The Scavenger Receptor Serves as a Route for Internalization of Lysophosphatidylcholine in Oxidized Low Density Lipoprotein-induced Macrophage Proliferation*

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We have recently demonstrated that the growth of murine macrophages is induced by oxidized low density lipoprotein (Ox-LDL) and that lysophosphatidylcholine (lyso-PC), a major phospholipid component of Ox-LDL, plays an essential role in its mitogenic effect. The present study was undertaken to further characterize the role of the macrophage scavenger receptor (MSR) in Ox-LDL-induced macrophage growth. The growth-stimulating effect of Ox-LDL on murine resident peritoneal macrophages was inhibited by maleylated bovine serum albumin (maleyl-BSA), a non-lipoprotein ligand for MSR but a poor carrier of lyso-PC, while maleyl-BSA itself failed to induce macrophage growth even in the presence of lyso-PC. Moreover, it competitively inhibited the endocytic uptake of 125I-Ox-LDL and the specific uptake of lyso-PC by MSR, whereas nonspecific lyso-PC transfer to cells was not affected. Furthermore, the Ox-LDL-induced cell growth of peritoneal macrophages obtained from MSR knockout mice was significantly weaker than that of macrophages obtained from their wild-type littersmates. Our results suggest that the MSR is an important and efficient internalization pathway for lyso-PC in Ox-LDL-induced macrophage growth.

Macrophage-derived foam cells, characterized by massive deposition of cytoplasmic cholesterol esters, are the key cellular elements in the early stage of atherosclerosis and play an essential role in the progression of this process (1). Macrophages are known to take up chemically modified low density lipoproteins (LDL), such as oxidized LDL (Ox-LDL) and acetylated LDL (acyt-LDL), through the scavenger receptor pathway and to become foam cells in vitro (2). Among the chemically modified LDL, Ox-LDL has been proposed as an atherogenic lipoprotein in vivo (3, 4). It is also known to have various atherogenic effects, e.g. acting as a chemoattractant for circulating monocytes (5), impairs endothelium-dependent arterial relaxation (6), and is cytotoxic to endothelial cells (7). We recently described a new property for Ox-LDL, the induction of macrophage growth (8), in which lysophosphatidylcholine (lyso-PC), a major phospholipid component of Ox-LDL, plays an important role in its mitogenic effect (9, 10). Based on several lines of evidence suggesting that macrophage-derived foam cells proliferated in situ in atherosclerotic lesions (11–13), it is possible that the Ox-LDL-induced macrophage growth may be linked to the enhanced progression of atherosclerosis.

In contrast to Ox-LDL, acetyl-LDL that binds to the macrophage scavenger receptor (MSR) but has a negligible amount of lyso-PC, does not have a mitogenic effect on mouse resident peritoneal macrophages (9) and on human monocyte-derived macrophages (10). Lyso-PC itself also had no mitogenic effect. However, incubation of macrophages with acetyl-LDL together with lyso-PC or phospholipase A2-treated acetyl-LDL, result in a significant enhancement of macrophage growth (9, 10). Based on these results, we considered that lyso-PC and a modified LDL are two essential factors for the induction of macrophage growth.

There are two different mechanisms involved in the transfer of lyso-PC from Ox-LDL to macrophages. One is the direct transfer from Ox-LDL to plasma cell membranes by aqueous diffusion or lipid exchange reaction. The other pathway is the endocytic uptake of Ox-LDL particles by MSR(s) leading to internalization of lyso-PC. At this stage, the exact route essential for the induction of macrophage growth is not known. To investigate this process, we investigated the role of MSR in Ox-LDL-induced macrophage growth using maleylated bovine serum albumin (maleyl-BSA), a non-lipoprotein ligand for MSR (14) but a less efficient carrier of lyso-PC than lipoproteins. Our results demonstrated that maleyl-BSA itself does not possess mitogenic activity even in the presence of lyso-PC. However, Ox-LDL-induced macrophage growth was inhibited by maleyl-BSA, under which the endocytic uptake of both 125I-Ox-LDL and lyso-PC-labeled Ox-LDL by these cells was also competitively inhibited. Involvement of MSR in Ox-LDL-induced macrophage growth was further examined with peritoneal macrophages obtained from the MSR gene knockout mice. Our results showed that the Ox-LDL-induced cell growth of these cells was significantly weaker than that of macrophages from their wild-type littersmates. These findings taken together support the notion that the MSR serves as an efficient pathway for internalization of lyso-PC, ultimately leading to macrophage growth.
Experimental Procedures

