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Selective uptake and efflux of cholesteryl linoleate in LDL by macrophages expressing 12/15-lipoxygenase

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Abstract

Oxidation of low density lipoprotein (LDL) is a critical step for atherogenesis, and the role of the 12/15-lipoxygenase (12/15-LOX) as well as LDL receptor-related protein (LRP) expressed in macrophages in this process has been suggested. The oxygenation of cholesteryl linoleate in LDL by mouse macrophage-like J774A.1 cells overexpressing 12/15-LOX was inhibited by an anti-LRP antibody but not by an anti-LDL receptor antibody. When the cells were incubated with LDL double-labeled by [^{3}H]cholesteryl linoleate and [^{125}I]apoB, association with the cells of [^{3}H]cholesteryl linoleate expressed as LDL protein equivalent exceeded that of [^{125}I]apoB, indicating selective uptake of [^{3}H]cholesteryl linoleate from LDL to these cells. An anti-LRP antibody inhibited the selective uptake of [^{3}H]cholesteryl ester by 62% and 81% with the 12/15-LOX-expressing cells and macrophages, respectively. Furthermore, addition of LDL to the culture medium of the [^{3}H]cholesteryl linoleate-labeled 12/15-LOX-expressing cells increased the release of [^{3}H]cholesteryl linoleate to the medium in LDL concentration- and time-dependent manners. The transport of [^{3}H]cholesteryl linoleate from the cells to LDL was also inhibited by an anti-LRP antibody by 75%. These results strongly suggest that LRP contributes to the LDL oxidation by 12/15-LOX in macrophages by selective uptake and efflux of cholesteryl ester in the LDL particle.

Keywords: 12/15-Lipoxygenase; Arachidonic acid; Cholesteryl linoleate; LDL oxidation; Macrophages; LDL receptor-related protein

12/15-Lipoxygenase (12/15-LOX) is the enzyme introducing one molecule of oxygen into unsaturated fatty acids in regiospecific and stereospecific manners [1–5]. The enzyme consists of leukocyte-type 12-LOX expressed in rats, mice, cows, and pigs, and 15-LOX-1 in humans and rabbits, catalyzing the formation of 12*S*-hydroperoxy and 15*S*-hydroperoxy acids from arachidonic acid, respectively [5]. The two enzymes not only share 70–90% amino acid sequence homology but also many common enzymatic features [4,5]. In particular, both enzymes can directly oxygenate linoleate esterified to cholesterol in low density lipoprotein (LDL) particles [6,7]. Oxidative modification of LDL is one of the critical steps for the development of atherosclerosis [8,9]. Since the macrophages expressing high level of the 12/15-LOX are accumulating in atherogenic lesions [10,11] and capable of LDL oxidation [12], the enzyme has been extensively studied with regard to the contribution of the atherogenic process [13,14]. It has been proposed that 12/15-LOX initiates the LDL oxidation by oxygenating cholesteryl linoleate in the LDL particle, and subsequent radical chain reactions modify the LDL to its oxidized form which is recognized by scavenger receptors in

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macrophages which become foam cells [4,7,13–15]. Recent studies showing that LDL oxidation and development of atherosclerosis were diminished in 12/15-LOX knockout mice clearly demonstrated that the enzyme was required for LDL oxidation [16,17].

We have studied the mechanism of oxidation of extracellular LDL by intracellular 12/15-LOX, and have demonstrated that binding of LDL to a LDL receptor-related protein (LRP), a cell surface receptor expressed on macrophages, is required for the LDL oxidation [18]. The receptor mediates the translocation of 12/15-LOX from cytosol to plasma membrane in macrophages which is an essential step for LDL oxidation [19]. The results lead to the hypothesis that cholesteryl linoleate in LDL is oxidized by the 12/15-LOX in the plasma membrane of macrophages. In fact, it is reported that cholesteryl ester in LDL which binds to LRP is selectively transferred to the plasma membrane without endocytosis and degradation of LDL using mouse Y1 adrenocortical cells [20].

In the present study, we have demonstrated a role of LRP in the selective uptake of cholesteryl linoleate from the LDL particle using mouse macrophage-like J774A.1 cells overexpressing the 12/15-LOX and mouse peritoneal macrophages. The efflux of cholesteryl linoleate from the cells to the LDL particle, which is a novel function of LRP, is also suggested. We propose that oxygenation of cholesteryl linoleate in LDL by the 12/15-LOX takes place in the plasma membrane of macrophages, and LRP plays a critical role in this process.

