REDD2 Gene Is Upregulated by Modified LDL or Hypoxia and Mediates Human Macrophage Cell Death


Objective—Cholesterol accumulation in macrophages is known to alter macrophage biology. In this article we studied the impact of macrophage cholesterol loading on gene expression and identified a novel gene implicated in cell death.

Methods and Results—The regulated in development and DNA damage response 2 (REDD2) gene was strongly upregulated as THP-1 macrophages are converted to foam cells. These results were confirmed by Northern blot of RNA from human monocyte-derived macrophages (HMDM) treated with oxidized LDL (oxLDL). Human REDD2 shares 86% amino acid sequence identity with murine RTP801-like protein, which is 33% identical to RTP801, a hypoxia-inducible factor 1-responsive gene involved in apoptosis. Treatment of HMDM with desferrioxamine, a molecule that mimics the effect of hypoxia, increased expression of REDD2 in a concentration-dependent fashion. Transfection of U-937 and HMEC cells with a REDD2 expression vector increased the sensitivity of the cells for oxLDL-induced cytotoxicity, by inducing a shift from apoptosis toward necrosis. In contrast, suppression of mRNA expression using siRNA approach resulted in increased resistance to oxLDL treatment.

Conclusion—We showed that stimulation of REDD2 expression in macrophages increases oxLDL-induced cell death, suggesting that REDD2 gene might play an important role in arterial pathology. (Arterioscler Thromb Vasc Biol. 2004; 24:1830-1835.)

Key Words: REDD2 ■ macrophages ■ atherosclerosis ■ hypoxia ■ oxLDL ■ necrosis ■ apoptosis

It is well-established that elevation of plasma low-density lipoproteins (LDL) is a risk factor for atherosclerosis, and that lesion-prone areas show increased intramural retention of lipoproteins by the extracellular matrix.1 Many of these lipoproteins are modified mainly through glycation, aggregation, protease degradation, and oxidation and as such become increasingly atherogenic.2,3 Several studies have indicated that a diversity of effects on macrophage function can be attributed to modified LDL. These include growth stimulation,4–6 proinflammatory effects such as expression of inflammatory cytokines,7 increase in cytotoxicity8 and in expression of metalloproteinases,9 inhibition of expression of inducible nitric oxide synthase,9,10 and effects on lipid metabolism and accumulation.11–13

The human leukemia cell line THP-1 has been used as a model for the monocyte–macrophage lineage.14 On treatment with phorbol esters, these cells take on the characteristics of activated macrophages, including decreased LDL receptor expression and increased expression of scavenger receptors. These changes in gene expression enable the cells to take-up modified lipoproteins and subsequently to develop a foam cell-like morphology. Using a powerful DNA microarray approach, Shiffman et al.15 have identified several upregulated genes in THP-1 cells loaded with oxidized LDL (oxLDL). In addition, Yuchang et al have also reported several differentially expressed genes in oxLDL-treated compared with nontreated THP-1 cells using the subtractive approach.16 In this study, we have combined the subtractive and the DNA microarray approaches and identified additional genes induced in cholesterol loaded THP-1 macrophages. Among the most significant upregulated genes, one expressed sequence tag (EST) gene was characterized and its potential role in inducing cell death was studied in U-937 monocytic cells and in HMEC endothelial cells.

Materials and Methods

Lipoprotein Isolation and Acetylation

LDL (d=1.03–1.053 g/mL) were isolated from freshly drawn blood from healthy normolipidemic volunteers as described.17 One mg protein/mL of LDL was acetylated with acetic anhydride as previ-
uously described. The level of acetylation was assessed by electrophoresis in cellulose acetate gels (Cellogel; SEBiA) and by the evaluation of the percent of acetylated amino acids according to the procedure of Habeck. These modified LDL particles had \( \approx 83\% \) acetylated lysine residues.