Chemicals—Palmitoyl-lysophosphatidylcholine (PLA₂) from \textit{Naja naja} venom were purchased from Sigma. \textit{methyl-}\textsuperscript{3}H\textit{/Thymidine (80 Ci/mmol), [\textit{3}H\textit{/palmitoyl-lyso-PC (60 Ci/mmol), lyso-phosphatidylcholine, and 1\textit{a}-\textit{14}C\textit{/palmitoyl\textsuperscript{1}H\textit{/lyso-PC (60 Ci/mmol) were purchased from DuPont NEN. Other chemicals were the best grade available from other commercial sources.

Lipoproteins and Their Modifications—Human LDL (d = 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation of human plasma of normolipidemic subjects obtained after overnight fasting (15). LDL was dialyzed against 0.15 M NaCl and 1 mM EDTA (pH 7.4). Acetyl-LDL was prepared by chemical modification of LDL with acetic anhydride as described previously by our laboratory (16). To prepare Ox-LDL, LDL was dialyzed against phosphate-buffered saline to remove EDTA. LDL (0.1 mg/ml) was then incubated for 20 h at 37 °C with 5 μM CuSO₄, followed by the addition of 1 mM EDTA and cooling (9). Acetylated LDL was dialyzed against phosphate-buffered saline and treated with PLA₂, as described by Quinn et al. (5). The concentrations of lyso-PC in lipoproteins were determined by the methods of Bartlett (17) as described previously (9). The concentration of proteins was determined by BCA protein assay reagent (Pierce) using bovine serum albumin (BSA) as a standard. Ox-LDL was iodinated with \textsuperscript{125}I according to the method of McFarlane (18). Ox-LDL and LDL were labeled with \textsuperscript{14}C\textit{/lyso-PC using a method of Albers et al. (19). Briefly, \textsuperscript{14}C\textit{/lyso-PC was dried under nitrogen and resuspended in 5 μl of ethanow. This solution was slowly added beneath the surface of 2 ml of Ox-LDL or LDL (1 mg/ml) with gentle stirring. The mixtures were incubated at 37 °C for 2 h to allow for equilibrium of \textsuperscript{14}C\textit{/lysophosphatidylcholine with the lipoproteins and then dialyzed extensively against 0.15 M NaCl and 1 mM EDTA (pH 7.4). Specific radioactivities of \textsuperscript{14}C\textit{/lyso-PC-Ox-LDL and LDL were 15,500 and 14,500 cpm/μg protein, respectively. The level of thiobarbituric acid-reactive substances in Ox-LDL was 80 nmol of MDA/mg protein, whereas those of LDL and acetylated LDL were within 3.0 nmol of MDA/mg protein. The level of endoxin associated with these lipoproteins was less than 1 pg/μg protein as measured by a commercially available kit (Toxicolor system; Seikagaku Corp., Tokyo, Japan). Moreover, macrophage growth was not induced by endoxin at a concentration less than 1 ng/ml in our experimental system.

Preparation of Maleylated Bovine Serum Albumin (Maleyl-BSA)—Twenty milligrams of defatted BSA in 10 ml of 0.1 M sodium pyrophosphate buffer (pH 9) was added dropwise on ice to 1 ml maleic anhydride in dioxane (total 750 μl). The pH was continuously adjusted to 9.0 using NaOH solution. After incubation on ice for 5 min, the mixture was dialyzed extensively at 4 °C against 0.15 M NaCl and 1 mM sodium phosphate buffer (pH 7.4) (20). To determine the capacity of maleyl-BSA to hold lyso-PC, 0.5 ml of 10 mM palmitoyl-lysophosphatidylcholine was added to 100 ml of RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum. After incubation for 7 days without a medium change, the adherent cells were collected on filters with Labomash LM-101 (Labo Science, Tokyo). The radioactivity and the material was collected on filters with Labomash LM-101 (Labo Science, Tokyo). The radioactivity of the material was counted using a liquid scintillation spectrophotometer (8).