Materials and methods

Materials. Lipoprotein-deficient serum (LPDS) was obtained from Sigma (St. Louis, MO), [1-^{14}C]arachidonic acid (2.1 GBq/mmol), and [1α,2α(*n*)-^{3}H]cholesteryl linoleate (1.1–2.2 TBq/mmol, 37 MBq/ml) from Amersham (Buckinghamshire, UK), 3,3′-dioctadecylindocarbocyanine-labeled LDL (DiI-LDL) from Molecular Probes (Junction City, OR), and IODO-GEN pre-coated iodination tubes and the micro BCA protein assay kit from Pierce (Rockford, IL). A rabbit polyclonal antibody against the LDL receptor was raised as described and purified to IgG using protein A–Sepharose [18]. An anti-LRP antibody [21] was a generous gift from Dr. J. Herz of University of Texas Southwestern Medical Center. The specificity of these antibodies is described [18,21]. Human LDL was prepared by sequential ultracentrifugation and dialyzed against phosphate-buffered saline at 4 °C for 24 h before each experiment as described previously [15,18]. A murine macrophage-like cell line J774A.1 was kindly provided by Dr. Y. Saeki of Shiga University of Medical Science. The cells did not express detectable 12/15-LOX enzyme activity [15]. An expression vector, pEF-BOS, having an elongation factor-1α promoter [22] was kindly provided by Dr. S. Nagata of Osaka University. J774A.1 cells transfected with the pEF-BOS vector carrying porcine leukocyte 12/15-LOX cDNA and mock-transfected cells were established as previously described [15]. Mouse peritoneal macrophages were collected from C57BL/6J mice as described previously [23], except that thioglycollate was not injected before harvesting the cells.

Labeling of LDL by [^{3}H]cholesteryl linoleate and ^{125}I. Cholesteryl ester transfer protein was partially purified from human plasma by a slight modification of the procedure of Morton and Zilversmit [24]. LDL (200 μg protein) was mixed with 7.4×10^5 Bq [^{3}H]cholesteryl linoleate, 2 nmol cholesteryl linoleate, 0.42 μmol of 5,5′-dithiobis (2-nitrobenzoic) acid, and the partially purified 10 μg of cholesteryl ester transfer protein with a specific activity of approximately 0.08 nmol/16 h/μg of protein as determined by transfer of [^{3}H]cholesteryl linoleate. The reaction mixture with the total volume of 300 μl in phosphate-buffered saline at pH 7.4 was incubated at 37 °C for 16 h in nitrogen atmosphere [25]. The [^{3}H]cholesteryl linoleate-labeled LDL was subsequently radioiodinated using 7.4×10^7 Bq of Na[^{125}I] by IODO-GEN according to the manufacturer's instructions. Free iodine was removed by gel filtration, followed by dialysis against phosphate-buffered saline at pH 7.4, and the double-labeled LDL was reisolated by ultracentrifugation. The specific activity of the double-labeled human LDL ranged from 52 to 173 dpm/ng of LDL protein for ^{125}I and from 30 to 105 dpm/ng of LDL protein for ^{3}H. Significant oxidation of the LDL during the labeling process was not observed [15].

Determination of selective uptake of LDL cholesteryl linoleate. 12/15-LOX-expressing cells were cultured in DMEM containing 10% LPDS in 24-well dishes at 37 °C for 48 h. Mouse resident peritoneal macrophages were plated in 24-well dishes in Medium 199 containing 10% LPDS followed by overnight incubation. An anti-LDL receptor antibody at 10 μg/ml or an anti-LRP antiserum at 10 μl/ml was added to the medium and the cells were further incubated at 37 °C for 2 h. The double-labeled LDL at 10 μg/ml was added, and the incubation was continued at 37 °C for 4 h. The washed cells were lysed with 300 μl of 0.1 M NaOH. The lysate was processed to determine ^{125}I radioactivity or organic solvent-extractable ^{3}H radioactivity [26]. The cellular protein from each well was measured using micro BCA protein assay kit according to the manufacturer's instructions with bovine serum albumin as a standard. The ^{125}I-protein association or extractable [^{3}H]cholesteryl linoleate association was expressed in nanogram of LDL protein equivalent per microgram of cellular protein [27]. Selective uptake of cholesteryl linoleate was calculated as the total [^{3}H]cholesteryl linoleate association subtracted by the ^{125}I-protein association [20,27,28]. Non-specific association was determined by the addition of 50-fold excess unlabeled LDL and subtracted from the total association to obtain the specific association. The culture medium was also harvested to determine the trichloroacetic acid-soluble ^{125}I radioactivity.

