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Regulation of acetylated low density lipoprotein uptake in macrophages by pertussis toxin-sensitive G proteins

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Abstract **Class A scavenger receptors (SR-A) mediate the uptake of modified low density lipoprotein (LDL) by macrophages. Although not typically associated with the activation of intracellular signaling cascades, results with peritoneal macrophages indicate that the SR-A ligand acetylated LDL (AcLDL) promotes activation of cytosolic kinases and phospholipases. These signaling responses were blocked by the treatment of cells with pertussis toxin (PTX) indicating that SR-A activates $G_{i/o}$-linked signaling pathways. The functional significance of SR-A-mediated $G_{i/o}$ activation is not clear. In this study, we investigated the potential role of $G_{i/o}$ activation in regulating SR-A-mediated lipoprotein uptake. Treatment of mouse peritoneal macrophages with PTX decreased association of fluorescently labeled AcLDL with cells. This inhibition was dependent on the catalytic activity of the toxin confirming that the decrease in AcLDL uptake involved inhibiting $G_{i/o}$ activation. In contrast to the inhibitory effect on AcLDL uptake, PTX treatment did not alter β-VLDL-induced cholesterol esterification or deposition of cholesterol. The ability of polyinosine to completely inhibit AcLDL uptake, and the lack of PTX effect on β-VLDL uptake, demonstrated that the inhibitory effect is specific for SR-A and not the result of non-specific effects on lipoprotein metabolism. Despite having an effect on an SR-A-mediated lipoprotein uptake, there was no change in the relative abundance of SR-A protein after PTX treatment. These results demonstrate that activation of a PTX-sensitive G protein is involved in a feedback process that positively regulates SR-A function.**—Whitman, S. C., A. Daugherty, and S. R. Post. **Regulation of acetylated low density lipoprotein uptake in macrophages by pertussis toxin-sensitive G proteins.** *J. Lipid Res.* **2000.** 41: **807–813.**

Supplementary key words atherosclerosis • endocytosis • cholesterol • ACAT • class A scavenger receptor • β-VLDL

An early step in the formation of atherosclerotic lesions is the accumulation of modified low density lipoprotein (LDL), formed by oxidation of both the protein and lipid components of LDL in the arterial wall (1–4). Modified LDL is taken up by macrophages via interaction with class A scavenger receptors (SR-A). Chemically modified forms of LDL, such as acetylated LDL (AcLDL), bind to SR-A with high affinity and are commonly used to assess the function of this receptor (5). In macrophages, the uptake of AcLDL via SR-A results in excessive cholesteryl ester accumulation and leads to lipid engorgement as characteristically seen in atherosclerotic lesions (6). The role of SR-A in mediating macrophage cholesteryl ester accumulation during incubation with AcLDL is demonstrated by the 80% reduction in esterification in SR-A-deficient mice (7). Moreover, SR-A deficiency decreases lesion development in atherosclerosis-susceptible mice (8, 9). While it seems clear that SR-A is a key component in the development and progression of atherosclerotic lesions, the molecular mechanisms by which SR-A function is regulated are not well understood.

Lipoprotein receptors are primarily thought to function in cholesterol transport and are not typically associated with transmembrane signaling processes. However, recent evidence indicates that specific lipoproteins, in particular modified LDL, activate intracellular signaling cascades (10–12). For example, incubation of human monocyte/macrophage cells with AcLDL results in the activation of tyrosine kinases and protein kinase C (10). Results from other studies in isolated peritoneal macrophages indicate that AcLDL increases intracellular Ca^{2+} and promotes the activation of cytosolic tyrosine kinases and phospholipases (12). In these studies, signal propagation was blocked by the treatment of cells with pertussis toxin (PTX), a highly specific inhibitor of heterotrimeric GTP binding proteins (G proteins) of the $G_{i/o}$ family (13). Together, these results demonstrate a link between scavenger receptors and activation of PTX-sensitive G proteins.