**Lipoprotein Oxidation**

Before oxidation, EDTA was removed by extensive dialysis of the LDL solution against EDTA free phosphate-buffered saline. Oxidation was initiated by incubating at 37°C with 5 \( \mu \)mol/L CuSO\(_4\) for 24 hours. Oxidation was stopped by adding 20 \( \mu \)mol/L EDTA. Native and oxLDL were screened for lipopolysaccharide (LPS) contamination by using a limulus lysate assay. All the different LDL preparations used in this study contained \(<0.75\) IU of LPS/mL. LDL contained 20\(\pm\)4 nmol peroxides/mg protein and 0.163 nmol thiobarbituric acid-reactive substances (TBARS)/mg protein, whereas ox-LDL contained 215.2\(\pm\)32.0 nmol peroxides/mg protein and 46.4\(\pm\)4 nmol TBARS/mg protein.

**Isolation and Culture of Human Monocytes**

Mononuclear cells were isolated frombuffy-coats of healthy normolipidemic donors. Cells were isolated after Ficoll gradient centrifugation and cultured in RPMI 1640 medium containing gentamicin (40 \( \mu \)g/mL), glutamine (0.05\%), and 10% pooled human serum at a density of 6\(\times\)10\(^5\) cells/well in 60-mm-well plastic culture dishes (Primaria; Polylabo). Differentiation of monocytes into macrophages occurred spontaneously by adhesion of cells to the culture dish after 12 days of culture. Cells were washed 3 times with phosphate-buffered saline and incubated for 24 hours with or without native LDL (nLDL), acLDL (100 \( \mu \)g protein/mL), oxLDL (100 \( \mu \)g protein/mL), or for 4 hours with desferrioxamine (DFO).

**5’ Rapid Amplification of cDNA Ends (RACE)**

Amplification of the 5’-end of this EST was performed using a 5’-RACE kit ( Gibco BRL, Gaithersburg, Md) according to the manufacturer’s instructions, using 5 \( \mu \)g of mRNA from human fetal liver, human uteri, and THP-1 macrophages, and a nested pair of EST sequence specific primers. After 1 round of 5’-RACE, the cDNA sequence was elongated to 2691 bp. BLAST analysis of the elongated sequence with more recent releases of GenBank identified the EST to be identical to human REDD2.

**Carotid Endarterectomy**

Carotid endarterectomy specimens were obtained from the Department of Cardiovascular Surgery (CHRU, Lyon, France), according to the protocol described by Legedz et al. from patients with severe carotid occlusive disease, and subjected to Stary classification. All samples were obtained by qualified hospital staff, and all procedures were approved by the local human ethics committee.

**RNA Analysis**

Total RNA from carotid endarterectomy specimens, HMDM, and THP-1 macrophages were extracted using the RNeasy kit (Qiagen). For Northern blot analyses, samples of total RNA (20 \( \mu \)g) were electrophoresed in 1% formaldehyde/agarose gel, blotted onto a nylon membrane (Nytran; Schleicher & Schuell, Keene, NH) and probed with a (\( \alpha ^{-}\)32P)dCTP-labeled polymerase chain reaction (PCR)-amplified 634 bp fragment of REDD2 cDNA, prepared using the sense primer 5’-AGGGAACAAGAGCCGTGACCACTGGTTGCAACTG-3’ and the antisense primer 5’-TATCCTTTATATTTTTCCTTCTTAG-3’. Gene expression was normalized to the control expression of GAPDH mRNA by rehybridizing the blot with a (\( \alpha ^{-}\)32P)dCTP-labeled 240-bp fragment of human GAPDH cDNA obtained by PCR amplification using the sense primer 5’-TGATGACATCAAGAAGTTGAG-TGGAC-3’ and the antisense primer 5’-TCTTTGGAGGCCATGTGGGC-3’, which was PCR amplified using the following primers 5’-CGAAGACGATCAGATACCGTCTGTAAG-3’ and 5’-AAGGCTATCACAGACCCTGTATTG-3’.

**Expression Plasmid Construct**

The pCI-cDNA REDD2 construct was prepared by subcloning the full-length REDD2 cDNA between the Mu)Lex63 inserts of expression pCI vector under the control of the CMV promoter (Promega, France). The cDNA fragment was obtained by PCR amplification using the upper primer 5’-AAG GAA ACA GAC GCG TTG AC-3’ and the lower primer 5’-TCA TGA AAT CTC TAG ATA TTT TCC CTT TTT AG-3’, which contained Mu 1 and Xbal sites, respectively. The integrity of the open reading frame was confirmed by sequencing of PCR products and by validating the size of the recombinant protein using the TNT T7 Quick coupled Transcription/Translation System (Promega, France).