[^{3}H]Cholesteryl linoleate efflux from 12/15-LOX-expressing cells and macrophages. Cellular efflux of cholesteryl linoleate to LDL was determined by the method of Gu et al. [29] with a slight modification. 12/15-LOX-expressing cells were plated in 24-well dishes with DMEM containing 10% FBS and incubated at 37 °C for 12 h. After medium was removed, the cells were incubated with 3.7×10^4 Bq/ml [^{3}H]cholesteryl linoleate in 0.5 ml DMEM containing 10% LPDS for 48 h. The washed cells were then incubated with or without the LDL at 37 °C for 6 h, if not indicated, in the presence or absence of an anti-LDL receptor antibody at 10 μg/ml or an anti-LRP antiserum at 10 μl/ml. Efflux of cellular cholesteryl linoleate was determined by measuring radioactivity in the medium. The cells were dissolved in 400 μl of 0.1 M NaOH, and the radioactivity in an aliquot was determined. The percentage of [^{3}H]cholesteryl linoleate efflux (percent of total [^{3}H]cholesteryl linoleate released into the medium) was calculated by dividing the medium-derived radioactivity by the sum of medium-derived plus cell-derived radioactivity [29,30]. Mouse peritoneal macrophages were also used for the efflux experiment under the same conditions as that with 12/15-LOX-expressing cells, except that the M199 was used as the medium and the incubation time with [^{3}H]cholesteryl linoleate was reduced to 24 h.

HPLC analysis. [^{3}H]Cholesteryl linoleate-labeled LDL (50 μg/ml) was incubated at 37 °C for 12 h with 12/15-LOX-expressing cells in 60-mm dishes in the presence of 10 μl/ml of an anti-LRP serum or of 10 μg/ml of an anti-LDL receptor antibody. The lipids in the medium were extracted as described above and applied to reverse-phase HPLC. HPLC was carried out using a TSK ODS-120T column (Tosoh,

250 mm × 4.6 mm, 5 μm particle size) with a solvent system of acetonitrile/2-propanol (1:1, by vol.) at a flow rate of 1 ml/min. Fractions of 1 ml were collected for 30 min and radioactivity was measured by liquid scintillation counting. Straight-phase HPLC was performed using a NovaPak silica column (Waters, 3.9 mm × 150 mm, 4 μm particle size) with a solvent system of *n*-hexane/2-propanol/acetic acid (100:0.5:0.1, by vol.) at a flow rate of 1 ml/min. UV absorbance at 235 nm was continuously monitored.

Results

Oxygenation of cholesteryl linoleate in LDL by 12/15-LOX-expressing cells requires LRP

We previously demonstrated that 12/15-LOX in macrophages mediated the oxygenation of cholesteryl linoleate in human LDL using macrophage-like J774A.1 cells overexpressing the enzyme [15]. The major oxygenated product in LDL incubated with the 12/15-LOX-expressing cells was 13*S*-hydroxyoctadeca-9*Z*,11*E*-dienoic acid which was identified by the analysis of the alkaline-hydrolyzed lipids in the medium by reverse-phase HPLC. To examine whether the oxygenation of cholesteryl linoleate in LDL by the 12/15-LOX in macrophages requires LRP, we labeled LDL with [^{3}H]cholesteryl linoleate using cholesteryl ester transfer protein and incubated the LDL with the J774A.1 cells overexpressing the enzyme in the presence or the absence of receptor-blocking antibodies. The lipids extracted from the incubation medium were analyzed without alkaline hydrolysis by reverse-phase HPLC. As shown in Fig. 1, a major radioactive peak which cochromatographed with authentic cholesteryl hydroxylinoleate was detected in the LDL-containing medium incubated with 12/15-LOX-expressing J774A.1 cells (Fig. 1B). To confirm the structure of the compound, the peak was collected and the alkaline-hydrolyzed products were further separated by straight-phase HPLC. The major peak cochromatographed with authentic 13-hydroxyoctadeca-9*E*,11*Z*-dienoic acid, although a minor product which cochromatographed with 9-hydroxyoctadeca-10*Z*,12*E*-dienoic acid was also observed (data not shown). Mock cells which did not have detectable LOX activity [15] did not oxygenate cholesteryl linoleate in LDL (Fig. 1A). An anti-LRP antibody significantly inhibited the oxygenation of [^{3}H]cholesteryl linoleate in LDL by the 12/15-LOX-expressing cells (Fig. 1C), whereas an anti-LDL receptor antibody did not affect the oxygenation (Fig. 1D). The results indicate that the oxygenation of cholesteryl linoleate in LDL by 12/15-LOX-expressing cells requires LRP.

