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The LDL receptor is the major pathway for β-VLDL uptake by mouse peritoneal macrophages

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Abstract

In order to determine the contribution of the low density lipoprotein receptor (LDL-R) to the removal of apoB-containing native lipoproteins by macrophages, we compared the uptake of β-VLDL in peritoneal macrophages (MPM) from wild type mice and mice lacking the LDL-R. The $d < 1.006$ g/ml lipoproteins obtained from apoE deficient mice fed a high fat diet were poorly degraded by macrophages and caused only a slight formation of CE in macrophages from both types of mice. On the other hand, $d < 1.006$ g/ml lipoproteins obtained from LDL-R deficient mice fed a high fat diet, β-VLDL with apoE, were avidly taken up by and markedly stimulated CE formation in wild type macrophages, but not in macrophages lacking the LDL-R. The degradation of ^{125}I-labeled-apoE-containing β-VLDL by wild type MPM was poorly inhibited by unlabeled human LDL, and β-VLDL without apoE had no effects. In conclusion, we propose that the in vitro uptake of native apoE-enriched lipoproteins by murine macrophages is primarily mediated by the LDL receptor and not by other apoE-recognizing receptor systems such as: the LDL receptor related protein, the VLDL receptor or the triglyceride-rich lipoprotein receptor.

Keywords: Foam cell formation; Knock-out mice; Receptor

1. Introduction

An early event in the development of an atherosclerotic lesion is the intimal accumulation of lipid-laden foam cells which originate from either monocyte-macrophages or vascular smooth muscle cells [1]. It is believed that foam cells are generated by cellular uptake of lipoproteins through pathways independent of the low density lipoprotein receptor (LDL-R), because massive accumulation of foam cells is found even in atherosclerotic lesions of patients who completely lack the LDL-R, i.e. homozygous familial hypercholesterolemia (see Refs. [2,-4] for review). Furthermore, the LDL-R is barely detectable in lesions from patients

Abbreviations: apo, apolipoprotein; BSA, Bovine Serum Albumin; CE, cholesterol ester; CHO, Chinese Hamster Ovary; DMEM, Dulbeco modified Eagle's medium; EDTA, Ethylenediaminetetra-acetic; FCS, fetal calf serum; FH, familial hypercholesterolemia; LDL, low density lipoproteins; LDL-R(−/−), LDL-R deficient; LPDS, lipoprotein deficient serum; LPL, lipoprotein lipase; LRP, low density lipoprotein receptor-related protein; MPM, mouse peritoneal macrophages; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate buffered saline; PMSF, phenylmethylsulphonylfluoride; SDS, Sodium Dodecyl Sulphate; TGRLP, triglyceride-rich lipoproteins; TLC, thin layer chromatography; WHHL, Watanabe Heritable Hyperlipidemic; VLDL, very low density lipoprotein; β-VLDL, β-migrating very low density lipoprotein; β-VLDL-E(+), *d* less than 1.006 g/ml lipoproteins obtained from the LDL receptor knockout mice fed an atherogenic diets; β-VLDL-E(+), *d* less than 1.006 g/ml lipoproteins obtained from apolipoprotein E knockout mice fed an atherogenic diets.

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with the LDL-R [5,6]. In support of this notion, numerous receptors, which recognize and take up denatured lipoproteins have been identified in macrophages. These include scavenger receptor A [7,8], CD36 [9], Fc receptor [10], macrosialin [11] and others [12]. Furthermore, modified lipoproteins have been demonstrated in atherosclerotic lesions [13,14].

In the development of atherosclerosis, lipoprotein retention precedes lipoprotein denaturation [15]. Therefore, the mechanisms by which lipoproteins deposited in the extracellular milieu are eliminated before they are denatured are no less important than are those of foam cell formation. Receptor systems, which mediate the uptake of native lipoproteins, account for the elimination by surrounding cells of native lipoproteins, which are retended in the extracellular matrix. The LDL-R is expressed in macrophages and mediates the uptake of LDL, β-VLDL and apoE-rich β-migrating VLDL [16–19] The LRP is widely expressed in various tissues including macrophages [20–22] and potentially mediates the uptake of lipoproteins that are rich in apoE or LPL [23–25]. Finally, the VLDL receptor (LR8) also binds to β-VLDL [26,27]. In addition to these receptors belonging to the LDL receptor gene family, other receptors such as lipolysis-stimulated receptor (LSR) [28] and triglyceride-rich lipoprotein receptor (TGRLP receptor) [29,30] have been postulated to be involved in the uptake of triglyceride-rich lipoproteins, although their molecular identity is yet to be determined. Among these receptors for native lipoproteins, LRP [3,31] and the VLDL receptor [32], but not the LDL-R [3,4], have been demonstrated to be present in atherosclerotic lesions, implicating their involvement in atherosclerosis.