**U-937 and HMEC Culture and Transient Transfections**

Mononuclear U-937 cells and human endothelial HMEC cells were cultured, respectively, in RPMI-1640 medium containing 25 mmol/L HEPES buffer and in MCB131 medium. Both media were supplemented with 10% fetal calf serum. Cells were incubated at 37°C in a humidified 5% CO\(_2\) atmosphere. The day before transfections, the cells were plated at a density of 2\(\times\)10\(^5\) cells/well in 6-well-well plates. The cells were then transfected overnight in the aforementioned medium with 5 \( \mu \)g of pCI-cDNA REDD2 plasmid using the Superfect reagent (Qiagen, France) according to the manufacturer’s protocol. The cells were then washed and incubated for an additional 8 to 12 hours in serum-free medium. Thereafter, the cells were stimulated with various concentrations of oxLDL for 24 hours. Under these experimental conditions, transfection efficiency determined with a GFP expression plasmid was estimated to be \( \approx 60\% \).

**Transfection of HMEC Cells With siRNA**

On the day of transfection, cells were at 50% to 70% confluence. Transfections with siRNA specific for REDD2 gene (Ambion, France) were performed using oligofectamine according to the manufacturer’s instructions (Invitrogen, France).

**Evaluation of Necrosis and Apoptosis**

Cytotoxic effect of oxLDL was determined on intact U-937 and HMEC cells by the MTT test and trypan blue exclusion test according to protocol described by Escargueil-Blanc et al. Apoptosis, postapoptotic necrosis, and primary necrosis were evaluated microscopically after staining with 2 vital fluorescent dyes, 0.6 \( \mu \)mol/L SYTO-13 (a permeant DNA intercalating green probe), and 15 \( \mu \)mol/L propidium iodide (a nonpermeant DNA intercalating orange probe), and counted by an inverted fluorescence microscope (Fluovert FU). Normal nuclei exhibited a diffuse green chromatin staining caused by SYTO-13 uptake, whereas apoptotic nuclei exhibited condensed green staining of intact or fragmented chromatin. Postapoptotic necrosis was characterized by orange-stained nuclei exhibiting apoptotic morphological features, whereas necrotic cells exhibited orange nuclei with loose chromatin and were also stained by trypan blue.

**Fluorogenic Assay for Caspase Activity**

Cells were lysed in ice-cold 10 mmol/L Tris-HCl buffer pH 7.4 (containing 10 mg/mL NP-40, 200 mmol/L NaCl, 5 mmol/L EDTA, 5 \( \mu \)g/mL leupeptin, 5 \( \mu \)g/mL aprotinin, and 100 mmol/L PMSF). The assay mixture contained 100 \( \mu \)L of the cell lysate and 100 \( \mu \)L of
40 mmol/L Ac-DEVD-AMC, and was incubated for 30 minutes, and the released fluorescent product AMC (AminoMethylCoumarin) was determined by fluorometry (excitation and emission wavelengths, 351 and 430 nm, respectively).

**Statistical Analysis**

Statistical analyses were evaluated by Student *t* tests and *P*<0.05 were considered significant.

**Results**

**Differentially Expressed Genes During THP-1 Macrophages Transformation into Foam Cells After AcLDL Treatment**

Among almost 4640 separate genes identified, 300 were confirmed as upregulated by cholesterol loading and 317 were downregulated 2-fold or more. Among these genes, one EST, which turned out to be identical to human REDD2 gene, was one of the most highly upregulated by acLDL treatment of THP-1 cells. REDD2 was of particular interest because of its sequence homology to RTP801, a gene upregulated by hypoxia that controls cell death.23 Northern blot analysis confirmed that REDD2 was also upregulated by acLDL in HMDM (Figure 1).