LRP contributes to selective uptake of cholesteryl ester in LDL to the plasma membrane

To investigate the detailed mechanism on the oxygenation of cholesteryl linoleate in the LDL particle by

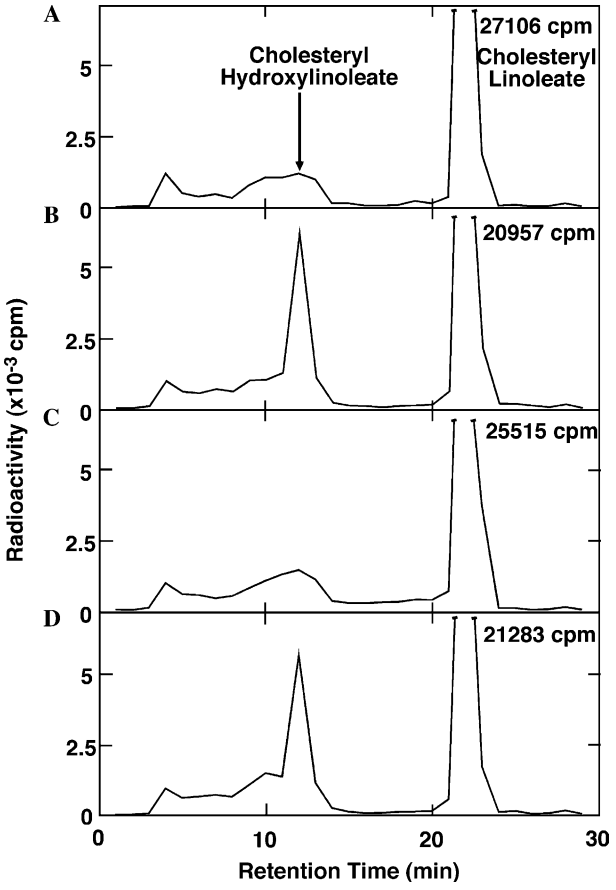

Fig. 1. HPLC analysis of lipids extracted from ^{3}H-labeled LDL incubated with 12/15-LOX-expressing cells. [^{3}H]Cholesteryl linoleate-labeled LDL (50 μg/ml) was incubated with mock cells (A) or 12/15-LOX-expressing cells (B–D) in the presence of an anti-LRP serum (C) or an anti-LDL receptor antibody (D). The lipids in the medium were extracted and separated by reverse-phase HPLC as described under Materials and methods. The radioactivity of ^{3}H in 1-ml fractions was determined for 30 min. The elution time of authentic cholesteryl hydroxylinoleate is shown by an arrow.

12/15-LOX-expressing cells, we incubated the DiI-labeled LDL with the cells. It has been demonstrated that incubation of DiI-labeled lipoprotein with the cells produces a punctate pattern distribution of fluorescence when the cells process the lipoprotein via receptor-mediated endocytosis in which lipoprotein is delivered to endosomes and lysosomes to be degraded [31,32]. On the other hand, the cells produce diffuse staining pattern of fluorescence over the entire cell surface when cholesteryl ester in the lipoprotein is selectively transferred into the plasma membrane without uptake or degradation of the lipoprotein particle [31,33]. After 2-h incubation of DiI-LDL with Chinese hamster ovary cells predominantly expressing the LDL receptor [31], the punctate pattern was observed under the fluorescent microscope as shown in Fig. 2A. In contrast, diffuse staining was observed when the DiI-LDL was incubated with mouse peritoneal macrophages expressing LRP but hardly expressing the LDL receptor (Fig. 2B) [34,35].