Cell Counting Assay—The peritoneal cells were adjusted to 4 × 10⁴ cells/ml and 1 ml of cell suspension was dispensed in each well of a 24-well tissue culture plate (16 mm in diameter, Falcon) and incubated for 90 min at 37 °C. The non-adherent cells were removed by washing three times with 1 ml of prewarmed medium A. More than 90% of adherent cells were judged to be macrophages by both Giemsa staining and carbon-particle uptake (8). These macrophages were cultured at 37 °C in 1 ml of medium A with or without the lipoproteins to be tested. After incubation for 7 days without a medium change, the adherent cells in triplicate wells were lysed in 1% (w/v) Triton X-100, and all naphthol blue-black-stained nuclei were counted in a hemocytometer as described previously (8).

Endocytic Degradation and Cell Association of \textsuperscript{125}I-Ox-LDL—Mouse macrophages (2 × 10⁴ cells) in 1.0 ml of medium A were seeded to each plastic culture dish (22 mm in diameter, Falcon) and incubated for 60 min at 37 °C. The monolayers thus formed were washed three times with 1.0 ml of medium A. Each well was incubated with \textsuperscript{125}I-Ox-LDL for 6 h at 37 °C in the absence or presence of the unlabeled ligands to be tested. Endocytic degradation was determined by trichloroacetic acid soluble radioactivity in the medium after precipitating free iodine with AgNO₃, as described previously (21). Cells were solubilized with 1.0 ml of 0.1 M NaOH and the cell-associated radioactivity was determined as described previously (22).

Experiments with Peritoneal Macrophages from the MSR Gene Knockout Mice—Mice lacking both type I and type II MSR were established by targeted disruption of exon 4 of the MSR gene in A3-1 ES cells according to the method described previously by Kurihara et al. (23). Immunos staining using 2F8 anti-MSR monoclonal antibody indicated that the homozygote mice for MSR deficiency completely lacked both type I and type II receptor proteins and that peritoneal macrophages obtained from mice indicated very low (18%) acetyl-LDL degradation compared with their wild-type littermates. These animals grow normal and fertile. Their establishment and phenotype will be reported in detail elsewhere.

We used the MSR knockout mice and their wild-type littermates that were born to heterozygous mice. Peritoneal macrophages (1 × 10⁶ to 2 × 10⁶) were obtained from each mouse. The cells obtained from 2 mice were used for one set of growth experiments (totally 7 sets) and those obtained from 3 mice were used for the cell-association and degradation of \textsuperscript{125}I-Ox-LDL, and finally 17 knockout mice and 17 mice of their wild-type littermates were used in this study. Experimental protocols for the cell growth assay by \textit{methyl-}\textsuperscript{3}H\textit{/thymidine incorporation and the cell counting were essentially identical to those for C3H/He mice as described above. The cell association of \textsuperscript{125}I-Ox-LDL with these cells was also determined in the same way, except the cell numbers were reduced from 2 × 10⁶/well (C3H/He mice) to 0.5 × 10⁶/well (the MSR knockout mice), because the MSR knockout mice and their wild-type littermates were limited in number.

Statistical Analysis—Data were expressed as mean ± S.D. Differences were examined by the paired Student’s t-test. A p value less than 0.05 denoted the presence of a significant difference.