**Dose-Dependent Induction and Kinetic Analysis of REDD2 mRNA in oxLDL-Treated HMDM**

We determined whether upregulation of REDD2 was specific to acLDL-loaded HMDM or could also occur in response to the more physiological particles oxLDL or nLDL. HMDM were incubated with increasing concentrations of oxLDL for 24 hours, followed by RT-PCR analysis with primers specific for REDD2 and for 18S rRNA. Expression of REDD2 in human macrophages gradually increased with increasing oxLDL concentrations. The maximum effect was observed at 100 μg/mL of oxLDL (not shown). At this concentration, REDD2 expression was significantly upregulated by oxLDL but not by nLDL (Figure 2). In addition, kinetic study of REDD2 gene expression was performed by stimulating U-937 or HMEC cells with 100 μg/mL of oxLDL. The results showed a gradual increase of REDD2 mRNA between 4 and 48 hours of treatment and the maximum effect was observed at 24 hours (data not shown).

**Evaluation of REDD2 mRNA in Atherosclerotic Plaques by RT-PCR**

To determine whether REDD2 mRNA was elevated in atherosclerotic versus normal arteries, and hence extrapolate the in vitro findings to a clinical setting, total RNA from patient carotid endarterectomies (n=5) was isolated and subjected to RT-PCR. Almost a 2-fold increase in REDD2 expression was found in atherosclerotic plaques relative to the level of expression in healthy segments of the same artery from the same patients (Figure 3).

**Desferrioxamine, a Hypoxia-Mimic Condition, Induces the Expression of REDD2 Gene**

Because murine REDD2 protein shares ~33% identity with murine RTP801, a recently identified gene reported to be

---

**Figure 1.** Northern blot analysis of REDD2 mRNA from acLDL-stimulated or unstimulated THP-1 macrophages. THP-1 cells were treated with PMA (160 nM, 72 hours) to induce their differentiation into mature macrophages and then incubated, in serum-free medium, in the presence or absence of acLDL (100 μg protein/mL) for 24 hours. Total RNA was extracted and samples of 20 μg were electrophoresed and blotted onto a nylon membrane. The Northern blot was hybridized with a 32P-labeled human REDD2 cDNA probe. The same blot was stripped and rehybridized with a 32P-labeled human GAPDH cDNA and exposed to X-ray film.

**Figure 2.** Effect of oxLDL, native LDL, and vehicle on the expression of the REDD2 gene in cultured HMDM. Human macrophages were treated for 24 hours with 100 μg/mL of oxLDL, nLDL, or phosphate-buffered saline. Total mRNA was isolated and used for semiquantitative RT-PCR analysis of REDD2 expression; 18S rRNA levels served as internal standard. The data in the bar graph are the quantified ratio of the signal for REDD2 to that of 18S rRNA, with nontreated cells set to 1. Data shown are mean±SD of 3 different experiments.

**Figure 3.** Expression of REDD2 mRNA in human atherosclerotic plaques using mRNA extracted from patient carotid endarterectomy samples. Total RNA from atherosclerotic plaques as well as macroscopically assessed nonaffected regions of the same samples, obtained from carotid endarterectomy (n=5), was subjected to RT-PCR analyses, using specific primers for REDD2 and 18S rRNA as internal standard. The bar graph shows the ratio of the REDD2 to 18S rRNA signals in the atherosclerotic plaques, with the normal artery samples of the same patient set to 1.
rapidly and sharply inducible in response to hypoxia, we decided to evaluate the effect of hypoxia on the expression of REDD2 gene in HMDM. The iron chelator, DFO, has been reported to mimic the effect of oxygen deprivation on a number of hypoxia responsive genes on various cell culture. Therefore, we treated HMDM with different concentrations of DFO for 4 hours and measured the mRNA levels of adrenomedullin and REDD2 (Figure 4). The adrenomedullin mRNA measurement, already known to be enhanced in cells treated with DFO, was performed to serve as a positive control. Treatment of HMDM with increasing concentrations of DFO gradually increased adrenomedullin (data not shown) and REDD2 (Figure 4), with maximal gene expression of both observed with 500 μmol/L DFO.