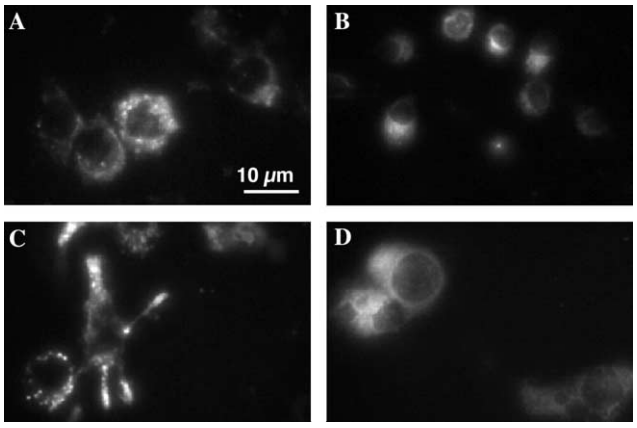

Fig. 2. Fluorescent pattern of Chinese hamster ovary cells, mouse peritoneal macrophages, and 12/15-LOX-expressing J774A.1 cells incubated with DiI-labeled LDL. The cells (10^5 per chamber) were incubated at 37 °C for 12 h in DMEM supplemented with 10% LPDS. DiI-LDL at 5 μg/ml was added into the culture medium of Chinese hamster ovary cells (A), mouse peritoneal macrophages (B), or 12/15-LOX-expressing J774A.1 cells in the presence of 10 μl/ml anti-LRP antiserum (C) or of 10 μg/ml anti-LDL receptor antibody (D). After 2-h incubation, cells were washed and observed under a fluorescent microscope. Bar = 10 μm.

J774A.1 cells express both LRP and LDL receptors [18,19], so we incubated the cells with DiI-LDL in the presence of an anti-LDL receptor antibody or an anti-LRP antibody which blocked the binding of LDL to each receptor [18]. The cells produced a punctate pattern in the presence of anti-LRP antibody (Fig. 2C), whereas the cells showed diffuse staining when the LDL receptor was blocked by the anti-LDL receptor antibody (Fig. 2D). The results strongly suggest that LRP on macrophages or the 12/15-LOX-expressing cells mediates selective transfer of the cholesteryl ester in the LDL particle to the plasma membrane.

To demonstrate selective uptake of cholesteryl ester in the LDL particle to the plasma membrane in a more direct way, the protein in the LDL particle containing [^{3}H]cholesteryl linoleate was labeled with ^{125}I. The double-labeled LDL was then incubated with 12/15-LOX-expressing cells or mouse peritoneal macrophages in the presence or absence of the blocking antibody for LRP or the LDL receptor. After being washed, the cells were lysed and processed to determine ^{125}I-protein association and organic solvent-extractable [^{3}H]cholesteryl linoleate association. To determine the amount of cholesteryl linoleate association due to selective uptake, protein association was subtracted from cholesteryl linoleate association, both in nanogram of LDL protein per microgram of cellular protein, and the value without antibodies was set at 100%. In mouse peritoneal macrophages which expressed LRP but hardly expressed the LDL receptor, the [^{3}H]cholesteryl linoleate association was 1.82 ± 0.01 ng LDL protein equivalent/μg of cellular protein. The amount exceeded the ^{125}I-LDL protein association which was 1.05 ± 0.02 ng LDL protein/μg of cellular protein by 1.7-fold (Figs. 3A and B). The