Although AcLDL interacts with SR-A to activate G proteins, no functional consequences have been attributed to this process. The results of the present study define the impact of $G_{i/o}$ activation on lipoprotein uptake. Overall,

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Abbreviations: LDL, low density lipoprotein; AcLDL, acetylated low density lipoprotein; SR-A, class A scavenger receptor; PTX, pertussis toxin; β-VLDL, β very low density lipoprotein; MPM, mouse peritoneal macrophages; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DiI-AcLDL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled acetylated low density lipoprotein.

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our results indicate that a feedback process involving ligand-dependent activation of a PTX-sensitive G protein positively regulates SR-A function.

EXPERIMENTAL PROCEDURES

Chemicals

DMEM medium with l-glutamine and high glucose and heat-inactivated fetal bovine serum (FBS) were purchased from Gibco-BRL (Grand Island, NY). Pertussis toxin and the isolated toxin B-oligomer were from Calbiochem (La Jolla, CA). Guinea pig polyclonal anti-SR-A sera (Mac 5.2) was a generous gift from Joseph Witztum (UCSD, CA) and rat monoclonal anti-SR-A antibody (2F8) was purchased from Serotec (Raleigh, NC). The monoclonal anti-β actin antibody was obtained from Sigma (St. Louis, MO). 1,1′-Dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate-labeled AcLDL (DiI-AcLDL) was purchased from Biomedical Technologies Inc. (Stoughton, MA). Assay kits for determination of total and unesterified cholesterol were purchased from Wako Pure Chemical (Richmond, TX).

Lipoprotein isolation and acetylation

LDL (d 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation (14) of EDTA-anticoagulated plasma obtained from healthy normolipidemic volunteers. LDL was dialyzed against 0.15 M NaCl and 1 mM EDTA (pH 7.4). Acetylated LDL (AcLDL) was prepared by chemical modification of LDL with acetic anhydride as described by Basu et al. (15) and confirmed by agarose gel electrophoresis and comparison to unmodified LDL. Beta-migrating VLDL (β-VLDL; d < 1.006 g/ml) was isolated by sequential ultracentrifugation of EDTA-anticoagulated plasma obtained from New Zealand White rabbits maintained on a 1.0% cholesterol-enriched diet (16). Lipoprotein preparations were sterilized by passage through 0.22-μm filters and stored at 4°C. Lipoprotein samples were analyzed for protein content by the method of Lowry et al. (17).

Cell culture

Resident peritoneal macrophages were collected from 6–8-week-old male outbred Cr:NIH (S) Swiss mice (NCI Charles River) by peritoneal lavage with 5 ml ice-cold sterile saline. Cells were resuspended in DMEM containing penicillin/streptomycin and 10% FBS and plated at a density of 1×10^6 cells/ml. After overnight incubation at 37 °C, non-adherent cells were removed by gently washing cells three times with serum-free DMEM. Adherent macrophages were then cultured in medium with FBS for 24 h prior to experimentation.

DiI-AcLDL association assays

Association of DiI-AcLDL was used to assess the effect of PTX treatment on modified lipoprotein uptake. Isolated peritoneal macrophages were treated with the indicated concentrations of PTX (A+B subunits) or the toxin's binding domain (B-oligomer) in DMEM plus FBS for 24 h. Cells were then washed, fluorescent lipoprotein (DiI-AcLDL, 5 μg/ml) was added, and incubations were continued for 2 h in serum-free DMEM. Unbound ligand was removed by washing twice with ice-cold phosphate-buffered saline (PBS, pH 7.4) and cells were fixed by incubating with paraformaldehyde (4% in PBS, pH 7.4) for 15 min on ice. Cells were scraped into PBS and cell-associated fluorescence was determined by fluorescence-activated cell sorting (FACS) analysis. Data are expressed as the percent of cells displaying a fluorescent intensity within the peak defined by DiI-AcLDL association with untreated cells.