Results

Negligible Capacity of Maleyl-BSA to Hold Lyso-PC—Prior to experiments using maleyl-BSA, the capacity of maleyl-BSA to hold lyso-PC was compared with that of acetyl-LDL under the present culture conditions. When 10 μg/ml acetyl-LDL was incubated at 37 °C for 2 h with 50 μg palmitoyl-lysophosphatidylcholine in 100 ml of RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum, 10.2 nmol of lyso-PC was associated with 10 μg of acetyl-LDL (Table 1). This value was higher than that of Ox-LDL (6.4 nmol of lyso-PC associated with 10 μg of Ox-LDL). In contrast, when 50 μg lyso-PC was incubated with 200 μg/ml maleyl-BSA under the same experimental conditions, 0.2 nmol of lyso-PC was associated with 200 μg of maleyl-BSA (Table 1).

From these results, it is evident that the capacity of maleyl-BSA to hold lyso-PC is negligibly weaker than that of acetyl-LDL.

Failure of Maleyl-BSA to Induce Macrophage Growth—We

\footnotesize{{H. Suzuki, Y. Kurihara, and T. Kodama, unpublished observation.}}
have recently demonstrated that two essential factors for induction of macrophage growth are lyo-PC and a ligand for MSR such as acetyl-LDL and Ox-LDL (9, 10). In a strict sense, however, it is not clear whether a direct transfer of lyo-PC from these modified LDL to the cell surface membranes is involved in growth stimulation or whether endocytic internalization of lyo-PC by MSR is required for growth induction. To solve this issue, the capacity of maleyl-BSA to induce macrophage growth was compared with that of acetyl-LDL in the absence or presence of palmitoyl-lyso-PC. As shown in Table II, maleyl-BSA itself had no effect on macrophage growth in the absence or presence of lyo-PC. Similarly acetyl-LDL alone showed negligible growth stimulating activity. In the presence of lyo-PC, however, it showed a significant mitogenic effect on macrophages (Table II). Moreover, PLA2-treated acetyl-LDL also showed a significant growth-stimulating activity for these macrophages (Table II). Since acetyl-LDL is an efficient carrier of lyo-PC compared with maleyl-BSA (Table I), lyo-PC may act as a growth inducer when it occurs with modified LDL. Thus, it seems reasonable to expect that internalization of lyo-PC, as a component of a modified LDL, by MSR pathway may be an essential step in macrophage growth. The likelihood of such process was further examined in the following series of experiments.

**Maleyl-BSA Inhibits Ox-LDL-induced Macrophage Growth**—We examined the effect of maleyl-BSA on Ox-LDL-induced macrophage growth. When macrophages were incubated simultaneously with Ox-LDL and maleyl-BSA, Ox-LDL-induced macrophage growth determined by [3H]thymidine incorporation was inhibited by 70% in a dose-dependent manner (Fig. 1). The cell counting assay also showed that maleyl-BSA significantly inhibited Ox-LDL-induced increase in cell number by 60% (Table III). When macrophages were incubated with 200 μg/ml maleyl-BSA, more than 98% of cells were viable as determined by both trypan blue staining and MITT method, which is based on the cellular reduction of MTT to MITT formazan. These results indicated a significant inhibitory effect of maleyl-BSA on Ox-LDL-induced macrophage growth.

**Mechanism of the Inhibitory Effect of Maleyl-BSA on Ox-LDL-induced Macrophage Growth**—To elucidate the exact mechanism of the inhibitory action of maleyl-BSA, we examined the effect of maleyl-BSA on endocytic uptake of Ox-LDL by macrophages. As shown in Fig. 2B, the cell association of 125I-Ox-LDL was inhibited by maleyl-BSA as effectively as unlabeled Ox-LDL, whereas unlabeled LDL and BSA had no effect. A similar inhibitory pattern was observed in the endocytic degradation of 125I-Ox-LDL (Fig. 2A), indicating that maleyl-BSA competitively inhibited cellular uptake of Ox-LDL by MSR.

