Macrophage oxidative modification of low density lipoprotein occurs independently of its binding to the low density lipoprotein receptor

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Abstract The oxidative modification of low density lipoproteins (LDL) by arterial wall cells is thought to contribute to atherogenesis. Monocyte/macrophages, among other arterial wall cells, oxidatively modify LDL to a form that is recognized by scavenger/oxidized LDL receptors. It has recently been suggested that LDL binding to the LDL receptor (B/E receptor) is essential for macrophage-mediated oxidation of LDL. In the present study, we compared the ability of resident peritoneal macrophages from LDL-R-deficient ($LDLR^{-/-}$) mice to oxidize LDL with that of resident peritoneal macrophages from C57B6 mice. The $LDLR^{-/-}$ macrophages oxidized LDL at least as rapidly as did the C57B6 macrophages both in F-10 medium and in Dulbecco's modified Eagle's medium supplemented with 1 μM copper (DMEM-Cu^{2+}). Studies were also conducted to examine the effect of preincubation of $LDLR^{-/-}$ and C57B6 macrophages with 10% lipoprotein-deficient serum (LPDS), which up-regulates LDL receptors, or with acetylated LDL (Ac-LDL), which increases cellular cholesterol and down-regulates LDL receptors. Preincubation with 10% LPDS had no significant effect on subsequent LDL oxidation by either type of cells in F10 medium, but the C57B6 cells did show a small (18%) but significant increase in LDL oxidation in DMEM-Cu^{2+}. Preincubation with 50 μg/ml Ac-LDL in F10 medium had no effect on LDL oxidation by either $LDLR^{-/-}$ or C57B6 macrophages. Preincubation with 100 μg/ml Ac-LDL had no effect on subsequent LDL oxidation by C57B6 cells but, unexpectedly, caused a modest (26%) fall in LDL oxidation by the receptor-negative cells. Using DMEM-Cu^{2+} medium, preincubation with Ac-LDL reduced LDL oxidation substantially (50–66%) but the effect was just as great in LDL-R negative cells (59–66%) as in C57B6 cells (50–58%), suggesting that the effect is not due to changes in LDL receptor density. It may be related somehow to the Ac-LDL-induced increase in cell cholesterol content. The data demonstrate that the absence of LDL receptors does not reduce the ability of macrophages to oxidize LDL and that LDL binding to LDL receptors is not an essential requirement for macrophage oxidation of LDL.—**Tangirala, R. K., M. J. T. Mol, and D. Steinberg.** Macrophage oxidative modification of low density lipoprotein occurs independently of its binding to the low density lipoprotein receptor. *J. Lipid Res.* 1996. **37:** 835–843.

Supplementary key words lipoprotein oxidation • LDL receptor-deficient macrophages • atherosclerosis • modified lipoproteins • resident peritoneal macrophages • LDL receptor-knockout mice • apoB/E receptor • lipid peroxides

Oxidative modification of LDL in arterial wall is thought to play an important role in the pathogenesis of atherosclerosis (1, 2). The three major arterial wall cells, endothelial cells, monocyte/macrophages, and smooth muscle cells, are all capable of oxidative modification of LDL. The cell-mediated oxidation of LDL induces a number of changes in its physico-chemical properties including an increased electrophoretic mobility, a reduction in unsaturated fatty acids, an increase in lipoperoxides, fragmentation of apoB-100, and conversion to a form that no longer binds to the native LDL receptor but binds to the unregulated scavenger/oxidized LDL receptor(s) (1, 2). The cell-mediated oxidation of LDL appears to be complex and the precise mechanisms involved in the LDL-cell interactions leading to cell-mediated oxidation of LDL remain to be established. Studies in cell culture systems suggest a number of mechanisms that could contribute to cell-mediated LDL oxidation, including the generation of superoxide anion (3–5), lipoxygenase reactions (6–8), myeloperoxidase reactions (9), or thiol production (10).

Abbreviations: LDL, low density lipoprotein; TBARS, thiobarbituric acid-reactive substances; PBS, phosphate-buffered saline; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; LPDS, lipoprotein-deficient serum; Ac-LDL, acetylated low density lipoprotein; $LDLR^{-/-}$, low density lipoprotein receptor-deficient; MDA, malondialdehyde; GLC, gas–liquid chromatography.

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In addition, cytokines (11), estrogens (12), calcium antagonists (13), and β-blockers (14) have been shown to affect cell-mediated oxidation of LDL.