Cholesterol esterification assays

The incorporation of [^{3}H]oleic acid into cholesteryl esters was used as a measure of macrophage-mediated metabolism of lipoproteins. Isolated peritoneal macrophages were treated with PTX in DMEM plus FBS for 24 h. Cells were then washed, lipoproteins (β-VLDL or AcLDL) were added, and incubations were continued for 5 h in serum-free DMEM containing 0.9 μCi [^{3}H]oleic acid (Amersham, Piscataway, NJ) complexed with fatty acid-free BSA in a molar ratio of 5:1. The cells were washed twice with ice-cold Tris buffer (pH 7.4) and lipids were extracted by two 30-min incubations with 1 ml hexane–isopropanol 3:2 (vol/vol) containing carrier lipid (triolein and cholesterol palmitate). Cell proteins were solubilized in 0.5 ml 0.1 N NaOH for 16 h at room temperature and protein content was determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA) with BSA as a standard. The lipid extracts were dried under nitrogen, resuspended in chloroform–methanol 2:1 (vol/vol), and resolved by thin-layer chromatography with petroleum ether–diethyl ether–acetic acid 84:15:1 (vol/vol/vol). Esterified cholesterol was identified by exposure to iodide vapor, scraped into EcoLite (ICN, Costa Mesa, CA), and the amount of cholesteryl [^{3}H]oleate was quantified by liquid scintillation counting using a Beckman LS 3801 counter. Results are expressed as nanomoles of cholesteryl [^{3}H]oleate formed/milligram of cell protein.

Cholesterol mass assays

For determination of cholesterol mass, adherent macrophages were treated (or untreated) with PTX for 24 h, washed with serum-free DMEM, and incubated for an additional 16 h with the indicated lipoproteins and PTX (treated cells) in DMEM supplemented with 5% lipoprotein-deficient serum. Cells were washed with ice-cold Tris buffer (pH 7.4) and lipid was extracted as described above using hexane–isopropanol without carrier lipid (18). Cell proteins were solubilized in 0.5 ml 0.1 N NaOH for 16 h at room temperature and protein content was determined as described above. Cholesterol and cholesteryl ester content in the lipid extracts were determined as described previously (19). Briefly, the extracted lipid was solubilized in Triton X-100 containing chloroform and converted to an aqueous suspension. Aliquots were transferred to 96-well microtiter plates and cholesterol and cholesteryl ester content was determined using commercial enzyme-based assay kits. Results are expressed as micrograms of cholesterol/milligram of cell protein.

Detection of SR-A protein

For determination of SR-A protein expression, peritoneal macrophages were incubated with or without PTX (100 ng/ml) for 24 h. Incubation medium was removed, cell lysates were prepared in reducing Laemmli buffer, proteins were resolved by 12% SDS-PAGE, and proteins were transferred to PVDF. Blots were probed sequentially with anti-SR-A sera (Mac 5.2) and anti-β-actin antibody followed by incubation with species-specific HRP-coupled secondary antibodies. Blots were incubated with chemiluminescence substrate (Supersignal; Pierce, Rockford, IL) and luminescent bands were detected using a Kodak Image Station 440.

Statistical analysis

For statistical analysis, data were analyzed by one-way ANOVA using GraphPad Prism program. When a statistical difference was indicated, results were analyzed with the appropriate post-test. For cholesterol esterification and mass assays, results from PTX-treated cells were compared to untreated cells using a Bonferroni's post-test.