We then examined the effect of maleyl-BSA on the transfer of lyo-PC from Ox-LDL to cells. When macrophages were incubated with [14C]lyo-PC-labeled Ox-LDL, the cell-associated radioactivity increased with time (Fig. 3). The presence of 20-fold excess of maleyl-BSA (on a protein basis) caused a significant 40% inhibition of cell association of [14C]lyo-PC. Since the LDL receptor is negligibly expressed in mouse macrophages, we determined the transfer of [14C]lyo-PC from LDL to cells as an index of the nonspecific transfer of [14C]lyo-PC to cells by the lipid exchange reaction that also occurred to Ox-LDL. When macrophages were incubated with [14C]lyo-PC-labeled LDL, the cell-associated [14C]lyo-PC increased rapidly and reached a plateau level (5% of added radioactivity) after incubation for 2 h. In contrast to Ox-LDL, the transfer of [14C]lyo-PC from LDL to cells was not affected by maleyl-BSA. The amount of [14C]lyo-PC transferred from LDL to cells was very close to that transferred from [14C]lyo-PC-labeled Ox-LDL.
TABLE III

Effect of maleyl-BSA on Ox-LDL-induced macrophage growth determined by counting of solubilized nuclei

Resident mouse peritoneal macrophages (4 × 10⁶) were incubated with 10 μg of protein/ml of Ox-LDL and/or 200 μg of protein/ml of maleyl-BSA. On day 6, counting of solubilized nuclei was performed as described under “Experimental Procedures.” Data are expressed as mean ± S.D. of triplicate counts.

| Sample                              | Cell number (% of nonloaded) |
|-------------------------------------|-----------------------------|
| 4 × 10⁻⁴/well                       |                             |
| Nonloaded                           | 3.4 ± 0.2 (100%)            |
| Ox-LDL                              | 7.8 ± 0.5 (229%)            |
| Maleyl-BSA                          | 3.3 ± 0.3 (97%)             |
| Ox-LDL and maleyl-BSA               | 5.2 ± 0.3 (157%)            |

*p < 0.001, compared with non-loaded control (Student’s t test).

*p < 0.001, compared with Ox-LDL.

Fig. 2. Effect of maleyl-BSA on endocytic uptake of ¹²⁵I-Ox-LDL by mouse macrophages. Mouse resident peritoneal macrophages (2 × 10⁶) were incubated at 37 °C for 6 h with 5 μg/ml ¹²⁵I-Ox-LDL in the absence or presence of the indicated protein concentrations of BSA (C), maleyl-BSA (●), LDL (○), or Ox-LDL (▲). Trichloroacetic acid soluble radioactivity in the medium (A) and cell associated radioactivity (B) were determined as described under “Experimental Procedures.” The raw values of endocytic degradation (A) and cell association (B) equivalent to 100% were 2.61 and 1.71 μg/6 h/mg of cell protein, respectively. Data represent the mean ± S.D. of four separate experiments.

LDL in the presence of maleyl-BSA, suggesting that maleyl-BSA might suppress [¹⁴C]lyso-PC transfer from Ox-LDL to cells to almost nonspecific level. Combined together, these results suggested that maleyl-BSA might inhibit “specific” cellular uptake of lyso-PC by MSR rather than “nonspecific” transfer to cell membrane.

Growth-stimulating Effect of Ox-LDL on Scavenger Receptor-deficient Macrophages—To confirm the involvement of MSR in Ox-LDL-induced macrophage growth, we compared the mitogenic effect of Ox-LDL on peritoneal macrophages from the MSR gene knockout mice with that on peritoneal macrophages from their wild-type littermates (24). These cells were incubated with 5 μg/ml ¹²⁵I-Ox-LDL at 37 °C for 18 h, washed with medium, and amounts of the radiolabeled ligand associated with these cells were determined. As shown in Fig. 4, the amount of ¹²⁵I-Ox-LDL associated with the MSR knockout macrophages was less than 25% of that of their wild-type littermate macrophages. Moreover, the cell association of ¹²⁵I-Ox-LDL with wild-type macrophages was inhibited by 70% by maleyl-BSA, whereas maleyl-BSA had no effect on the cell association of ¹²⁵I-Ox-LDL with the MSR knockout macrophages (Fig. 4). Fig. 5 and Table IV show the effect of Ox-LDL on cell growth of these macrophages. Upon [³H]thymidine incorporation assay, Ox-LDL significantly induced the growth of wild-type macrophages (Fig. 5, A and C), whereas the growth-stimulating effect of Ox-LDL for the MSR-deficient macrophages was significantly weaker (Fig. 5, B and D). Upon the cell counting assay, 7 days incubation with 20 μg/ml Ox-LDL caused a 2-fold increase in the wild-type macrophages but only a 1.3-fold increase in the MSR knockout macrophages (Table IV). PLÁ₂-treated acetyl-LDL also induced cell growth of wild-type macrophages, whereas its effect on the MSR knockout macrophages was negligible (Table IV). Moreover, in wild-type macrophages, the inhibitory effect of maleyl-BSA on PLÁ₂-treated acetyl-LDL-induced cell growth was much more prominent as compared with that on the Ox-LDL-induced cell growth (Table IV). In contrast to the marked difference between these two macrophage populations in their response to the growth-stimulating effect of Ox-LDL and PLÁ₂-treated acetyl-LDL, their responses to GM-CSF were equally effective when determined under identical conditions (Fig. 5 and Table IV). It is likely from these results, therefore, that the MSR
might play an important role in Ox-LDL-induced macrophage growth.