The binding of LDL to the LDL receptor (apoB/E receptor) has been implicated by Aviram and Rosenblat (15) as an essential requirement for macrophage-mediated oxidation of LDL. They reported that monoclonal antibodies directed against either the LDL receptor or the LDL receptor-binding site on apoB-100 inhibited mouse peritoneal macrophage-mediated oxidation by 52–95% (15). In contrast, in a recently published study, Cathcart, Li, and Chisolm (16) showed that the extent of LDL oxidation by activated human monocytes and monocytic U937 cells is not affected by down-regulation of LDL receptors. They point out that species differences and differences in incubation conditions might account for the discordant results. A murine model for familial hypercholesterolemia, lacking functional LDL receptors, has been recently generated by Ishibashi et al. (17) using gene-targeting techniques. These LDL-receptor knockout mice develop spontaneous hypercholesterolemia and atherosclerosis (17, 18). The macrophage-rich regions of atherosclerotic lesions of these mice also contained oxidation-specific epitopes detected immunohistochemically (19). These observations suggest that macrophages in the mouse do oxidize LDL despite the LDL receptor deficiency. In the present study, we have directly evaluated the effect of LDL receptor deficiency by using peritoneal macrophages from LDL-receptor knockout mice.

MATERIALS AND METHODS

Materials

Cell culture media, RPMI, Ham's F10, and DME media without phenol red were purchased from Gibco Laboratories (Grand Island, NY). Fetal calf serum, BSA, EDTA, and $CuSO_4$ were purchased from Sigma Chemical Co. (St. Louis, MO). Agarose electrophoresis gels were obtained from Ciba Corning Diagnostic Corp. (Palo Alto, CA). Carrier-free $Na^{125}I$ was from Amersham (Chicago, IL).

Animals and cells

Male C57B6 mice were purchased from Simonsen Laboratories (Gilroy, CA). Homozygous, male LDL receptor-deficient ($LDLR^{-/-}$) mice (with a C57BL/6 × 129Sv background) (17, 18), were from a breeding colony established from animals obtained from Jackson Laboratories (Bar Harbor, ME). Both C57B6 mice and $LDLR^{-/-}$ mice were maintained on regular chow diet. Resident peritoneal macrophages were harvested from male mice (2–3 months old weighing 24–32 g) without thioglycollate stimulation. The cells harvested by peritoneal lavage were plated in 6-well plates (Costar, Cambridge, MA) at a cell density of $2–4 \times 10^6$ cells per well and incubated overnight in RPMI medium supplemented with 10% fetal calf serum at 37°C in humidified air/CO_2 (95:5). After incubation, cells were rinsed 3 times with RPMI and used for LDL oxidation and degradation studies under conditions described below. In some experiments, cells were preincubated for 18 h in RPMI medium supplemented with either 10% human lipoprotein-deficient serum (LPDS) or 10% FBS containing acetyl LDL (Ac-LDL) (50 and 100 μg Ac-LDL protein/mL).

LDL isolation and iodination

LDL (d 1.019–1.063 g/mL) was isolated from pooled normal human plasma (20) and stored at 4°C with 1 mM EDTA. LDL was radioiodinated (sp act 285,000 cpm/μg LDL protein) using the Iodogen method (21). Both labeled and unlabeled LDL samples were extensively dialyzed against EDTA-free PBS at 4°C to remove EDTA before using LDL for incubation with cells.

Incubation conditions and oxidation of LDL by macrophages

Oxidative modification of LDL by resident peritoneal macrophages was studied in two different media: Ham's F10 medium (F10) and DMEM supplemented with 1 μM $CuSO_4$ (DMEM-Cu^{2+}). In the initial experiments, LDL (100 μg LDL protein/mL, 1 mL per well) was incubated in F10 medium with cells for 10 or 22 h at 37°C. To examine the effect of incubation medium on the extent of LDL oxidation, parallel time course experiments of LDL oxidation by C57B6 and $LDLR^{-/-}$ macrophages were conducted in F10 and DMEM-Cu^{2+}. Control wells, without cells, were also incubated under identical conditions. After incubation, media were removed and aliquots were analyzed for LDL oxidation. The cells in wells were dissolved by the addition of 0.2 N NaOH and aliquots were analyzed for cell protein by the method of Lowry et al. (22).

Measurement of LDL oxidation

TBARS. The extent of LDL oxidation was measured by the determination of TBARS (23). Analysis of TBARS was performed on 100-μL aliquots of LDL samples using malondialdehyde (MDA) as standard. No-cell controls were included and the TBARS values for no-cell controls were subtracted from the respective experimental TBARS values. Data are expressed in nanomoles of MDA produced per milligram cell protein (not per milligram LDL protein).