β-VLDL is an atherogenic lipoprotein that is found in the plasma of animals, which are fed a high cholesterol diet. In vitro, β-VLDL has been shown to cause the conversion of macrophages into foam cells [16]. Murine models of both LDL-R [33] and apoE deficiency [34,35] have been created by targeted disruption of these genes in embryonic stem cells, providing valuable tools for the detailed analysis of the pathways involved in the uptake of native lipoproteins [36,37]. In the current study, we made use of these animals to re-examine the roles of the LDL-R and apoE in cellular cholesterol homeostasis in mouse peritoneal macrophages (MPM) from wild-type and LDL-R knockout mice.

2. Materials and methods

2.1. cDNA

A plasmid containing mouse LDL-R cDNA [33] was digested with *Eco*RI, religated and used as a template to synthesize an antisense riboprobe spanning from nt233 to nt801 of the cDNA sequence. Polyclonal antibody against the human LDL-R was a generous gift from Drs Ho, Goldstein and Brown.

2.2. Animals

Generation of the mutated mice lacking the LDL-R [33] and apoE [34] was described in the indicated references. These animals were allowed access to food and water ad libitum. Two diets were used: (i) a normal chow (MF from Oriental Yeast, Tokyo); and (ii) an atherogenic diet which contained 1.25% cholesterol 7.5% cocoa butter, 7.5% casein, and 0.5% cholic acid (Oriental Yeast, Tokyo). The wild-type mice had a genetic background similar to that of the other two mutant mice; hybrids of C57BL6 and 129Sv.

2.3. RNase protection assay

The ^{33}P-labeled riboprobes for the LDL-R and β-actin were synthesized from linearized plasmid templates by reverse transcriptase using MAXI scripts™ (Ambion, Austin, TX). The specific activity of the β-actin riboprobe was one tenth that of the LDL receptor riboprobe. An RNase protection assay was performed using RPAII™ (Ambion, Austin, TX) according to the manufacturer's instruction.

2.4. Macrophage collection

One ml of 5% thioglycollate broth was injected into the peritoneal cavities of mice aged between 2 and 6 months. After 4 days, the peritoneal cavities were lavaged with 10 ml of ice-cold saline. The cells were washed three times with PBS and resuspended in DMEM to give a concentration of 10^6 cells/ml. A total of 1 ml/well and 10 ml/dish were used for plating in 12 well-plates and in 55 mm^2 dishes (Corning), respectively. After incubation at 37°C for 2 h, the non-adherent cells were removed by washing three times with pre-warmed PBS and the adherent cells were incubated with DMEM containing 5 mg/ml of LPDS for 36 h at 37°C in an atmosphere of 5% CO_2 and 95% air unless otherwise stated.

2.5. Immunoblot analysis

MPM from five mice were scraped in 1 ml of ice-cold Buffer A containing 20 mM Tris–HCl, pH 8.0, 1 mM $CaCl_2$, 150 mM NaCl and a cocktail of protease inhibitors (1 mM PMSF, 1 mM *o*-phenanthroline, 1 mM leupeptin, 5 μg/ml aprotinin and 1 μg/ml pepstatin) and homogenized in a Potter–Elvehjem homogenizer. The homogenate was centrifuged at $2000 \times g$ for 10 min and the resulting supernatant was centrifuged at $100\,000 \times g$ for 60 min. Pellets were solubilized in an ice-cold buffer

B containing 250 mM Tris–maleate pH 6.0, 2 mM $CaCl_2$ and 1% (v/v) Triton X-100 supplemented with the mixture of protease inhibitor. Resuspended pellets were centrifuged at 100 000 × *g* for 60 min. The supernatant, the Triton X-100 soluble fraction, was stored at −70°C for up to 2 weeks. Thirty micrograms of protein was subjected to 7% SDS-polyacrylamide gel electrophoresis. After transfer to a nitrocellulose membrane (Hybond ECL™, Amersham), the LDL receptor was detected with the polyclonal antibody using the ECL Western blotting system (Amersham) according to the manufacturer's procedure.

2.6. Lipoproteins

Mice were fed an atherogenic diet for 2 weeks, and blood was collected from the retro-orbital venous plexus into tubes containing EDTA after an overnight fast. The $d < 1.006$ g/ml lipoproteins were isolated by ultracentrifugation at a density of 1.006 g/ml for 16 h. β-VLDL containing apoE (β-VLDL-E(+)) and β-VLDL without apoE (β-VLDL-E(−)) were prepared from the LDL-R deficient mice and apoE knock-out mice, respectively. Blood was drawn from healthy volunteers and LDL ($d = 1.019$–1.063 g/ml) and LPDS ($d > 1.21$ g/ml) were prepared by step-wise ultracentrifugation as described [38]. After re-centrifugation, the lipoprotein and LPDS solutions were dialyzed against 2 l of 2 mM sodium phosphate, pH 7.4, 150 mM NaCl, 0.01% EDTA and 0.01% NaN_3. After dialysis against saline, protein concentrations were determined using a BCA™ protein assay reagent kit (Pierce, IL), Total cholesterol, free cholesterol, triglycerides and phospholipids were determined using the determiner TC-555, determiner L FC, determiner TG-S555 and determiner L PL (Kyowa Medex, Tokyo, Japan), respectively. Lipoproteins were radioiodinated by the iodine monochloride method as described [39]. LDL were acetylated as described [40]. These lipoproteins were used within 1 week after preparation.