Role of REDD2 in U-937 Monocytic Cells

Because hypoxia is known to induce a variety of cellular responses, including necrosis and apoptosis, we transiently transfected U-937 cells with a REDD2 expression vector and examined the influence of REDD2 on oxLDL-induced cytotoxicity. Our results indicated an increase in cell mortality by necrosis in the absence of oxLDL (2.5±0.4% for superfect transfection reagent treated cells [control] versus 8±4% for cells transfected with the REDD2 expression vector) (Figure 5A). In the presence of oxLDL, the cellular toxicity was more pronounced in cells overexpressing REDD2 compared with control cells. The percent of cell death after treatment with 100, 200, or 300 μg/mL of oxLDL was 14.7±4.1, 35±4.4, and 48.7±8.5, respectively, in control cells versus 44.6±8.4, 61.0±9.6, and 82.6±14.0, respectively, in REDD2-transfected cells (Figure 5A). Similar results were obtained using the MTT test (Figure 5B). Moreover, microscopic observations of U-937 after staining by SYTO and propidium iodide showed no difference between untreated and oxLDL-treated (100 μg/mL, 24 hours) control cells. In contrast, we clearly observed an increase in cell sensitivity to oxLDL in cell transiently transfected with the REDD2 expression vector, occurring by apoptosis or postapoptotic necrosis (orange staining) (online Figure I, please see http://atvb.ahajournals.org).

Role of REDD2 in HMEC Endothelial Cells

Because U-937 cell type does not allow determining adequately if REDD2 transfection induces apoptosis or postapoptotic necrosis in the presence of oxLDL, we have repeated the transient transfection experiments on HMEC endothelial cells, which mainly die by apoptosis in the presence of oxLDL. As shown in Figure 5D and as observed in U-937 cells (Figure 5B), REDD2 transfection increased the sensitivity of HMEC to oxLDL cytotoxicity. In control cells, oxLDL elicited apoptosis and postapoptotic necrosis, but not primary necrosis after 24 hours (32% apoptotic cells versus 4% primary necrotic cells), as assessed by low LDH release (Figure 5C), morphological features of chromatin condensation, pyknosis, and nuclear fragmentation (online Figures I and II). Moreover, caspase activity was enhanced by oxLDL after 16 hours of incubation (Figure 5D). In contrast, in cells transiently transfected by REDD2, oxLDL elicited a shift toward necrosis (19% of apoptotic cells versus 30% of primary necrotic cells) on the basis of LDH release (2-fold increase).

Figure 4. Effect of DFO on the expression of the REDD2 gene in HMDM. HMDM cultured for 12 days were washed twice with phosphate-buffered saline and treated for 4 hours, in serum-free medium, with increasing concentrations of DFO. REDD2 mRNA levels were determined by semiquantitative RT-PCR. The results are the means±SD of 3 separate experiments (*P<0.05).

Figure 5. Influence of REDD2 overexpression on oxLDL-mediated cell death in U-937 monocytic cells and HMEC endothelial cells. Cells were transfected with 5 μg/mL of REDD2 gene construct or with Superfect transfection reagent and empty vector (control) and after 24 hours, treated with 0, 100, 200, and 300 μg protein/mL of oxLDL for 24 hours or 16 hours for caspase. Cytotoxicity was evaluated by trypan blue staining (A), MTT method (B,D), LDH measurements (C), and caspase activity (D). Results are mean±SD of 4 separate experiments.
increase by comparison to the basal level) (Figure 5C), and of morphological features showing an absence of cytoplasmic and nuclear condensation (online Figure II). In addition, caspase activation was decreased by REDD2 transfection by comparison to control cells (Figure 5D).

To clarify the role of REDD2 in cell death, experiments using siRNA approach were performed. Expression of REDD2 gene was significantly reduced (to the basal level) when cells were transfected with specific siRNA for REDD2 gene (data not shown). In this experimental condition, oxLDL induced almost only apoptosis, an effect comparable to that observed on control cells (Figures 5 and 6) and (online Figure I and II).