LDL fatty acid composition. The extent of LDL oxidation was also determined by measuring the depletion of

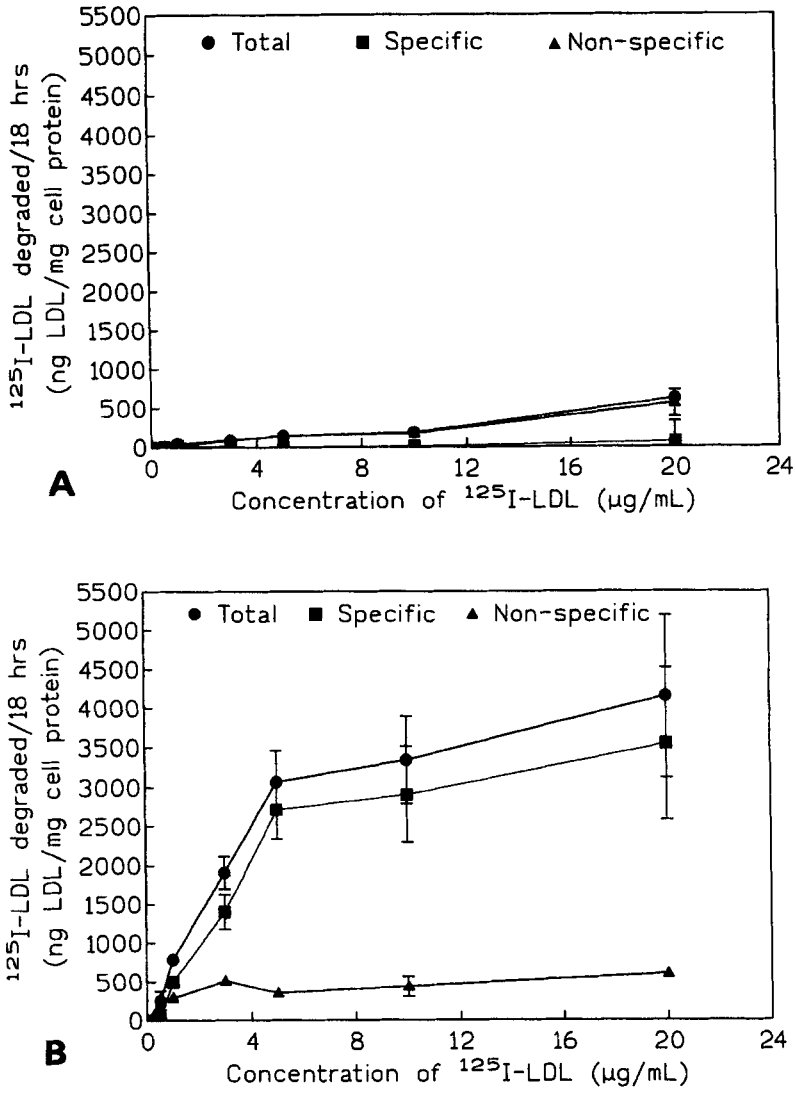

Fig. 1. Native ^{125}I-labeled LDL degradation by resident peritoneal macrophages. LDL receptor activity on resident peritoneal macrophages isolated from homozygous, LDL receptor-knockout mice (A) and C57B6 mice (B) were incubated at 37°C for 18 h in the presence of 10% LPDS with indicated concentrations of ^{125}I-labeled LDL both in the presence (Non-specific) and absence (Total) of a 30-fold excess of unlabeled native LDL. The trichloroacetic acid-soluble, non-iodide radioactivity was determined. The values in the no-cell control wells were subtracted from the experimental values. Specific degradation due to LDL receptor-mediated uptake was calculated by subtracting values for non-specific degradation in the presence of 30-fold excess unlabeled LDL from values for total degradation. Each point represents the mean ± SD of triplicate determinations. Where not apparent the error bars are smaller than the symbols.

LDL linoleate (18:2) and arachidonate (20:4) in oxidized LDL lipid extracts by gas–liquid chromatography. Lipids from oxidized LDL samples were extracted by a modified method of Folch, Lees, and Sloane Stanley (24), transmethylated, and analyzed by GLC (Model 3700, Varian Associates, Sugarland, TX) using a column of 10% Silar 5CP on Gas Chrom QII, 100/120 mesh (Altech Associates), as described previously (25). The absolute quantity of total fatty acids in the sample was determined by the addition of an internal standard of pentadecanoic acid (15:0) to each sample before lipid extraction. The amount of fatty acids with carbon chains longer than 20 was less than 3% of the total, so each run was truncated after elution of the C20 peaks.

Agarose electrophoresis

The electrophoretic mobility of the LDL samples incubated with cells was determined by agarose gel electrophoresis. LDL samples were loaded at 4.0 μg protein/well and run at 100 volts for 30 min. After drying, the gels were stained with Oil Red O to visualize lipoprotein bands.

Macrophage LDL receptor assay

LDL receptor expression was assayed in terms of the rate of degradation of ^{125}I-labeled LDL (26, 27). Resident peritoneal macrophages plated in 12-well plates at 2×10^6 cells/well were rinsed three times with RPMI and incubated with increasing concentrations of ^{125}I-labeled LDL (0.5–20.0 μg protein/well) both in the presence and absence of a 30-fold excess unlabeled LDL. After 18 h, the medium was assayed for trichloroacetic acid-soluble and silver nitrate-soluble (non-iodide) radioactivity (28). Appropriate control incubations in wells containing no cells were conducted and the control values were subtracted from experimental values. The cell protein

in the wells was determined by the method of Lowry et al. (22) after dissolving the cells in 0.2 N NaOH.