RESULTS

Pertussis toxin treatment decreases DiI-AcLDL association with macrophages

As an initial approach to determine whether $G_{i/o}$ proteins regulate AcLDL interaction with SR-A, MPM were isolated from NIH-Swiss mice and treated for 24 h with increasing concentrations of either the PTX (A+B subunits) or the toxin-binding domain (B-oligomer). The B-oligomer mediates cell association and facilitates entry of the A-subunit into the cell. The A-subunit possesses the catalytic activity required for ADP-ribosylation of α-subunits of heterotrimeric $G_{i/o}$ proteins, a modification that prevents receptor-mediated activation of the G protein (13). Cells were incubated with the fluorescent SR-A ligand, DiI-AcLDL, for 2 h at 37°C. Unbound ligand was removed by repeated washing, cells were fixed with paraformaldehyde, and the amount of ligand associated with cells was determined by FACS analysis. As shown in **Fig. 1**, treatment of macrophages with PTX (A+B subunits) substantially inhibited DiI-AcLDL association with MPM. This inhibitory effect of PTX was maximal at a toxin concentration of 100 ng/ml (32 ± 5%; n = 10) with no further increase in inhibition observed at concentrations as high as 500 ng/ml. This concentration range is consistent with that shown previously to specifically inhibit $G_{i/o}$ function in intact macrophages (20). Therefore, in all further experiments PTX was used at a concentration of 100 ng/ml.

Decreased AcLDL association with PTX-treated macrophages depends on inhibition of $G_{i/o}$

The dependence of this inhibitory effect on AcLDL uptake on PTX-mediated ADP-ribosylation of the α-subunits of $G_{i/o}$ proteins is demonstrated by the lack of effect of the toxin's B-oligomer. Although minimal (<10%) inhibition of DiI-AcLDL association was observed after incubation with high concentrations of the B-oligomer, this result is consistent with the presence of holotoxin (≤1%) in the B-oligomer preparation (as reported by the supplier). These data support the conclusion that $G_{i/o}$ activation is important in regulating a significant component of AcLDL uptake by macrophages.

Inhibition of $G_{i/o}$ specifically decreases AcLDL metabolism

To determine whether the decreased association of DiI-AcLDL with PTX-treated macrophages reflected reduced SR-A function, the effect of PTX treatment on the ability of AcLDL to increase cholesteryl [^{3}H]oleate accumulation was quantified. Treatment of macrophages with PTX decreased the incorporation of [^{3}H]oleate into cholesteryl ester (**Fig. 2**) after incubation with AcLDL. In contrast to the inhibitory effect on AcLDL metabolism, PTX treatment did not alter cholesteryl [^{3}H]oleate deposition in macrophages incubated with β-VLDL, a lipoprotein that stimulates cholesterol esterification through an alternative receptor pathway. The lack of a PTX effect on β-VLDL metabolism indicates that PTX treatment does not have a general inhibitory effect on intracellular lipoprotein trafficking and processing.

Native and modified lipoproteins are thought to internalize via receptor-mediated endocytosis in clathrin-coated pits and metabolized in lysosomes (21, 22). However, it has been suggested that AcLDL internalization by SR-A involves multiple pathways (23, 24). To confirm that the decreased AcLDL-induced cholesteryl ester deposition in PTX-treated macrophages reflected decreased lipoprotein internalization and not enhanced uptake via a pathway that did not involve lipoprotein metabolism in lysosomes, lipoprotein-induced increases in cholesterol mass were determined. PTX treatment decreased AcLDL-induced increase in total cellular cholesterol mass, but did not alter β-VLDL-induced changes (**Fig. 3A**). The de-