2.7. Uptake and degradation of the radioiodinated lipoproteins

The cells were incubated with a medium containing radioiodinated lipoprotein with or without unlabeled lipoproteins and 5 mg/ml of BSA for 5 h. The medium was removed and the radioiodinated lipoproteins degraded by the cells were measured according to Basu et al. [40].

2.8. Cholesterol ester formation assay

Cholesterol ester (CE) formation from [1-^{14}C]oleate was determined essentially as described [40]. Briefly, the cells were incubated in a medium containing lipoproteins, the [1-^{14}C]oleate-albumin complex and 5 mg/ml of BSA at 37°C for 24 h. Cells were washed twice with PBS containing 2 mg/ml BSA and once with PBS.

CE was extracted with hexane-isopropanol (3:2) to which [^{3}H]cholesteryl-oleate and unlabeled oleic acid were added as the internal standard and carrier, respectively. The organic phase was evaporated to dryness under flowing N_2. Cholesterol [1-^{14}C]oleate was separated on silica coated TLC plates (#5583, Merck, FRG) using a solvent system composed of heptane/ethyl ether/acetic acid (90:30:1; v/v/v), their position was identified using a BAS2000 phosphoimager (Fuji Film, Tokyo, Japan). The spots corresponding to CE were scraped and their radioactivities were quantified by liquid scintillation. Cellular protein was dissolved in 0.1 N NaOH and its concentration was determined by the Lowry method [41].

2.9. Cellular cholesterol mass measurement

Lipids were extracted from cultured monolayers of MPM by hexane-isopropanol. TC and FC were determined by fluorometric microassay according to a modified method of Heider and Boyett [42]. Cellular protein was dissolved in 0.1 N NaOH and its concentration was determined by the Lowry method [41].

2.10. Statistics

A two-tailed Student's *t*-test was used to assess the significance of difference between two groups.

3. Results

3.1. Lipoproteins

The results of the SDS/PAGE analysis of the apolipoprotein composition of the lipoproteins used for the experiments are shown in Fig. 1(A). ApoB-100, apoB-48 and apoE were the major apolipoprotein constituents of the β-VLDL-E(+) and the apoB-48 and apoB-100 concentrations were similar. In contrast, the β-VLDL-E(−) contained apoB-48, apoA-IV and apoA-I, but lacked apoE. Upon agarose gel electrophoresis (Fig. 1(B)), β-VLDL-E(+) had β-mobility, while β-VLDL-E(−) migrated faster and had mobility between the β and pre-β positions, suggesting that β-VLDL-E(−) was charged more negatively than β-VLDL-E(+). The ratios of free cholesterol, cholesterol ester and phospholipids to the proteins of β-VLDL-E(+) were similar to those of β-VLDL-E(−) (Table 1). The ratio of triglycerides were higher in β-VLDL-E(+) however this difference was not statistically significant ($P = 0.064$).

3.2. Expression of the LDL-R

RNase protection assays were performed to confirm the expression of the LDL-R mRNA (Fig. 2). After treatment with LPDS for 36 h, a band corresponding to the LDL-R was observed in the wild type MPM (614 bp). The mRNA for the disrupted LDL-R was detected in the LDL-R(−/−) MPM (401 bp) and was markedly up-regulated (between 50- and 100-fold) compared to the band for the wild-type mRNA.

Immunoblot analysis was performed to estimate the LDL-R protein expression in the wild type MPM (Fig. 3). Without treatment with LPDS, the LDL receptor protein was not detectable. After treatment with LPDS for 36 h, LDL receptor expression was substantially induced.

3.3. Degradation of ^{125}I-β-VLDL-E(+) and ^{125}I-β-VLDL-E(−) by MPM

The cellular degradation of ^{125}I-β-VLDL-E(+) and ^{125}I-β-VLDL-E(−) were compared in MPM obtained from the LDL-R(−/−) and wild type mice (Fig. 4). The MPM possessing functional LDL-R degraded substantial amounts of ^{125}I-β-VLDL-E(+). In contrast, the degradation of ^{125}I-β-VLDL-E(+) in LDL-R(−/−) MPM was less than 10% of that in wild type MPM.