Effect of conditions affecting cellular cholesterol and LDL receptor level on LDL oxidation

To compare LDL oxidation by macrophages under conditions of maximal and minimal LDL receptor expression, resident peritoneal macrophages were plated at 2×10^6 cells/well in 6-well plates in RPMI medium supplemented with 10% FBS. The cells were allowed to adhere and were then rinsed 3 times with serum-free medium and further incubated for 18 h in RPMI medium supplemented with *a)* 10% FBS (control), *b)* 10% human lipoprotein-deficient serum (LPDS), *c)* 10% FBS with Ac-LDL (50 and 100 μg Ac-LDL protein/mL). The incubation of cells with Ac-LDL was conducted in the presence of 10% serum to inhibit any cell-mediated oxidation of Ac-LDL and to avoid any prior exposure of the cells to oxidation products during the preincubation. After preincubation, the cells were rinsed 3 times with serum-free medium, and further incubated with native LDL (100 μg LDL protein/mL) either in F10 medium or DMEM-Cu^{2+}, and the LDL oxidation was measured.

Statistical analysis

Results were analyzed by Student's unpaired *t*-tests. Data are expressed as mean ± SD.

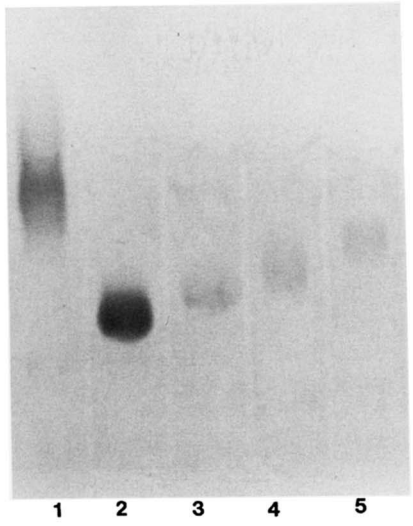

Fig. 2. Agarose gel electrophoretic mobility of LDL incubated with $LDLR^{-/-}$ and C57B6 macrophages. LDL samples (100 μg/mL) were incubated for 22 h with resident peritoneal macrophages in Ham's F10 medium, loaded on an agarose gel at 4–8 μg/well, and run at 100 volts for 30 min. The gel was dried and stained with Oil Red O. Lane 1, copper-oxidized LDL; lane 2, native LDL; lane 3, no-cell control; lane 4, LDL incubated with C57B6 macrophages; lane 5, LDL incubated with $LDLR^{-/-}$ macrophages.

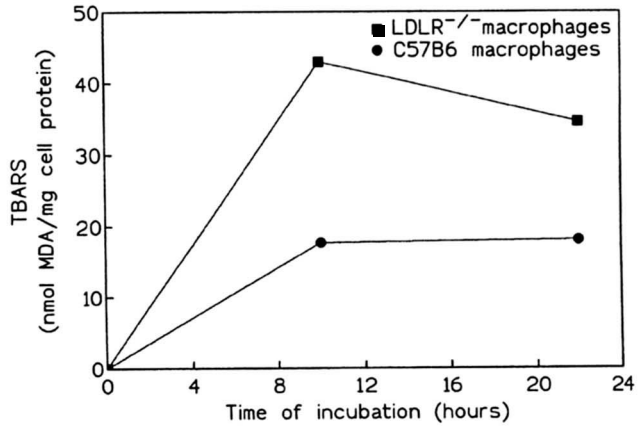

Fig. 3. LDL oxidation by resident $LDLR^{-/-}$ and C57B6 macrophages. LDL (100 μg/mL)) was incubated for 10 and 22 h with the macrophages (2×10^6 cells/well) in Ham's F10 medium. The TBARS content of the medium was determined after indicated times. The values of control plates incubated in the absence of cells (no-cell control) were subtracted from experimental values. After 10 and 22 h incubation the average TBARS for no-cell controls were 4.0 and 16.9 nmol MDA/mg LDL protein and the average cell protein/well were 62 and 49 μg, respectively. Each data point is an average of duplicate values from a representative experiment that varied less than 5%.

RESULTS

Macrophage LDL receptor status

The LDL receptor status of both $LDLR^{-/-}$ and C57B6 peritoneal macrophages was verified by measuring degradation of ^{125}I-labeled LDL. As shown in **Fig. 1A,** no specific degradation of LDL was observed in the case of $LDLR^{-/-}$ macrophages whereas C57B6 macrophages showed specific degradation that was competed by a 30-fold excess of unlabeled LDL (Fig. 1B), confirming the absence of detectable, functional LDL receptor activity on $LDLR^{-/-}$ macrophages.

