), indicating that other cytotoxic mechanisms are also triggered by these agents. In this study, cytotoxic effects of these agents were examined in 2% (cell culture) or 0.5% (organ culture) FBS. Endothelial cells were more resistant to OxLDL in the presence of higher concentration of serum (> 10%) or after pretreatment with high serum medium (data not shown). The presence of high
density lipoprotein (33) or survival factors (34) in serum may modulate endothelial cell sensitivity to OxLDL-induced cytotoxicity.

Copper-oxidized LDL has been widely used to study the biological activity of OxLDL (10, 11, 35, 36), but a recent study suggests that copper-oxidized LDL may not represent the relevant form of in vivo modified LDL (37), indicating that one must exert caution in extrapolating in vitro findings with copper-oxidized LDL to the in vivo state. However, circulating OxLDL can be detected in blood of patients with hyperlipidemia using antibody raised against copper-oxidized LDL (38), and monoclonal autoantibodies from apolipoprotein E–deficient mice recognize epitopes in copper-oxidized LDL (39). OxLDL and its components have also been detected in atherosclerotic plaque of humans (3), apolipoprotein E3-leiden transgenic mice (40), and Watanabe heritable hyperlipidemic rabbits (3, 4). Taken together, these findings demonstrate that OxLDL is present in vivo and suggest that our results provide insights about the pathophysiology of OxLDL toxicity in endothelial cells.

Oxidized lipids influence the Fas/FasL pathway in endothelial cells in at least two ways. First, they increase cell surface expression of FasL with no detectable effect on the level of cell surface Fas expression. Second, they sensitize endothelial cells to death signals from the Fas receptor. This conclusion is indicated by the finding that an agonistic anti-Fas antibody can kill endothelial cells in the presence, but not absence, of oxidized lipids when endogenous FasL is inactivated by a neutralizing antibody. Endothelial cells are normally refractive to apoptosis in response to Fas ligand overexpression (18). Therefore, these data suggest that oxidized lipids do not kill endothelial cells by upregulating FasL alone, but instead, by increasing the responsiveness of endothelial cells to Fas ligation.

There are several mechanisms that may account for the increased sensitization of endothelial cells to Fas-mediated cell death by OxLDL. First, OxLDL may alter the levels of the bcl-2 family of proteins, which function as positive and negative regulators of apoptosis, including Fas-mediated apoptosis in some (41, 42) but not all cell types (43, 44). Second, OxLDL may activate the Fas pathway through receptor aggregation, as has been reported for ultraviolet radiation–induced apoptosis (45). Third, OxLDL might bring about changes in the intracellular Fas-signaling pathway. Candidate targets include the FADD-binding suppressors of apoptosis that have been reported by several groups (46–48), but the function of these molecules is controversial with some groups proposing that they act as death activators (49, 50). Finally, other factors have been reported to modulate Fas-mediated cell death including sentrin (51), GD3 ganglioside (52), and nitric oxide (53, 54), which themselves may be modulated by OxLDL.

Recent evidence suggests that cells expressing both FasL and Fas can become dramatically sensitized to the Fas-mediated apoptosis in response to specific stimuli and injuries. There is increasing evidence that alterations in cancer cell sen-
sitivity to Fas-mediated apoptosis is a key feature controlling tumor progression (15–17), and perturbations in the Fas/FasL cell suicide pathway appear to be important in determining the viability of transformed cells (55–57). Ultraviolet- or γ-radiation–induced apoptosis is also reported to be mediated by an activation of the Fas/FasL pathway (45, 58), and it is well established that T lymphocyte number is controlled by a delayed sensitization to Fas/FasL–mediated suicide after activation (59). The data presented here suggest that OxLDL enhances the sensitivity of endothelial cells to Fas-mediated cell suicide leading to a disruption of the endothelium. Because the status of endothelial cells is critical for vessel wall homeostasis (20), alterations in endothelial cell sensitivity to Fas-mediated apoptosis may play a role in vascular disease.

At this time it is difficult to assess the overall impact of the endothelial Fas/FasL system in atherogenesis. On one hand, endothelial FasL can function to inhibit leukocyte extravasation by inducing apoptosis in mononuclear cells invading the vessel wall in the absence of normal inflammatory stimuli (18). In this context, FasL may have a protective role, as normal leukocyte adhesion to the endothelium and extravasation is an early event in atherogenesis (20). On the other hand, oxidized lipids can increase the sensitivity of endothelial cells to death signals from the Fas receptor. As injuries to endothelium trigger inflammatory processes, sensitization to Fas-mediated apoptosis may contribute to atherosclerosis that results from exaggerated hyperlipidemia. Further studies on Fas, Fas ligand, and the Fas-signaling pathway in endothelial cells may provide a mechanistic rationale for inflammatory cell accumulation that is characteristic of fibroproliferative disorders of the vessel wall.

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References


2. Steinberg, D. 1997. Low density lipoprotein oxidation and its pathologi


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References


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References


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Acknowledgments
This work was supported by National Institutes of Health grants AG15052 and HLO5962 to K. Walsh.

References


38. Tamai, O., H. Matsuoka, H. Itabe, Y. Wada, K. Kohno, and T. Imai-zumi. 1996. Single LDL apheresis improves endothelium-dependent vasodilata-


41. Rodriguez, I., K. Matsuraka, K. Khatib, J.C. Reed, S. Nagata, and P. Vas
salli. 1996. A bcl-2 transgene expressed in hepatocytes protects mice from ful
millant liver destruction but not from rapid death induced by anti-Fas antibody injection. *J. Exp. Med.* 183:1031–1036.

42. Lacronique, V., A. Mignon, M. Fabre, B. Viollet, N. Rouquet, T. Mo
1996. Bcl-2 protects from lethal hepatic apoptosis induced by an anti-Fas anti


44. Strasser, A., A.W. Harris, D.C.S. Huang, P.H. Krammer, and S. Cory.
1995. Bel-2 and Fas/APO-1 regulate distinct pathways to lymphocyte apopto

1997. Ultraviolet radiation-induced apoptosis is mediated by activation of CD-
95 (Fas/APO-1). *J. Biol. Chem.* 272:25783–25786.


50. Muller, M., S. Strand, H. Hug, E.-M. Heinemann, H. Walczak, W.J.
Hofmann, W. Stremmel, P.H. Krammer, and P.R. Galle. 1997. Drug-induced apoptosis in hepatoma cells is mediated by the CD95 (APO-1/Fas) receptor/


t of the CD95 (APO-1/Fas) receptor/ligand system in drug-induced apopto

1997. Radiation and stress-induced apoptosis: a role for Fas/Fas ligand interac