In wild type MPM, ^{125}I-β-VLDL-E(−) were significantly less degraded than ^{125}I-β-VLDL-E(+) and the level was comparable to that of ^{125}I-β-VLDL-E(+) degraded by LDL-R(−/−)MPM. There was no difference in the degradation of ^{125}I-β-VLDL-E(−) between the two types of cells. These results indicate that both the LDL-R on the cells and apoE on the lipo-

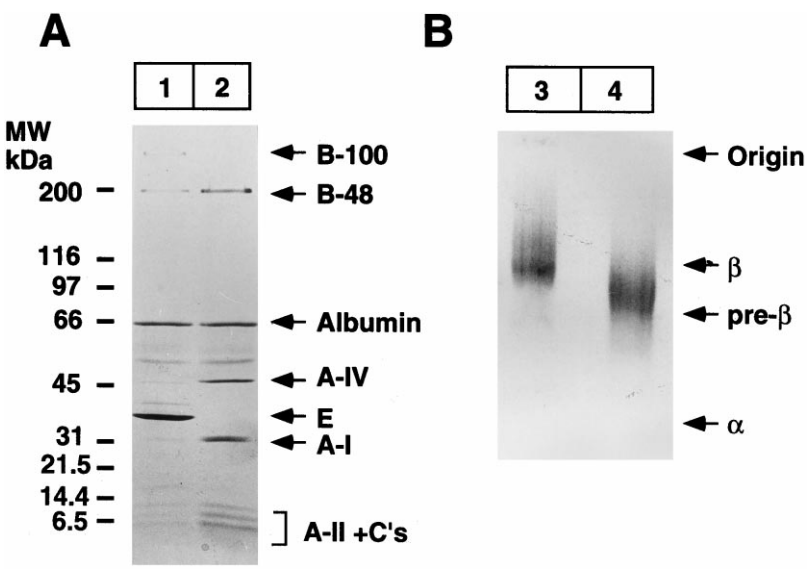

Fig. 1. Biochemical characteristics of β-VLDL-E(+) and β-VLDL-E(−). β-VLDL-E(+) and β-VLDL-E(−) were isolated by ultracentrifugation at a density of 1.006 g/ml from plasma obtained from LDL-R deficient and apoE deficient mice which had been fed an atherogenic diet for 2 weeks. Twenty-five micrograms of the lipoproteins were delipidated with ethanol/ether and subjected to 5–15% gradient polyacrylamide SDS gel electrophoresis (A). Proteins were visualized with Coomassie Brilliant Blue R250. Rainbow marker (Amersham) was used to calibrate the molecular weight. Lane 1, β-VLDL-E(+); lane 2, β-VLDL-E(−). One microliter of the concentrated $d < 1.006$ g/ml lipoproteins were subjected to agarose gel electrophoresis (B). Lipids were visualized with Fat Red 7B. The migratory positions are indicated. Lane 3, β-VLDL-E(+); lane 4, β-VLDL-E(−).

Table 1
Lipid composition of β-VLDL used in the cell studies[a]

Lipoprotein	Lipid/protein ratio				
	TC	FC	CE	TG	PL
β-VLDL-E(+)	3.11 ± 0.73	0.67 ± 0.11	2.43 ± 0.63	1.33 ± 0.73	1.01 ± 0.19
β-VLDL-E(−)	3.98 ± 1.14	0.81 ± 0.21	3.17 ± 0.95	0.24 ± 0.01	0.95 ± 0.27

[a] The $d < 1.006$ g/ml lipoproteins were isolated by ultracentrifugation at a density of 1.006 g/ml for 16 h. β-VLDL ($d < 1.006$ g/ml) containing apoE [β-VLDL-E(+)] and β-VLDL without apoE [β-VLDL-E(−)] were prepared from five LDL-R deficient mice and five apoE knock-out mice, respectively. The protein, total cholesterol (TC), free cholesterol (FC), triglyceride (TG) and phospholipid (PL) concentrations were determined as described in Section 2. The cholesterol ester values were obtained by subtracting free from total cholesterol values. Results represent the mean in mg lipid/mg protein ratio ± S.D. of five separate samples.

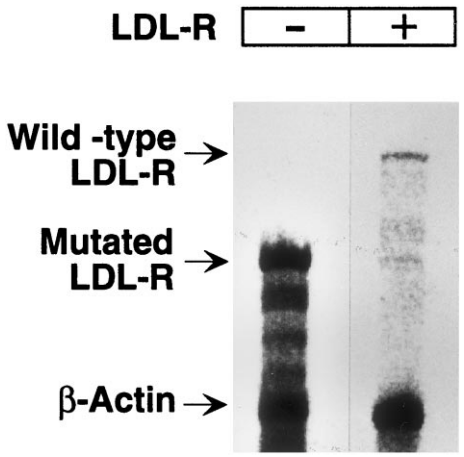

Fig. 2. RNase protection assay for the LDL receptor mRNA. MPM were incubated with DMEM containing 5 mg/ml of LPDS. Ten micrograms of total RNA was used for RNase protection assay for mouse LDL receptor and β-actin. Seven percent polyacrylamide gel was used for separation of the protected probes. The sizes of the protected RNA are 614, 401 and 245 bp, for the wild-type, mutated LDL receptor and β-actin, respectively.