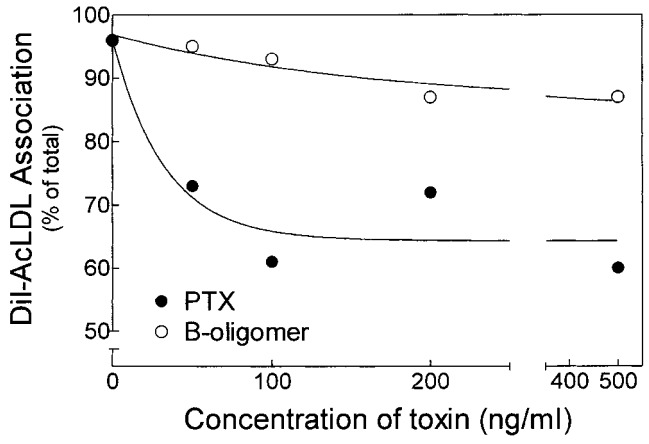

Fig. 1. PTX reduces uptake of AcLDL in MPM via ADP-ribosylation of $G_{i/o}$. The association of fluorescently labeled AcLDL (DiI-AcLDL) was determined in MPM that were untreated or treated for 24 h with the indicated concentration of PTX holotoxin (closed circles) or the cell-binding domain (B-oligomer, open circles) prior to incubation with DiI-AcLDL (5 μg/ml) for 2 h. The amount of fluorescence associated with cells was determined by FACS analysis.

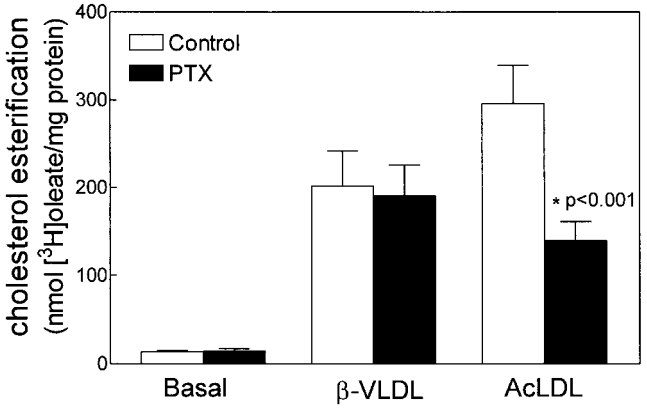

Fig. 2. PTX treatment inhibits AcLDL-, but not β-VLDL-induced cholesterol esterification in MPM. Cholesterol esterification was determined in MPM that were untreated or treated for 24 h with PTX (100 ng/ml) prior to incubation with 50 μg/ml of β-VLDL or AcLDL and a [^{3}H]oleic acid–albumin complex for 5 h. Cellular esterified cholesterol was isolated by thin-layer chromatography and cholesteryl [^{3}H]oleate was quantified. Histograms and bars represent mean ± SEM of at least three different experiments conducted in triplicate.