Oxidative modification of LDL by $LDLR^{-/-}$ macrophages

Initially experiments were conducted to compare LDL oxidation by resident $LDLR^{-/-}$ and C57B6 peritoneal macrophages by incubation of LDL (100 μg of LDL protein/mL) for 10 and 22 h in F10 medium. As indicated by the relative increase in electrophoretic mobilities on agarose gels, LDL incubated with $LDLR^{-/-}$ macrophages was oxidized, if anything, to a greater degree than the LDL incubated with C57B6 macrophages (**Fig. 2**). In F10 medium, LDL incubated with $LDLR^{-/-}$ macrophages contained much more TBARS than LDL incubated with C57B6 macrophages (**Fig. 3**). Although TBARS produced by resident macrophages during LDL oxidation was variable from experiment to experiment (n = 3), a relatively higher TBARS value was consistently observed in the case of LDL incubated with $LDLR^{-/-}$

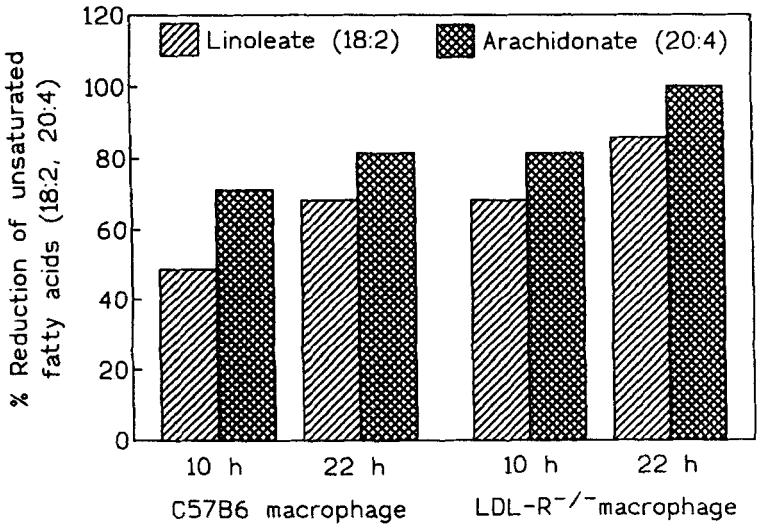

Fig. 4. Percent reduction in LDL linoleate (18:2) and arachidonate (20:4) due to oxidation modification by resident LDLR$^{-/-}$ and C57B6 macrophages. LDL samples (100 μg/mL), incubated under conditions described in Fig. 2, were analyzed by GLC for the mass of 18:2 and 20:4 remaining after 10 and 22 h of incubation with cells as described in Methods. The mass of 18:2 and 20:4 in the native LDL samples was taken as 100%. Each bar is an average of duplicate values from a representative experiment.

macrophages. In addition, LDL oxidation measured in terms of the depletion of LDL polyunsaturated fatty acids, linoleate (18:2) and arachidonate (20:4), also showed that LDLR$^{-/-}$ macrophages oxidize LDL at least as well as C57B6 macrophages (**Fig. 4**).

Incubation media and time course of LDL oxidation

The time course of LDL oxidation by LDLR$^{-/-}$ and C57B6 macrophages showed substantial TBARS production by 4 h, followed by a slower further increase over 20 h in both F10 medium and DMEM-Cu^{2+} (**Fig. 5, A and B**). Both types of macrophages oxidized LDL to a greater degree in DMEM-Cu^{2+} than in F10 medium. However, the overall pattern of LDL oxidation by LDLR$^{-/-}$ and C57B6 macrophages was essentially similar in F10 and DMEM-Cu^{2+}. Thus, the rate of LDL oxidation by LDLR$^{-/-}$ macrophages is not affected by LDL receptor deficiency.

Macrophage LDL oxidation under conditions affecting LDL receptor activity and cellular cholesterol

Oxidation of LDL by LDLR$^{-/-}$ and C57B6 macrophages was determined after exposure of cells to conditions influencing LDL receptor expression and cellular cholesterol levels. They were incubated first for 18 h with 10% LPDS or 10% FBS supplemented with Ac-LDL (50 and 100 μg Ac-LDL protein/mL). The cells were then incubated for another 18 h with native LDL (100 μg LDL protein/mL) either in F10 medium or DMEM-Cu^{2+} to assess LDL oxidation. As in the preceding experiments, LDLR$^{-/-}$ macrophages that had been preincubated with 10% FBS oxidized LDL at least as well as C57B6 macrophages. In fact, the LDLR$^{-/-}$ macrophages, compared to C57B6 macrophages, consistently produced more TBARS although the differences were small. Up-regulation of LDL receptors by preincubation of C57B6 macrophages for 18 h in LPDS had no effect on LDL oxidation in F10 medium (**Fig. 6A**). However, in DMEM-Cu^{2+} the C57B6 macrophages preincubated in LPDS showed a small but significant increase in TBARS (18%; $P < 0.05$) (Fig. 6B). The oxidation of LDL by LDLR$^{-/-}$ macrophages in F10 and DMEM-Cu^{2+} was unaffected by their preincubation in 10% LPDS. Despite the up-regulation of LDL receptors, C57B6 macrophages produced relatively lower TBARS than LDLR$^{-/-}$ macrophages.