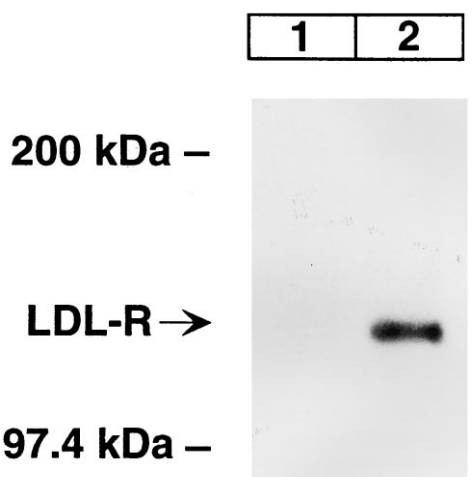

Fig. 3. Western blot analysis for LDL receptor protein. MPM were treated with DMEM containing either 10% (v/v) FCS (1) 5 mg/ml of LPDS (2) or for 36 h. The membrane fractions were prepared and 30 μg were subjected to 7% polyacrylamide SDS gel electrophoresis. Immunoblot analysis was performed as described in Section 2. Rainbow marker (Amersham) was used to calibrate molecular weight.

proteins are required for the specific uptake and degradation of $d < 1.006$ lipoproteins. The amounts of ^{125}I-LDL degraded by wild type MPM was two-fold larger than that of ^{125}I-β-VLDL-E(−) by the same cells (data not shown). Yet, the LDL-R(−/−) MPM did not degrade any human ^{125}I-LDL above background levels and this degradation could not be displaced by excess unlabeled LDL (data not shown).

Competition assays were performed to determine the specificity of the degradation of the lipoproteins (Fig. 5). The degradation of ^{125}I-β-VLDL-E(+) by wild type MPM was almost completely inhibited by excess amounts of unlabeled β-VLDL-E(+), while even 125-fold excess of β-VLDL-E(−) had no inhibitory effects on its degradation (Fig. 5(A)). These results indicate that the degradation process for β-VLDL-E(+) primarily depends upon the presence of apoE. As expected, 125-fold excess of human LDL reduced the degradation by 50%. This confirms that human LDL have lower affinity for receptors expressed by MPM than β-VLDL-E(+) [17]. On the other hand, the degradation of ^{125}I-β-VLDL-E(−) by wild type MPM, which was substantially lower than that of ^{125}I-β-VLDL-E(+), was displaced by both unlabeled β-VLDL-E(−) and β-VLDL-E(+) to a similar extent, but not by LDL, suggesting that the degradation process for β-VLDL-E(−) involves a molecule(s) common to both β-VLDL-E(−) and β-VLDL-E(+) (Fig. 5(B)).

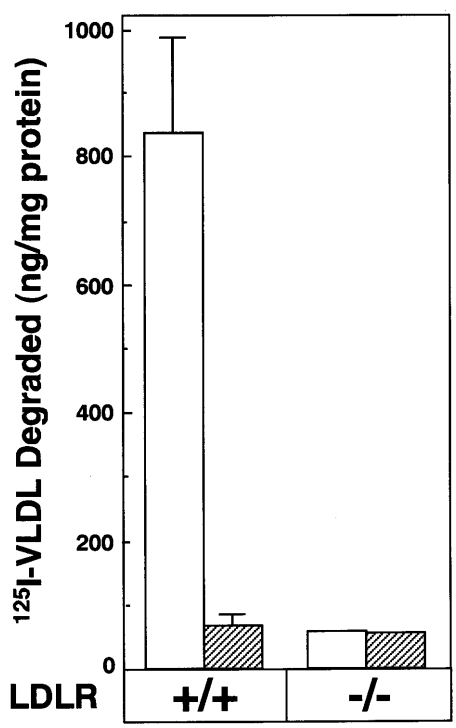

Fig. 4. Cellular degradation of ^{125}I-β-VLDL-E(+) and ^{125}I-β-VLDL-E(−) in MPM. MPM were prepared from mice as described in Section 2. After incubation with DMEM containing 10% (v/v) FCS for 36 h, the cells were incubated with DMEM containing 5 mg/ml of LPDS for 36 h. A total of 2 μg/ml of ^{125}I-β-VLDL-E(+) (open bar) and ^{125}I-β-VLDL-E(−) (hatched bar) were incubated with and without 100 μg/ml of unlabeled lipoproteins for 5 h. Data are the mean ± S.D. of four wells for the wild type MPM, and the mean of two wells for the LDL-R(−/−) MPM. The total degradation by the wild type macrophages was inhibited by 85% with an excess amounts of unlabeled β-VLDL-E(+) (data not shown), indicating that a specific endocytic pathway is involved in the uptake and degradation of the ^{125}I-β-VLDL-E(+).