**DISCUSSION**

We have recently demonstrated that two factors, lyso-PC and modified LDL that are recognized by MSR, are required for the induction of macrophage growth (9, 10). The present study was undertaken to elucidate the route for lyso-PC transfer to cells in order to induce macrophage growth. Our results showed that (i) maleyl-BSA, a ligand for MSR but a poor carrier of lyso-PC, did not induce macrophage growth even in the presence of lyso-PC, whereas acetyl-LDL induced cell growth in the presence of lyso-PC; (ii) when macrophages were incubated with Ox-LDL and maleyl-BSA, Ox-LDL-induced macrophage growth was inhibited; (iii) maleyl-BSA also inhibited specific cellular uptake of Ox-LDL and that of lyso-PC by the MSR but not nonspecific lyso-PC transfer to cells; (iv) the growth of the MSR-deficient macrophages was induced by Ox-LDL but the response of these cells to Ox-LDL was significantly weaker than the response of their wild-type littermate macrophages; (v) PLA2-treated acetyl-LDL showed a significant growth stimulating effect for wild-type macrophages, whereas its mitogenic effect was almost the same or slightly weaker than that of Ox-LDL (Table I). The reason for this is not clear. There could be two possibilities. First, the receptors other than the MSR might be involved in the internalization of lyso-PC in Ox-LDL-induced macrophage growth. In fact, the results of the recent studies showed that Ox-LDL is also recognized by other receptors such as Fc receptor, CD36, and SR-BI (26–36). Second, in addition to lyso-PC, oxidized compounds such as oxidized sterols and aldehydes or degraded apoB, which are generated during oxidation of LDL (2), could also serve as mitogens or enhancers for the macrophage growth. Recently, Heery et al. (36) reported that oxidized phospholipid other than lyso-PC could induce SMC proliferation via platelet-activating factor receptor. In this connection, the present study showed that the growth stimulating effect of Ox-LDL on macrophages obtained from the MSR knockout mice was low but significant, whereas PLA2-treated acetyl-LDL did not have such an effect (Table IV). Furthermore, the inhibitory effect of maleyl-BSA on cell growth of the wild-type macrophages was almost complete for PLA2-treated acetyl-LDL-induced cell growth, but partial (60%) for the Ox-LDL-induced macrophage growth (Table IV). Thus, these two possibilities might be taken into account to further elucidate the mechanism of the Ox-LDL-induced macrophage growth.

Maleyl-BSA inhibited Ox-LDL-induced macrophage growth probably by interfering with specific uptake of lyso-PC by MSR. However, in addition to inhibiting the specific uptake of Ox-LDL, maleyl-BSA is also expected to prevent the accumulation of cholesteryl esters (CE) in macrophages. Since phagocytic uptake and intracellular accumulation of CE led to proliferation of "starch-induced" murine macrophages (38), it is possible that CE accumulation itself may be a factor involved in Ox-LDL-induced macrophage growth. However, incubation of macrophages with 20 μg/ml Ox-LDL together with 80 μg/ml apoA-I, an efficient cholesteral acceptor from macrophage foam cells (39), inhibited CE accumulation by 60% whereas macrophage growth was not affected, as demonstrated in our previous study (9). These results suggest that intracellular accumulation of CE is not an essential event for Ox-LDL-induced cellular growth in mouse resident peritoneal macrophages.