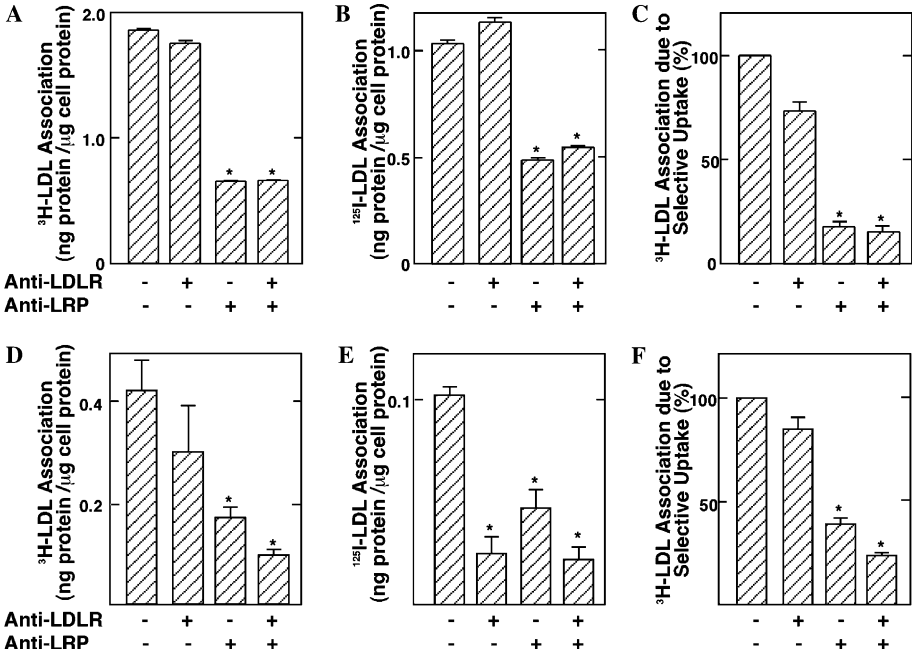

Fig. 3. Selective uptake of cholesteryl linoleate to membranes by LRP in macrophages (A–C) and the 12/15-LOX-expressing cells (D–F). [^{3}H]Cholesteryl linoleate association (A,D), ^{125}I-LDL protein association (B,E), and [^{3}H]cholesteryl linoleate association due to cholesteryl ester selective uptake (C,F) in the presence or absence of an anti-LDL receptor antibody or an anti-LRP antiserum are determined as described under Materials and methods. Non-specific protein association was measured in the presence of 50-fold excess unlabeled LDL and subtracted from total association for each condition. In (C,F), the control value without an antibody was set as 100%. Data are expressed as means ± SEM and asterisks show significant difference from the cells without antibody treatment by ANOVA ($P < 0.05$, $n = 3$). LDLR, LDL receptor.

[^{3}H]cholesteryl linoleate association due to selective uptake was inhibited by 81% by the anti-LRP antibody (Fig. 3C), whereas the anti-LDL receptor antibody did not show any significant effect on the association of either [^{3}H]cholesteryl linoleate or ^{125}I-LDL protein (Figs. 3A and B). The results agreed with the previous reports that macrophages hardly expressed the LDL receptor [34,35]. The 12/15-LOX-expressing J774A.1 cells had LRP as well as the LDL receptors [18,19]. We investigated the selective uptake of [^{3}H]cholesteryl linoleate by using the 12/15-LOX-expressing cells (Figs. 3D–F). Although the amount of [^{3}H]cholesteryl linoleate associated with the cells (0.43 ± 0.06 ng LDL protein equivalent/μg of cellular protein) was smaller than that associated with the macrophages, the amount exceeded the association of ^{125}I-LDL protein (0.10 ± 0.01 ng LDL protein/μg of cellular protein) by 4.3-fold (Figs. 3D and E). The [^{3}H]cholesteryl linoleate association due to selective uptake was inhibited by the anti-LRP antibody by 62%, whereas the anti-LDL receptor antibody did not significantly affect the selective uptake (Fig. 3F). These results taken together indicate that LRP is at least one of the receptors mediating the selective uptake of cholesteryl linoleate in LDL by macrophages and the 12/15-LOX-expressing cells. Trichloroacetic acid-soluble ^{125}I radioactivity in the double-labeled LDL-containing medium incubated with the cells in the presence of the anti-LDL receptor antibody was not significantly different from that in the absence of either antibody (data not shown), suggesting that the release of ^{125}I from endocytosed and degraded LDL via the LDL receptor was minimum in our experimental condition.

LRP contributes to efflux of cholesteryl ester from the cells to the LDL particle

To establish the role of LRP in the efflux of cholesteryl linoleate to the LDL particle by the 12/15-LOX-expressing cells and macrophages, we labeled the cells with [^{3}H]cholesteryl linoleate. The washed cells were then incubated with or without LDL. As shown in Fig. 4A, time-dependent efflux of [^{3}H]cholesteryl linoleate from the 12/15-LOX-expressing cells to the medium was observed only when the medium contained LDL and reached around 20% of the total cellular radioactivity at 6 h after addition of LDL. The maximum efflux was observed with LDL at 50 μg/ml, and the efflux decreased at higher or lower concentration of LDL (Fig. 4B). The efflux of [^{3}H]cholesteryl linoleate was inhibited by 75% in the presence of the anti-LRP antibody, whereas the anti-LDL receptor antibody did not significantly affect the efflux (Fig. 4C). Essentially the same results were obtained with mouse peritoneal macrophages (Fig. 4D). The results indicate that the efflux of cholesteryl linoleate from the cells to the LDL particle is mediated, at least in part, by LRP.