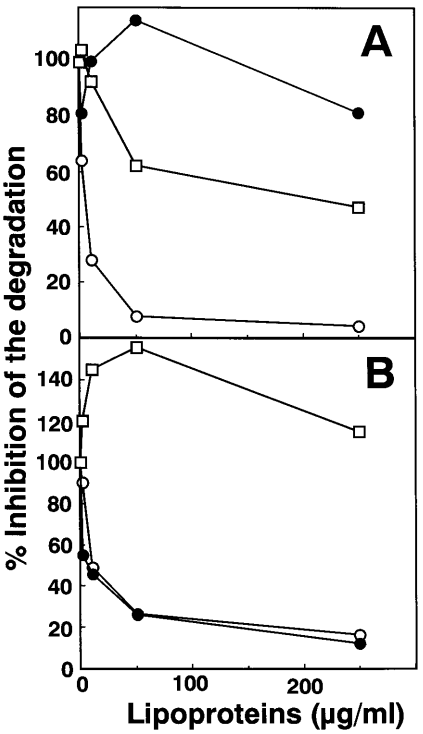

Fig. 5. Inhibition of the degradation of ^{125}I-β-VLDL-E(+) (A) and ^{125}I-β-VLDL-E(−) (B) by unlabeled β-VLDL-E(+), β-VLDL-E(−) and LDL in MPM. A total of 2 μg/ml of ^{125}I-β-VLDL-E(+) (A) and ^{125}I-β-VLDL-E(−) (B) were incubated without and with 2, 10, 50, 250 μg/ml of unlabeled β-VLDL-E(+) (open circle), β-VLDL-E(−) (closed circle) and LDL (square) for 5 h. Data are presented as the means of duplicate wells. The amounts of ^{125}I-β-VLDL-E(+) and ^{125}I-β-VLDL-E(−) degraded without excess unlabeled lipoproteins were 351 and 64 ng/mg, respectively.

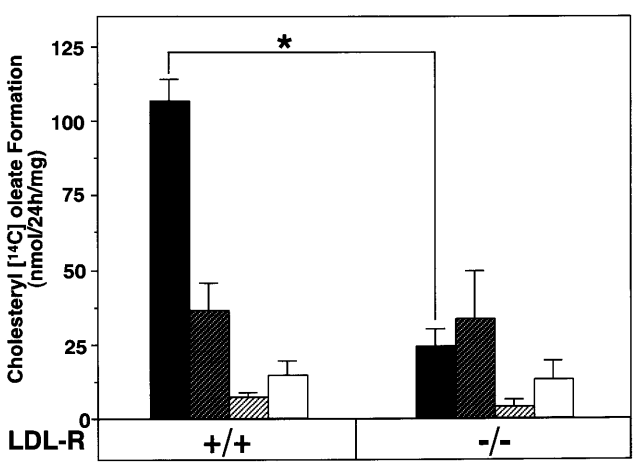

Fig. 6. Stimulation of cholesterol ester formation by β-VLDL-E(+), β-VLDL-E(−), LDL or acetyl LDL. MPM were harvested from wild type and LDL-R deficient mice. After incubation with DMEM containing 5 mg/ml LPDS for 36 h, the cells were incubated with 10 μg/ml of β-VLDL-E(+) (solid), β-VLDL-E(−) (black hatched), LDL (white hatched), acetyl LDL (white) and [1-^{14}C]oleate-albumin complex for 24 h. The values are presented as the means ± S.D. of four wells. *$P < 0.001$.

3.4. Cholesterol ester formation in MPM stimulated by lipoproteins

CE formation stimulated by LDL, acetyl LDL, β-VLDL-E(+) or β-VLDL-E(−) was compared between wild type and LDL-R(−/−) MPM (Fig. 6). β-VLDL-E(+) substantially stimulated CE formation in wild type MPM, but not in LDL-R(−/−) MPM. In LDL-R(−/−) MPM, however, there was no difference in CE formation between β-VLDL-E(+) and β-VLDL-E(−). Similarly, β-VLDL-E(−) induced comparable CE formation in both types of macrophages. Although β-VLDL, irrespectively of the presence of apoE, caused CE accumulation to occur in a dose-dependent saturable manner in wild type macrophages, its effects on CE accumulation in the LDL-R(−/−) MPM was linear (data not shown). Compared to β-VLDL-E(+), acetyl LDL and native LDL induced smaller CE formation.

3.5. Cellular cholesterol mass in MPM stimulated by lipoproteins

Wild type and LDL-R(−/−) macrophages were incubated with either β-VLDL-E(+) or β-VLDL-E(−) and CE mass was measured (Fig. 7). While β-VLDL-E(+) remarkably increased the CE mass in wild type MPM, it had only a small effect in LDL-R(−/−) MPM. β-VLDL-E(−) induced poor accumulation of CE mass irrespective of the expression of the LDL-R by the macrophages. Free cholesterol mass was increased by the addition of β-VLDL independently of the MPM phenotype or the origin of the lipoprotein.