The effect of Ac-LDL uptake (and down-regulation of LDL receptors) on LDL oxidation by C57B6 and LDLR$^{-/-}$ macrophages was studied in cells preincubated for 18 h with Ac-LDL (50 and 100 μg Ac-LDL protein/mL in 10% FBS). The preincubated macrophages contained several inclusions, presumably lipid droplets. In F10 medium, Ac-LDL had no significant effect on LDL oxidation by C57B6 macrophages, whereas LDLR$^{-/-}$ macrophages showed a significant reduction (26%) (Fig. 6A). In contrast, LDL oxidation in DMEM-Cu^{2+} by both LDLR$^{-/-}$ and C57B6 macrophages (preincubated with Ac-LDL) was substantially reduced (by 59–66% and 50–58%, respectively; $P < 0.05$). However, the degree of inhibition was quantitatively similar in LDLR$^{-/-}$ and C57B6 macrophages showing that the effects appear to be unrelated to changes in LDL receptor levels.

DISCUSSION

In this study, using peritoneal macrophages from homozygous LDLR-deficient (LDLR$^{-/-}$) mice, we have demonstrated that, under the conditions used, the ca-

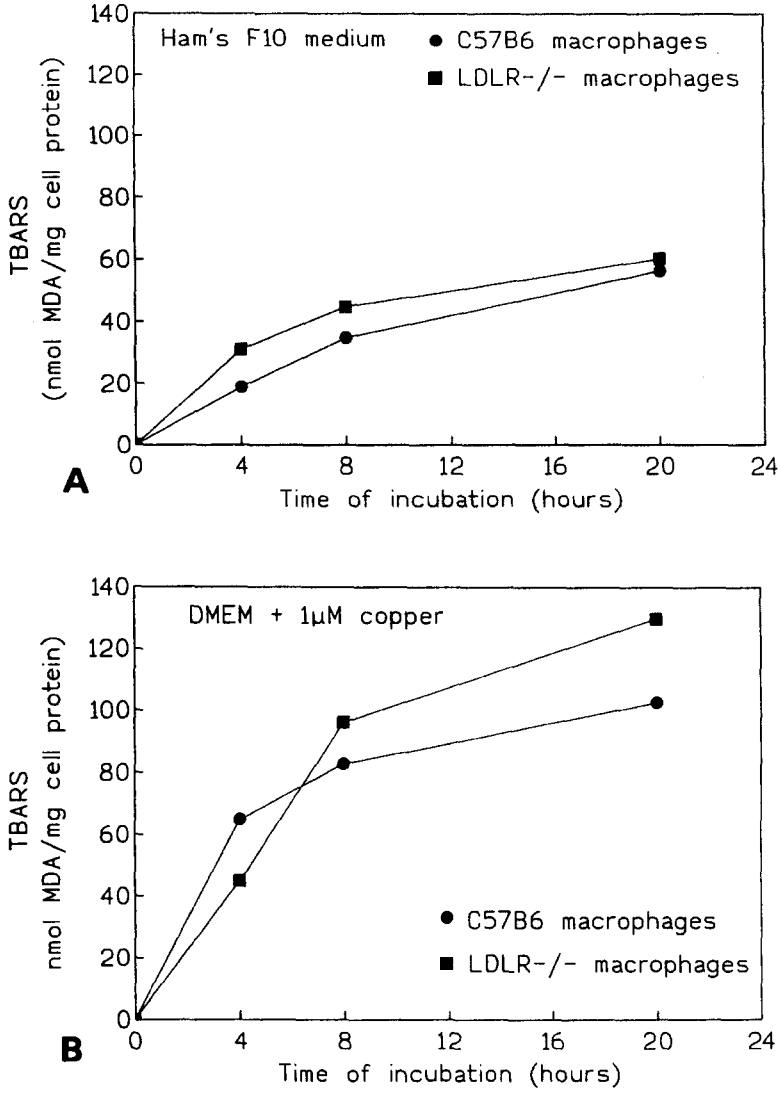

Fig. 5. Comparison of time course of LDL oxidation by LDLR$^{-/-}$ and C57B6 macrophages. LDL samples (100 μg/mL) were incubated with resident peritoneal macrophages for 4, 8, and 20 h in Ham's F10 medium (A) and DMEM supplemented with 1 μM copper (B) and the extent of oxidation was analyzed by measuring the TBARS content. The values of no-cell control were subtracted from the TBARS values obtained for LDL incubated with cells. The TBARS for no-cell controls after 4, 8, and 20 h incubation were 5.8, 8.7, and 23.3 nmol MDA/mg LDL protein in Ham's F10 and 4.4, 8.7, and 16.2 nmol MDA/mg LDL protein in DMEM supplemented with 1 μM copper, respectively. The average cell protein of the wells incubated with Ham's F10 and DMEM supplemented with 1 μM copper were 72.5 and 97.0 μg, respectively. Each value is the average of duplicate determinations.