Lyso-PC is known to have various atherogenic functions, including (i) a chemotactic activity for circulating monocytes (40); (ii) induction in endothelial cells of a variety of cell adhesion molecules such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 (41); (iii) induction in endothelial cells of growth factors such as platelet-derived growth factor and heparin-binding epidermal growth factor-like pro-
Resident murine peritoneal macrophages (4 × 10^6) obtained from the scavenger receptor gene knockout mice (+/-) and its wild-type littermates (+/+ ) were incubated with 20 μg of protein/ml of Ox-LDL or PLA2-treated acetyl-LDL in the presence or the absence of 200 μg of maleyl-BSA, or 23 ng/ml GM-CSF. On day 7, counting of solubilized nuclei was performed as described under “Experimental Procedures.” Data are expressed as mean ± S.D. of triplicate counts.

| Cell          | Sample             | Cell number (% of nonloaded) |
|---------------|--------------------|-----------------------------|
| C57/BL6 (+/-) | Nonloaded          | 3.5 ± 0.3 (100%)            |
|               | Ox-LDL             | 6.6 ± 0.4* (194%)           |
|               | Ox-LDL with maleyl-BSA | 4.8 ± 0.3* (137%)         |
|               | PLA2-treated acetyl-LDL | 6.1 ± 0.4* (185%)        |
|               | PLA2-treated acetyl-LDL with maleyl-BSA | 3.9 ± 0.2* (110%)   |
|               | GM-CSF             | 7.3 ± 0.5* (209%)           |
|               | Nonloaded          | 3.3 ± 0.2 (100%)            |
|               | Ox-LDL             | 4.3 ± 0.2* (130%)           |
|               | Ox-LDL with maleyl-BSA | 4.1 ± 0.4 (124%)          |
|               | PLA2-treated acetyl-LDL | 3.7 ± 0.3 (112%)         |
|               | PLA2-treated acetyl-LDL with maleyl-BSA | 5.8 ± 0.3 (109%)     |
|               | GM-CSF             | 6.0 ± 0.4* (182%)           |

*p < 0.001, compared with non-loaded control (Student’s t test).
*p < 0.05, compared with Ox-LDL.
*p < 0.01, compared with non-loaded control.
*p < 0.05, compared with PLA2-treated acetyl-LDL.
*p < 0.05, compared with non-loaded.
*p < 0.01, compared with non-loaded control.

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Fig. 6. Schematic representation of lyso-PC internalization by the scavenger receptor and subsequent induction of macrophage growth. A, when macrophage are incubated with Ox-LDL, lyso-PC, a major phospholipid component of Ox-LDL, is transferred to cells by two independent pathways. The first involves a nonspecific transfer of lyso-PC to plasma membrane by lipid exchange reaction while the second involves endocytic internalization of lyso-PC by MSR. B, when macrophages are incubated with Ox-LDL in the presence of excess amounts of maleyl-BSA, endocytic uptake of Ox-LDL as well as that of lyso-PC is inhibited whereas nonspecific lyso-PC transfer to plasma membrane is unaffected. Macrophage growth is initiated only when lyso-PC of Ox-LDL is effectively endocytosed by MSR. It is likely that internalized lyso-PC plays an essential role in generating intracellular signal for macrophage growth.

demonstrated that mouse macrophage growth was induced by phorbol esters. Consistent with this notion, Ox-LDL is known to induce accumulation of inositol triphosphate in human smooth muscle cells (46) and mouse macrophages (47) and increase the concentration of cytosolic calcium in mouse macrophages (47). However, further studies are required to define the functional link between protein kinase C and Ox-LDL-induced macrophage growth.
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