4. Discussion

It has been shown that β-VLDL-E(+) strongly stimulates CE accumulation in macrophages including MPM [16]. By using blocking antibody against the LDL-R, Koo et al. [17] and Ellsworth et al. [18] have shown that the uptake of β-VLDL-E(+) by MPM is largely mediated by the LDL-R. However, other receptors (i.e. LSR [28] and TGRLP receptor [29,30]) have been postulated to be involved in the uptake of triglyceride-rich lipoproteins, although their molecular identity is yet to be determined. The use of MPM obtained from mice whose LDL-R is genetically ablated, is ideal to address the question of whether the LDL-R pathway is involved in the uptake of β-VLDL-E(+). Comparison of the degradation of ^{125}I-β-VLDL-E(+) between wild type and LDL-R(−/−) MPM clearly indicates that the LDL-R pathway accounts for as much as 80–90% of the degradation of β-VLDL-E(+) (Fig. 4).

Previously, Whitman et al. [43] described that β-VLDL prepared from apoE deficient mice stimulates

CE accumulation only slightly in J774A.1 cells, which do not express endogenous apoE. This confirmed the results of other studies using human lipoprotein in which apoE amount or ability to bind to receptors play an important role in the CE accumulation in macrophages [44–47]. In line with this, our current results showed that the presence of apoE on lipoproteins is absolutely required for LDL-R-mediated degradation of ^{125}I-β-VLDL (Fig. 4). Furthermore, β-VLDL-E(+), but not β-VLDL-E(−), completely inhibited the degradation of ^{125}I-β-VLDL-E(+), and human LDL had intermediate effects (Fig. 5). These results implicate that the other receptor pathways, which recognize apoE, such as the LRP and VLDL receptors, play negligible roles in the uptake of apoE-rich lipoproteins by MPM.

Lipoprotein lipase is secreted by MPM [48] and potentially may mediate the direct uptake of lipoproteins by bridging the lipoprotein to the receptor [49]. However our current results indicate that the contribution of the bridge function [50] of LPL is minimal in terms of overall uptake of the apoE-rich lipoproteins compared with the LDL-R-dependent pathway.

Similar studies addressing the role of the LDL-R have been performed with cells from other species such as humans and rabbits with natural mutations in the LDL-R. Koo et al. [19] used monocyte-derived macrophages from homozygous FH individuals and concluded that the LDL-R accounts for the uptake of β-VLDL-E(+) in these cells. Daugherty and Rateri [51] used alveolar macrophages from WHHL rabbits, an animal model of FH, however the LDL-R in WHHL rabbits retains binding affinity to β-VLDL-E(+) [52] and this limits the interpretation of the results.

Although the degradation of β-VLDL-E(−) is lower than that of β-VLDL-E(+) in wild type MPM, the mechanism involved remains an interesting question. Zsigmond et al. [53] reported that serum VLDL in apoE(−/−) mice fed a chow diet are resistant to lipolysis by LPL, and this may affect their uptake by macrophages. However, the poor induction of cholesterol ester formation by β-VLDL-E(−) compared to β-VLDL-E(+) may not be explained by increased triglyceride content since in our experimental condition where mice were fed a high fat diet, triglycerides were not higher in β-VLDL-E(−). Another potential explanation for the degradation of the β-VLDL-E(−) is that radiolabeled apolipoprotein may be selectively taken up and therefore the degradation data do not reflect lipoprotein particle endocytosis. However β-VLDL-E(−) at 50 μg/ml induced a significant increase in CE mass independently of the LDL-R expression by the MPM (Fig. 7). Therefore, we assume that degradation reflects,