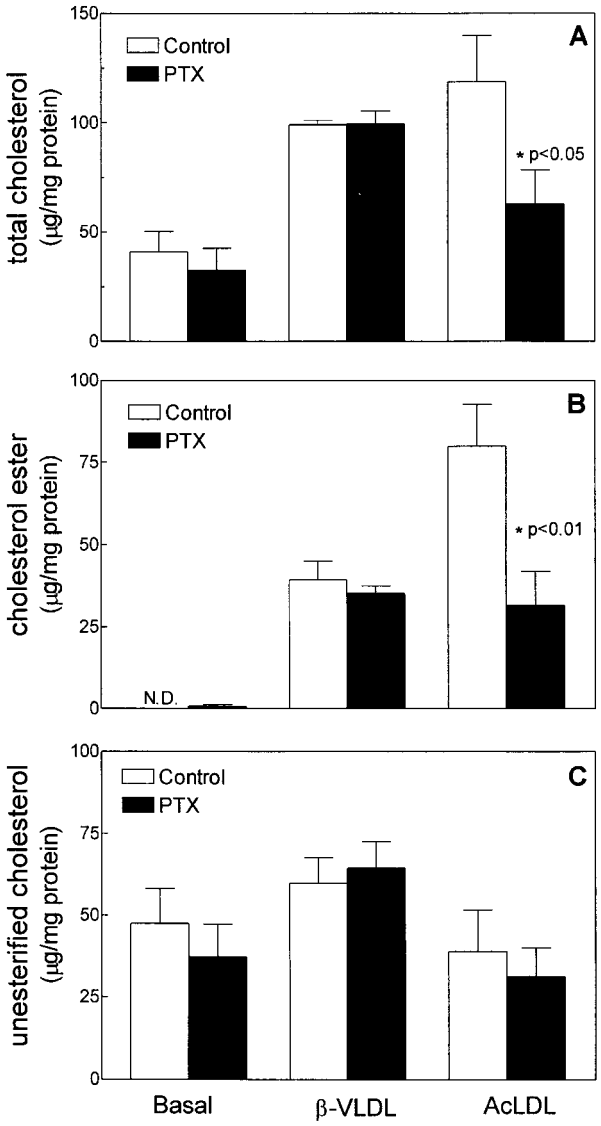

Fig. 3. PTX treatment decreases esterified cholesterol mass in AcLDL, but not in β-VLDL-treated MPM. Total and unesterified cellular cholesterol mass was determined in MPM that were untreated or treated with PTX (100 ng/ml) for 24 h prior to incubation with β-VLDL (10 μg/ml) or AcLDL (50 μg/ml) for 16 h. Cellular unesterified and total cholesterol were determined by enzymatic kits. Esterified cholesterol mass was determined by subtraction of the unesterified mass from the total mass. Histograms and bars represent mean ± SEM (n = 3).

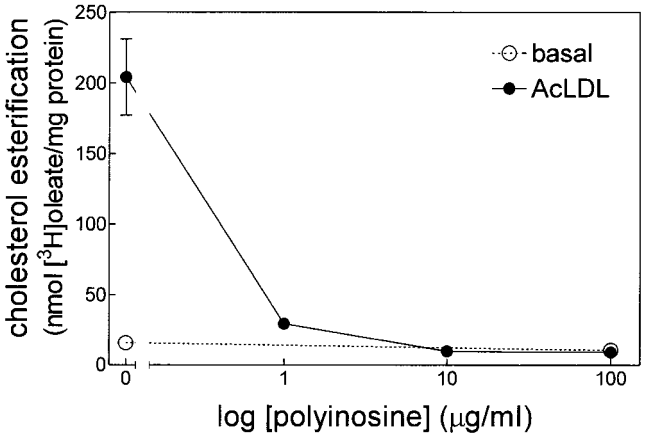

Fig. 4. Polyinosine inhibits the PTX-sensitive and insensitive pathways of AcLDL uptake. Cholesterol esterification was determined in MPM that were incubated with the indicated concentration of polyinosine, a competitive antagonist for SR-A. AcLDL (50 μg/ml) and a [^{3}H]oleic acid–albumin complex was added and cells were incubated for 5 h. Cellular esterified cholesterol was isolated by thin-layer chromatography and cholesteryl [^{3}H]oleate was quantified. The symbols represent the mean and the bars represent the range of triplicate determinations from a representative experiment (for some points the bars are smaller than the symbol).

crease in cholesterol mass in PTX-treated cells resulted from reduced cholesteryl ester content (Fig. 3B) and not from a reduction in the mass of unesterified cholesterol mass (Fig. 3C). Together, our results indicate that activation of $G_{i/o}$ proteins is specifically involved in regulating SR-A-mediated uptake. Further, our data indicate that SR-A-mediated uptake of AcLDL involves two pathways: one that is PTX-sensitive and the other PTX-insensitive.

$G_{i/o}$ inhibition decreases AcLDL uptake via SR-A

Although AcLDL is a selective ligand for SR-A, macrophages may express multiple scavenger receptor types that bind AcLDL. Therefore, it is possible that the decreased AcLDL uptake after PTX treatment reflects an inhibition of one or more receptor types. To test this possibility, AcLDL uptake was assessed in the absence or presence of the selective SR-A antagonist, polyinosine (25). As shown in **Fig. 4**, polyinosine reduced AcLDL-induced cholesteryl [^{3}H]oleate deposition to basal levels, indicating that AcLDL uptake by MPM is mediated by SR-A. Similarly, incubations of macrophages isolated from NIH-Swiss mice with the specific SR-A monoclonal antibody (2F8) reduced AcLDL uptake to ⩽5% of control values (26). Given the extent to which SR-A mediates AcLDL uptake in MPM, it appears that $G_{i/o}$ specifically regulates SR-A function in macrophages. Based on these results, we conclude that SR-A-mediated activation of a $G_{i/o}$ protein enhances internalization of AcLDL, suggesting a novel mechanism for regulating SR-A function.