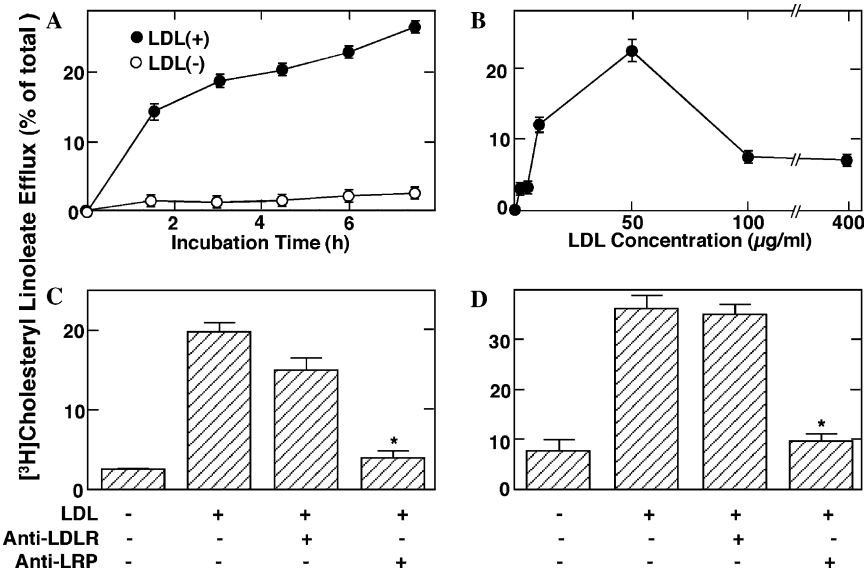

Fig. 4. LRP mediates cholesteryl linoleate efflux from the 12/15-LOX-expressing cells and macrophages. (A) Cells labeled with [^{3}H]cholesteryl linoleate were incubated with 50 μg/ml LDL (closed circles) or without LDL (open circles) for indicated time. ^{3}H released to the medium was measured and expressed as the percentage of the total ^{3}H as described under Materials and methods. Data are expressed as means ± SEM ($n = 3$). (B) Cells labeled with [^{3}H]cholesteryl linoleate were incubated with an indicated concentration of LDL for 6 h. Values are subtracted from those without LDL at each point and expressed as means ± SEM ($n = 3$). (C,D) Effects of antibodies on [^{3}H]cholesteryl linoleate efflux from the 12/15-LOX-expressing cells (C) and macrophages (D) are shown. The cells labeled with [^{3}H]cholesteryl linoleate were incubated with or without LDL for 6 h in the absence or presence of an anti-LDL receptor antibody (10 μg/ml) or an anti-LRP antiserum (10 μl/ml). ^{3}H efflux was expressed as described above. Data are expressed as means ± SEM and asterisks show significant difference from the LDL-treated cells without antibody by ANOVA ($P < 0.05$, $n = 3$). LDLR, LDL receptor.

Discussion

We have demonstrated that LRP plays a critical role in the process of oxygenation of cholesteryl linoleate in LDL by 12/15-LOX-expressing macrophage-like J774A.1 cells (Fig. 1). Stereospecific oxygenation of the linoleate moiety esterified to cholesterol by the 12/15-LOX-expressing cells was previously established [15]. The possibility of leakage or excretion of the enzyme from the cells was ruled out because no enzyme activity was detected in the culture medium [15]. Furthermore, the oxidized LDL generated by the 12/15-LOX-expressing cells was recognized by scavenger receptors [15]. These results support the requirement of the cell surface LDL-binding receptor for intracellular 12/15-LOX-mediated LDL oxidation by macrophages. We demonstrated that LRP was essentially involved in LDL oxidation by macrophages expressing high level of 12/15-LOX [18]. It was reported that the stereospecific oxygenation products of cholesteryl linoleate were detected in the human atherogenic plaque where macrophages were accumulating [36,37]. The results strongly suggest that 12/15-LOX in macrophages contributes to the oxygenation of cholesteryl linoleate in the LDL particle in vivo.