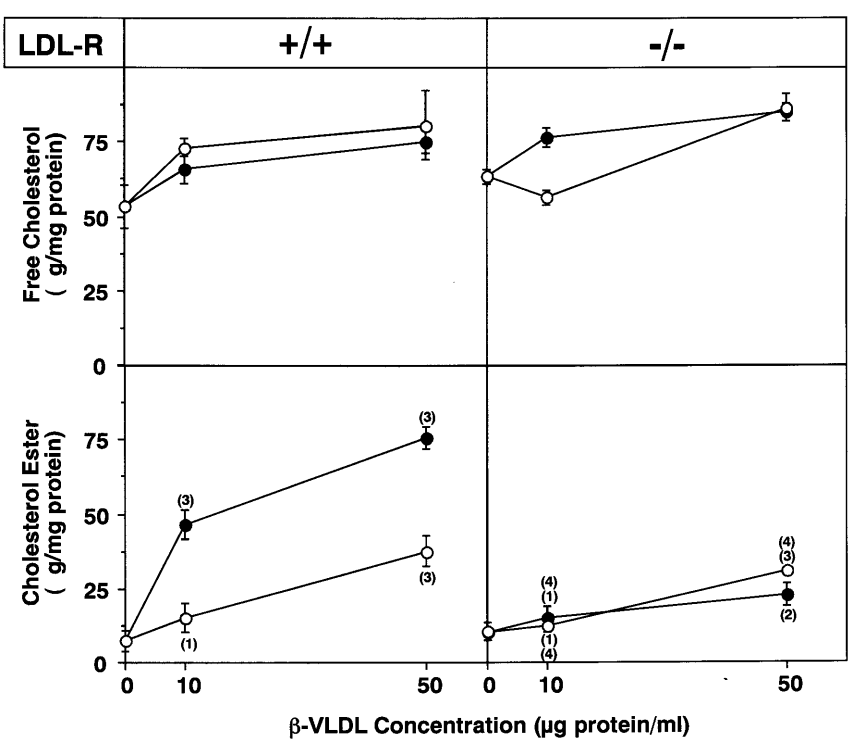

Fig. 7. Cholesterol ester mass measurement in MPM. MPM were harvested from wild type and LDL-R deficient mice. After incubation with DMEM containing 5 mg/ml LPDS for 36 h, the cells were incubated with 10 or 50 μg/ml of β-VLDL-E(+) (closed circle), or β-VLDL-E(−) (open circle) for 24 h. Lipid extraction and quantification were performed as described in Section 2. The values are presented as the means ± S.D. of three wells: (1) difference not significant compared to no β-VLDL addition; (2) $P < 0.05$ compared to no β-VLDL addition; (3) $P < 0.001$ compared to no β-VLDL addition; and (4) difference not significant compared to wild type MPM.

at least partially, lipoprotein uptake. Since both β-VLDL-E(+) and β-VLDL-E(−), but not LDL, displaced the degradation of ^{125}I-β-VLDL-E(−) in a similar manner, molecules common to these lipoproteins mediate its degradation; apoB-48, apoA-I and apoA-IV are likely candidates (Fig. 1).

Further studies are needed to know whether the receptor(s) responsible for the uptake of β-VLDL-E(−) are identical to certain known receptors such as the TGRLP receptors [29]. Recently, Hendriks et al. [54] have reported that VLDL from apoE-deficient mice were taken up by J774 macrophages, leading to an increase in CE mass, and they speculated that the triglyceride-rich lipoprotein (TGRLP) receptor proposed by Gianturco et al. accounted for the uptake. However we cannot rule out the possibility that slight oxidation or aggregation of the β-VLDL-E(−) lipoproteins might occur during preparation and storage and may in part be responsible for the LDL-R independent uptake of the lipoproteins.

In agreement with the results of the lipoprotein degradation, the stimulation of CE formation as well as the accumulation of CE by β-VLDL-E(+) was similar to that of β-VLDL-E(−) in the LDL-R(−/−) MPM (Figs. 6 and 7). Because apoE is secreted by the LDL-R(−/−) MPM [36], these results indicate that the secreted apoE did not further enrich the β-VLDL-E(+) leading to uptake by the LRP that is expressed by these cells (data not shown). Furthermore, the CE formation and accumulation stimulated by β-VLDL-E(−) in wild type MPM were similar to those in LDL-R(−/−) MPM (Figs. 6 and 7). This indicates that macrophage-secreted apoE does not interact with β-VLDL-E(−) nor does it direct β-VLDL-E(−) to the LDL-R pathway as postulated in the secretion-recapture hypothesis [49,55,56]. In contrast, Kowal et al. [23] reported that β-VLDL enriched with apoE stimulates CE formation in ldlA7 cells, CHO cells lacking the LDL-R. Potential explanations to reconcile the discrepancy between Kowal et al.'s and our experiments are as follows: (1) the amounts of apoE secreted by MPM may not be sufficient to convert β-VLDL-E(−) to be active in the stimulation of CE formation; and (2) MPM may express a lower level of LRP than CHO cells.

In conclusion, we demonstrate that the LDL-R is the primary mechanism for β-VLDL uptake in mouse peritoneal macrophages and the presence of apoE at the surface of the β-VLDL is critical for the uptake process. Furthermore the slight uptake of β-VLDL by LDL-R(−/−) MPM indicates that other potential secondary mechanisms such as the LRP, β-VLDL receptor or the TGLRP receptor which may or may not require apoE for β-VLDL uptake, play only a minor role.

Since lipoprotein retention has been described as an important event in the initiation of the development of atherosclerotic lesions, the LDL-R may be an important factor to remove native lipoproteins inside the arterial wall prior to denaturation. The cellular cholesterol-related regulation of the LDL-R may suggest a beneficial role since the down-regulation of its expression would allow cells to avoid becoming overwhelmed beyond their capacity of accommodating increased cholesterol. On the other hand, with LDL-R down-regulation native lipoprotein would become trapped by the extracellular matrix and be more susceptible to oxidative modification, resulting in cellular uptake by scavenger receptors, which are not subject to regulation by intracellular cholesterol.

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