PTX treatment does not alter SR-A expression

One possible explanation for the decreased uptake of AcLDL after PTX treatment is decreased SR-A expression. To assess this possibility, total cell lysates were prepared from MPM treated with PTX and cellular protein resolved by SDS-PAGE. SR-A was detected by immunoblotting using a SR-A-specific polyclonal antisera (Mac5-2). As shown in **Fig. 5**, treatment of cells with PTX did not alter the relative abundance of immunodetectable SR-A present in MPM cell lysates. The amount of β-actin present in each cell extract was used to normalize the abundance of SR-A. Thus, $G_{i/o}$ inhibition decreases the uptake of AcLDL by a mechanism that does not involve decreased expression of SR-A.

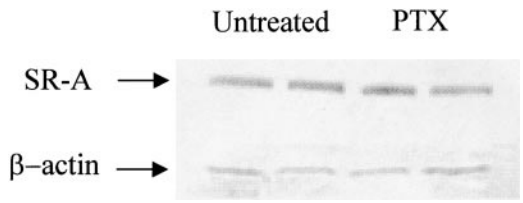

Fig. 5. Pertussis toxin treatment does not alter the relative abundance of immunodetectable SR-A in MPM. Duplicate isolates of MPM were incubated with or without pertussis PTX (100 ng/ml) for 24 h. Cell protein was separated by 12% SDS-PAGE and proteins were transferred to PVDF. Blots were probed sequentially with anti-SR-A antisera and anti-β-actin antibody followed by incubation with species-specific HRP-coupled secondary antibodies. Bands were detected by chemiluminescence.

DISCUSSION

The finding that AcLDL, but not β-VLDL, uptake by macrophages is PTX sensitive indicates a specific association between SR-A function and $G_{i/o}$ proteins. Although previously suggested that PTX-sensitive signaling pathways are activated by modified lipoproteins, the functional significance of activating these pathways on SR-A function has not been addressed. Our results demonstrate that inhibiting these signaling pathways with PTX substantially reduces the uptake of the scavenger receptor ligand AcLDL. In contrast, PTX treatment did not affect the uptake of β-VLDL. In addition, polyinosine and the SR-A-specific antibody 2F8 completely attenuated AcLDL uptake, indicating that decreased AcLDL uptake after PTX treatment resulted from the specific inhibition of SR-A. Together, our data indicate that activation $G_{i/o}$ is involved in a feedback process that enhances SR-A-mediated uptake.

Cell-surface receptors that interact with heterotrimeric G proteins generally have a characteristic seven-membrane spanning topology. However, recent studies indicate that other structurally diverse receptors can also interact with G proteins. For example, the LDL receptor-related protein (LRP) was recently shown to interact with and activate a G protein (11). Likewise, the thrombospondin receptor (CD47), a member of the IgG receptor superfamily, and the glycosylphosphatidylinositol (GPI)-anchored glycoproteins CD14 and CD59 were shown to activate PTX-sensitive G proteins (27–29). Like these receptors, the putative structure of SR-A is very different from the characteristic seven-membrane spanning topology of receptors that signal through $G_{i/o}$ proteins. Nevertheless, our results indicate that SR-A interacts with $G_{i/o}$ and that $G_{i/o}$ signaling pathways are activated in response to SR-A ligand.