We investigated the detailed mechanism in the oxygenation of cholesteryl linoleate by the cells. LRP was required not only for the 12/15-LOX translocation from the cytosol to the plasma membrane [19] but also for the selective uptake of cholesteryl linoleate from the LDL particle to the membranes (Figs. 2 and 3). The results support the hypothesis that cholesteryl linoleate derived from the LDL particle is oxygenated by 12/15-LOX on the plasma membranes in macrophages as well as the 12/15-LOX-expressing cells. The selective uptake of cholesteryl ester in LDL by LRP was previously reported using mouse adrenocortical Y1 cells [20]. It was reported that the selective uptake was apoE-dependent using a clone of Y1 cells which was engineered to express apoE4 under the control of tetracycline-regulated promoter. However, the selective uptake remained without apoE expression at the level of 30–50% as compared with the condition expressing apoE [20,38]. LDL contains apoB as the predominant apoprotein component and the amount of apoE is minimal [39,40]. Furthermore, the LRP-dependent translocation of 12/15-LOX in the 12/15-LOX-expressing cells was observed not only in the incubation with LDL [19] but also with apoB (H. Zhu et al., unpublished observation). These results strongly suggest that LRP recognizes apoB when LDL binds to LRP on the cells [41]. The ligand bound to LRP might be processed either by selective uptake or receptor-mediated endocytosis depending on the moiety recognized by LRP [42,43].

The amount of [^{3}H]cholesteryl linoleate as well as ^{125}I-LDL protein associated with the macrophages was larger than that associated with the 12/15-LOX-expressing cells, suggesting that the total expression level of receptors which bound to the native LDL was higher in macrophages (Fig. 3). The selective uptake of [^{3}H]cholesteryl linoleate was not completely inhibited by the anti-LRP antibody, particularly in the 12/15-LOX-expressing cells, suggesting the expression of other receptors which processed LDL by a selective uptake pathway in addition to LRP. Actually, scavenger receptor class B type I (SR-BI) is reported as another receptor which selectively transfers cholesteryl linoleate from the LDL particle to the plasma membrane [27,38], and the receptor is expressed in 12/15-LOX-expressing cells as well as macrophages [18,30]. However, decrease in expression level of SR-BI by antisense oligodeoxyribonucleotides against the receptor did not affect the level of 12/15-LOX-mediated LDL oxidation by 12/15-LOX-expressing cells [18]. Furthermore, translocation of the enzyme in the 12/15-LOX-expressing cells was not mediated by SR-BI [19]. The results indicate that SR-BI, which may play a role in selective uptake of cholesteryl ester from LDL in macrophages and the 12/15-LOX-expressing cells, is not implicated in LDL oxidation by the cells.

We found that the efflux of radioactivity from the [^{3}H]cholesteryl linoleate-labeled cells to the LDL-containing medium was dependent on the amount of LDL added to the medium and incubation time (Figs. 4A and B). The results strongly suggest that LDL is an acceptor molecule of cholesteryl linoleate released from the cells as shown previously using high density lipoprotein and the cells expressing its receptor [29,30]. The efflux was inhibited by the anti-LRP antibody but not significantly by the anti-LDL receptor antibody (Figs. 4C and D), indicating that LRP is at least one of the receptors mediating the efflux of cholesteryl linoleate from the cells to the LDL particle. The reason why the efflux of [^{3}H]cholesteryl linoleate was decreased at higher concentration of the acceptor LDL is not clear. It may be possible that there is some equilibration of cholesteryl ester between LDL and the cells, and the rate of uptake might exceed that of efflux at higher concentration of LDL in the medium.

LRP is a multifunctional receptor playing diverse biological roles including functions in lipid metabolism, the homeostasis of proteinases and proteinase inhibitors, cellular entry of viruses and toxins, activation of lysosomal enzymes, cellular signal transduction, and neurotransmission [42]. The present report demonstrates the multiple roles of LRP in the process of oxygenation of cholesteryl linoleate in the LDL particle by the 12/15-LOX-expressing cells and macrophages. Further investigations are necessary to elucidate the signals and molecular mechanism of LRP-mediated 12/15-LOX translocation, selective uptake of cholesteryl linoleate in LDL, and efflux of the cholesteryl linoleate to the LDL particle.

Acknowledgments

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