**Discussion**

In this study, by using the combination of a subtractive library and a DNA microarray approaches, we selected 1 cDNA fragment that could not be annotated and was initially referred to as EST. For this EST, acLDL loading of THP-1 cells increased expression of a 2.5-kb mRNA ~4-fold (Figure 1). Given the uniqueness of this sequence, we decided to full-length clone its cDNA and to explore its relevance to macrophage biology and potential implications in atherosclerosis. The identity of the EST was determined by BLAST analysis of the GeneBank database with the full-length cDNA sequence obtained by 5’ RACE. The EST turned out to be identical to human REDD2, also known as Smhs1-like gene or RTP801-like gene. Although the function of this gene is not known, the expression of the RTP801 gene, which has 33% amino acid sequence identity with REDD2/Smhs1-like gene/RTP801-like gene was reported to be induced in response to hypoxia in cultured cells and in vivo and to play a role in apoptosis.

To explore the biology of REDD2 in macrophages and its potential significance in atherosclerosis, further experiments were conducted using oxLDL in place of acLDL and HMDM in place of THP-1 cells. The expression of this gene was found to be increased in HMDM in the presence of oxLDL in a concentration-dependent manner (not shown), whereas nLDL particles were without effect (Figure 2). Using the RT-PCR semiquantitative approach, the expression of REDD2 was found to be expressed 2-fold greater in human carotid atheroma in comparison to nonatheromatous segments of the same arteries (P<0.05) (Figure 3).

Our results indicated, for the first time to our knowledge, that REDD2 gene expression is upregulated in macrophages stimulated with oxLDL or acLDL and in atherosclerotic plaques. The related gene RTP801 is upregulated under conditions of ischemia and oxidative stress; it is possible that treatment of macrophages with modified LDL may also induce an ischemic or oxidative stress resulting in upregulation of REDD2. To test this, we created a hypoxic condition by treatment of HMDM with the iron chelator DFO, which has been reported to mimic the effect of oxygen deprivation on a number of hypoxia responsive genes. The results showed that increasing concentrations of DFO gradually increased the expression of REDD2 gene (Figure 4). Taken together, these data strongly suggest that REDD2 is a hypoxia-responsive gene and that this may be, in part, the mechanism of its upregulation in modified LDL-treated macrophages. It is important to note that while preparing this manuscript, several genes have been reported to be upregulated by hypoxia in primary human monocyte-derived macrophages, including VEGF, GLUT-1, and matrix metalloproteinase-7. Nevertheless, REDD2 gene was not reported in this later study. This was likely caused by the absence of REDD2 cDNA on the nylon array used. These inducible hypoxia genes in macrophages might be important in the pathology of arterial diseases such as atherosclerosis. Björnhenh et al reported the existence of zones of hypoxia deep in atherosclerotic plaque, which probably caused an impaired oxygen diffusion capacity as a consequence of the thickness of the lesion.

In this article, we reported for the first time to our knowledge an increase of the expression of REDD2 in response to oxLDL in HMDM (Figure 2). Upregulated expression of this gene was reproduced under conditions that mimic hypoxia (Figure 4), establishing a potential role of oxLDL in inducing a hypoxic-like condition in macrophages. Furthermore, results from transfection experiments of U-937 and HMEC cells suggested that REDD2 gene sensitizes cells to oxLDL cytotoxicity, by inducing a shift from apoptosis toward necrosis (Figures 5 and 6 and online Figures I and II). Mechanism and type of cell death occurring in atherosclerotic areas may be important, because apoptotic cells are rapid engulfed and cleared, whereas necrotic cell debris may trigger a local inflammatory response.

**Acknowledgments**

This study was supported by grants from Inserm and Fondation Leducq. The authors thank V. Garcia for transfection experiments. Generation of substrated/normalized cDNA libraries from THP-1 cells exhibiting macrophages and foam cell phenotypes, as well as identification of regulated genes, were performed at Aventis Pharma.
References


REDD2 Gene Is Upregulated by Modified LDL or Hypoxia and Mediates Human Macrophage Cell Death


Arterioscler Thromb Vasc Biol. 2004;24:1830-1835; originally published online August 12, 2004;
doi: 10.1161/01.ATV.0000142366.69080.c3

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 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/24/10/1830

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2004/10/11/24.10.1830.DC1

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/
Figure I

- oxLDL

A

+ oxLDL

B

C

D