pacity of macrophages to oxidize LDL is not reduced by the absence of LDL receptors. In fact, LDLR$^{-/-}$ macrophages oxidized LDL at a slightly higher rate than did C57B6 macrophages. In the present studies we used not only F10 medium (containing 0.01 μM copper and 1.53 μM iron), which is routinely used for studies of cell-mediated oxidation (29–33), but also used the medium previously used by Aviram and Rosenblat (15), namely, DMEM with 1 μM copper added (0.28 μM iron). In both media we observed similar overall rates of LDL oxidation by LDLR$^{-/-}$ and C57B6 macrophages, showing that under the conditions used interaction of LDL with LDL receptors is not a prerequisite for macrophage LDL oxidation. These results do not necessarily rule out the importance of other kinds of LDL–cell interaction in the oxidation of LDL.

While these studies were in progress, Cathcart et al. (16) published their studies addressing the same issue but using human monocytes and human-derived monocytic U937 cells. Like Aviram and Rosenblat (15), they used various interventions that would increase or decrease expression of LDL receptors and correlated those changes with changes in the rate of LDL oxidation. Cathcart et al. (16) concluded that LDL-LDL receptor interaction did not play a significant role in controlling the rate at which the cells oxidized LDL. They also pointed out that the presence of 1 μM copper in the medium induced monocytes to oxidize LDL without the need for prior activation and suggested that this might be one of the reasons for the difference in their results and those of Aviram and Rosenblat (15).

In the present studies, we used the same medium used by Aviram and Rosenblat (15) to allow a more direct comparison with their results. As shown in Fig. 6B, using DMEM containing 1 μM copper, preincubation in LPDS did cause a small (18%) but statistically marginally sig-