Although it is commonly thought that SR-A internalizes lipoproteins via receptor-mediated endocytosis in clathrin-coated pits, previous results suggest that AcLDL uptake involves multiple processes. By assessing the rate of ^{125}I-labeled AcLDL internalization, Fong, Fong, and Cooper (23) showed that uptake involved two kinetic processes: the first with an apparent $t_{1/2} \sim 4$ min and the second with a $t_{1/2} > 30$ min. Consistent with these findings, Zha et al. (24) demonstrated that macrophages internalize AcLDL via two distinct pathways. One pathway is shared by the LDL receptor and likely represents a classic clathrin-coated pit endocytic process. The second pathway accounted for >40% of the AcLDL uptake and was distinct from that used by LDL. Uptake via this pathway involved prolonged cell-surface localization of the AcLDL particle within a region of membrane displaying microvilli-like projections. While these authors did not exclude the possibility that the second pathway involved clathrin-coated pits, they demonstrated that uptake via both pathways was mediated by SR-A. Although it has yet to be determined whether lipoprotein uptake by either of these uptake pathways is PTX sensitive, our results demonstrating that SR-A mediates AcLDL uptake by both $G_{i/o}$-dependent and -independent pathways suggest such a possibility.

Internalization of cell surface receptors is largely dependent on internalization signals contained within the cytoplasmic domain. Several sequences that regulate receptor endocytosis have been identified including: *a*) tyrosine-containing motifs with the consensus of YXXΦ, where Φ represents a bulky hydrophobic residue (e.g., NPXY in the LDL receptor (30), and YXRF in the transferrin receptor (31, 32)); *b*) dileucine motifs (e.g., insulin receptor (33, 34), Glut4 transporter (35), and IgG Fc receptor (36)); *c*) clusters of acidic amino acids (e.g., furin receptor (37, 38)); *d*) β-arrestin binding domains (e.g., G protein-coupled receptors)(39); and *e*) phosphorylation sites (e.g., G protein-coupled receptors, tyrosine kinase receptors (38, 40–47)). SR-A proteins, of which there are two splice variants (Types I and II), are trimeric membrane proteins with each monomer comprised of six (Type I) or five (Type II) distinct domains. Each monomer of the SR-A protein has a short (50 amino acid) amino-terminal cytoplasmic tail (5, 48, 49). To date, an internalization motif has not been identified in the cytoplasmic portion of SR-A and little is known regarding the cytoplasmic signals that regulate macrophage uptake of SR-A ligands.

The mechanisms by which $G_{i/o}$ signaling pathways modulate SR-A-mediated lipoprotein uptake are not clear, but may represent an important regulatory process in the uptake of modified lipoprotein and in the development of atherosclerosis. Based on our current understanding of receptor endocytosis and G protein regulation of this process, at least three possible explanations can be identified. First, $G_{i/o}$ proteins may regulate the localization of SR-A to the plasma membrane. Previous results have associated the redistribution of SR-A from an intracellular pool to the plasma membrane with an increase in the uptake of modified lipoprotein (50). A second mechanism that is consistent with our results is that interaction of SR-A with a $G_{i/o}$ protein enhances the ability of receptor to bind ligand. A reduced ability of SR-A to bind AcLDL was observed previously after treatment of macrophages with the protein phosphatase inhibitor okadaic acid (51). The reduced binding appeared to reflect receptor sequestration and/or inactivation, but did not result from a loss of cell-surface receptors. Thus, our results would be consistent with the notion that interaction of SR-A with $G_{i/o}$ regulates receptor sequestration/inactivation. Third, acti-

vation of a signaling cascade by $G_{i/o}$ proteins may regulate the process of receptor-mediated endocytosis. The finding that inhibition of intracellular protein kinases decreases AcLDL internalization (51) is of particular interest because AcLDL reportedly increases protein kinase activity through activation of a PTX-sensitive G protein (10, 12, 52). Thus, $G_{i/o}$-mediated activation of one or more protein kinases might feedback to enhance SR-A internalization. In summary, our results indicate that a feedback process involving the ligand-dependent activation of a PTX-sensitive G protein positively regulates SR-A-mediated lipoprotein uptake.

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