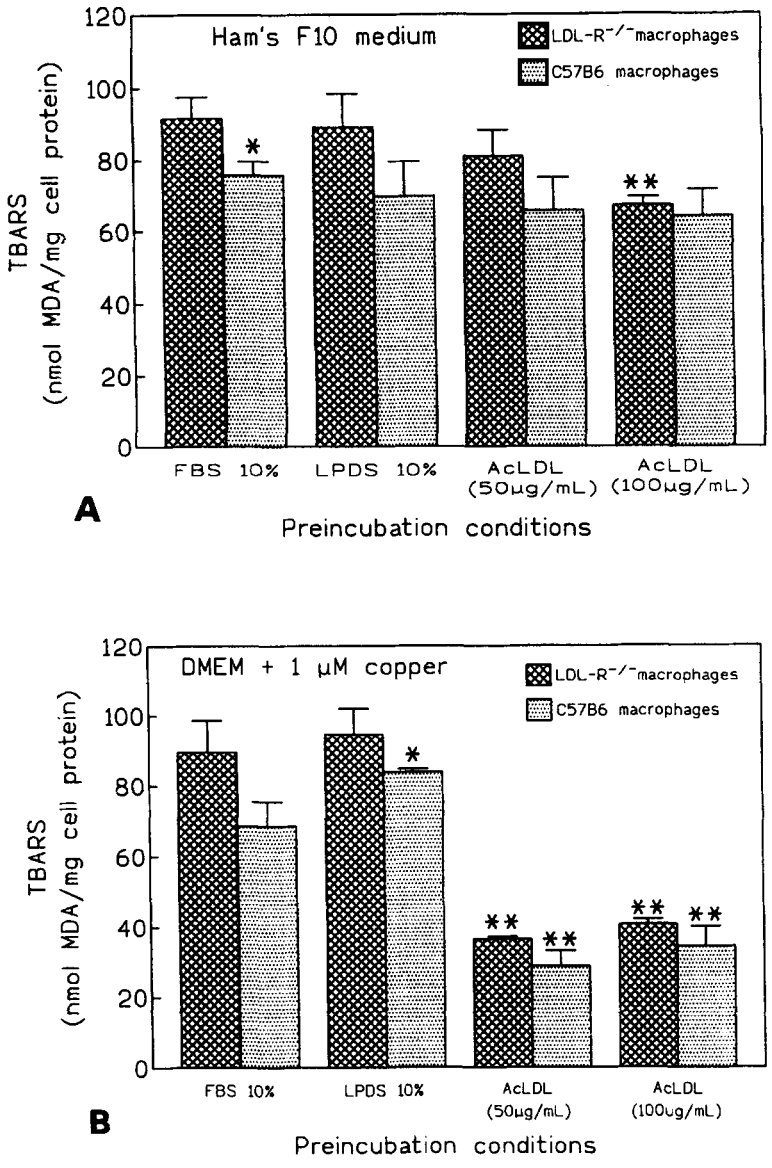

Fig. 6. Effect of preincubation of LDLR$^{-/-}$ and C57B6 macrophages under conditions affecting cellular cholesterol and LDL receptor activity on LDL oxidation. Resident LDLR$^{-/-}$ and C57B6 macrophages were incubated for 18 h in RPMI medium supplemented with 10% FBS, 10% LPDS, or Ac-LDL (50 and 100 µg Ac-LDL protein/mL). After the preincubation the cells were rinsed and further incubated with LDL (100 µg LDL protein/mL) in Ham's F10 medium (A) and in DMEM supplemented with 1 µM copper (B) for 18 h at 37°C. The samples were analyzed for LDL oxidation by measuring TBARS. The values for no-cell controls in Ham's F10 and DMEM supplemented with 1 µM copper were 19.0 and 10 nmol MDA/mg LDL protein, respectively, and subtracted from corresponding experimental values. The average cell protein for wells containing LDLR$^{-/-}$ and C57B6 macrophages in Ham's F10 were 29.3 and 34.4 µg, and in DMEM supplemented with 1 µM copper, 32.8 and 35.5 µg, respectively. Each bar represents mean ± SD (n = 3). A: *Significantly different ($P < 0.05$) from LDLR$^{-/-}$ (FBS 10%); ** significantly different ($P < 0.05$) from LDLR$^{-/-}$ (FBS 10%). B: *Significantly different ($P < 0.05$) from C57B6 (FBS 10%); **significantly different ($P < 0.05$) from FBS 10%.

nificant ($P < 0.05$) increase in the rate of LDL oxidation. An even greater effect was seen after preincubation of the cells with a high concentration of Ac-LDL (100 µg/mL). In this case, the inhibition of LDL oxidation was more than 50%, similar to the values reported by Aviram and Rosenblat (15). Previous studies have shown that the uptake of Ac-LDL does not occur by way of the native LDL receptors but by the scavenger receptor(s) (34, 35) and Ishii et al. (36) have shown that peritoneal macrophages from LDL receptor-deficient rabbits degrade Ac-LDL at the same rate as peritoneal macrophages from normal rabbits. The key point is that the magnitude of the inhibition of LDL oxidation was almost exactly the same in the cells that lack the LDL receptors. Thus, the phenomenon seen by Aviram and Rosenblat may relate directly or indirectly to changes in cell cholesterol content rather than to changes in LDL receptor activity per se.

It seems reasonable to suppose that binding of LDL to the plasma membrane might enhance the rate of its oxidation by, for example, facilitating the transfer of lipid peroxides from the cell to the LDL particle or by increasing the ambient concentration of free radicals released at the cell surface. Even if that is the case, it should be recalled that there is a great deal of nonspecific binding of LDL to the cell and the binding need not be of high affinity in order to speed up the rate of LDL oxidation. In other words, nonspecific binding may be ample and the increment due to specific binding to LDL receptors may represent a small increment. Another point that may be relevant here is made by analogy with the transfer of cholesterol from cells to HDL.

According to the work of Phillips, Rothblat and coworkers (37, 38), this process does not require postulating a tight binding to a cell receptor. They concluded that the movement of cholesterol molecules through the unstirred water layer at the surface of the cell to HDL in the ambient solution would be fast enough to account for the observed rates of cholesterol release. The fact that oxidized LDL is found in the atherosclerotic lesions of LDL-deficient animal models (19, 39) is consistent with our conclusion that the LDL receptor is not obligatory for LDL oxidation.

Although the difference was never large, the rate of LDL oxidation by $LDLR^{-/-}$ macrophages was consistently a little higher than that by the C57B6 macrophages. The experiments illustrated in Fig. 6B show that increasing the cell cholesterol content decreases the rate of LDL oxidation, in both C57B6 macrophages and $LDLR^{-/-}$ macrophages, and we have suggested that changes in cell cholesterol content in some fashion modulate rates of LDL oxidation. The reduction in oxidation of LDL by Ac-LDL-loaded cells may be due to inactivation of prooxidant enzymes (such as 15-lipoxygenase) and/or a decrease in the release of lipoperoxides due to increased plasma membrane cholesterol and decreased membrane fluidity. The $LDLR^{-/-}$ macrophages might be expected to have a somewhat lower cholesterol content than cells with intact LDL receptors and this might account for their somewhat greater ability to oxidize LDL. In summary, the data show that LDL receptor deficiency does not reduce macrophage oxidation of LDL. It should be noted that the different results obtained by Aviram and Rosenblat (15) may be due to differences in the experimental conditions, for example, use of J774 macrophages in most of their studies and elicited rather than resident peritoneal macrophages.

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