Ceramide in Lipid Particles Enhances Heparan Sulfate Proteoglycan and Low Density Lipoprotein Receptor-related Protein-mediated Uptake by Macrophages*

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Arterial wall sphingomyelinase (SMase) has been proposed to be involved in atherogenesis. SMase modification of lipoproteins has been shown to occur in atherosclerotic lesions and to facilitate their uptake by macrophages and foam cell formation. To investigate the mechanism of macrophage uptake enhanced by SMase, we prepared lipid emulsions containing sphingomyelin (SM) or ceramide (CER) as model particles of lipoproteins. SMase remarkably increased the uptake of SM-containing emulsions by J774 macrophages without apolipoproteins. The emulsion uptake was negatively correlated with the degree of particle aggregation by pretreatment with SMase, whereas the uptake of CER-containing emulsions was significantly larger than SM-containing emulsions, indicating that enhancement of uptake is due to the generation of CER molecules in particles but not to the aggregation by SMase. Heparan sulfate proteoglycans (HSPGs) and low density lipoprotein receptor-related protein (LRP) were crucial for CER-enhanced emulsion uptake, because heparin or lactoferrin inhibited the emulsion uptake. Confocal microscopy also showed that SMase promoted both binding and internalization of emulsions by J774 macrophages, which were almost abolished by lactoferrin. Apolipoprotein E further increased the uptake of CER-containing emulsions compared with SM-containing emulsions. These findings suggest the generation of CER in lipoproteins by SMase facilitates the macrophage uptake via HSPG and LRP pathways and plays a crucial role in foam cell formation. Thus, CER may act as an important atherogenic molecule.

Human plasma sphingomyelin (SM) levels have been reported to be a risk factor for coronary artery disease (1). The lipoprotein surface is mainly formed by phosphatidylcholine (PC) and SM together with cholesterol and apolipoproteins. The liver synthesizes sphingolipids de novo and incorporates the newly synthesized sphingolipids into very low density lipoproteins (VLDL) (2). The SM/PC ratio in VLDL is 0.25 (3). Unlike PC, SM is not degraded by lipoprotein lipase, hepatic lipase, or lecithin/cholesterol acyltransferase (4, 5), and the transfer of SM among lipoproteins as well as between lipoproteins and cell membranes is slower than that of other phospholipids (6). Thus, SM becomes enriched in VLDL remnants, and the SM/PC ratio in low density lipoproteins (LDL) is quite high (0.5) (7). Lipoprotein SM concentration is raised by lipopolysaccharide, dietary cholesterol, casein, and olive oil (8–11), and the SM/PC ratio was higher in all lipoproteins in apoE knockout mice compared with wild-type mice (12).

The subendothelial retention of atherogenic lipoproteins, LDL, and triglyceride-rich lipoproteins is a key event in the initiation of atherosclerosis. LDL extracted from human atherosclerotic lesions is highly enriched in SM compared with plasma LDL (7). Sphingomyelinase (SMase) hydrolyzes SM to phosphorylcholine and ceramide (CER), a lipid second messenger in apoptosis, cell differentiation, and cell proliferation (13). At least seven different SMases have been identified (14). Acid lysosomal SMase is found ubiquitously in tissues and defective in types A and B Niemann-Pick disease (15). A variety of cell types present in atherosclerotic lesions secrete SMase (16). Secretory SMase arises from the acid SMase gene via differential protein trafficking of a common protein precursor (17). SMase secretion by endothelial cells is stimulated by inflammatory cytokines, such as interleukin-1β and interferon-γ (16). The CER content of aggregated LDL in atherosclerotic lesions is 10- to 50-fold higher than that of plasma LDL (18). Treatment of LDL particles with SMase from Bacillus cereus has been shown to induce both aggregation and fusion of the particles, which depend on the accumulation of CER within the particles (18–20). Hydrolysis of LDL by SMase facilitated their uptake by macrophages and induced cholesteryl ester accumulation (foam cell formation) (19, 21). The formation of ceramide from SM could thus represent a critical step in atherosclerosis. However, no studies have addressed the effect of the SMase-induced lipid composition change on interactions between lipoproteins and cell surface receptors.

Emulsion particles have been used as protein-free models for plasma lipoproteins. We previously demonstrated that incorporation of SM into emulsion surface reduced the apolipoprotein E (apoE)- or lipoprotein lipase (LPL)-mediated emulsion uptake by HepG2 cells (22). In this study, we have examined the

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The abbreviations used are: SM, sphingomyelin; PC, phosphatidylcholine; LDL, low density lipoprotein; VLDL, very low density lipoprotein; SMase, sphingomyelinase; CER, ceramide; apoE, apolipoprotein E; LPL, lipoprotein lipase; HSPGs, heparan sulfate proteoglycans; LRP, LDL receptor-related protein; BSA, bovine serum albumin; PMC oleate, 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-ethanolamine; DLS, dynamic light scattering; FL, fluorophorid; MEM, Dulbecco’s modifed Eagle’s medium; FBS, fetal bovine serum; ConA, concanavalin A; TO, triolein.

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effect of SM hydrolysis with SMase on the aggregation of emulsion particles and the uptake by J774 macrophages. Our results show that the increase in SM-containing emulsion uptake by SMase is dependent on the formation of CER but not on the particle aggregation and that the CER-enhanced emulsion uptake involves the interaction with heparan sulfate proteoglycans (HSPGs) and LDL receptor-related protein (LRP).

**EXPERIMENTAL PROCEDURES**

**Materials—** Egg yolk PC was generously provided by Ashai Kasei Co. (Tokyo, Japan). Triolein (TO), egg yolk SM, SMase (from *B. cereus*), heparin, bovine milk LPL, choline oxidase, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). 1-Pyrenemethyl 3β-(cis-9-octadecenoyloxy)-22,23-bisnor-5-cholate (PC oleate) and cholesteryl-4,4-difluoro-5-(2-pyrrolyl)-4-bora-3-sindicate-3-dodecanoate (cholesteryl-BODIPY) were obtained from Molecular Probes (Eugene, OR). Bovine lactoferrin, alkaline phosphatase, and peroxidase were purchased from Wako Pure Chemicals (Osaka, Japan). Rat anti-mouse class A scavenger receptor (SRA) was from Harlan (Borona, NC). All other chemicals used were of regent grade.

**Preparation of Emulsions—** The lipid emulsions were prepared by the method described previously using a high pressure emulsifier (Nanoemulzer System YSNM-2000; ARS; Yokohama Co., Nagoya, Japan) (23). Briefly, mixtures of TO, PC, SM, and CER were suspended in Heps buffer (137 mM NaCl, 6 mM KCl, 6.1 mM D-glucose, and 10 mM Hepes; pH 7.4) and successively emulsified under 100 MPa of pressure at 40–60 °C. Depending on the purpose of the experiment, PC oleate or cholesteryl-BODIPY, which has been used as a tracer of lipoprotein endocytosis (24, 25) and as a general non-exchangeable membrane marker (26, 27), was added into the lipid mixtures at a ratio of 1 or 0.1 mol% of TO, respectively. After removing contaminating vesicles by ultracentrifugation, homogenous emulsion particles were obtained. The mean particle diameter of each emulsion was about 120 nm, except for large TO-PC/SM (2/1) emulsions (about 230 nm), determined from dynamic light scattering (DLS) measurements using a HeNe Laser (137 mW; 633 nm; Laser Technologies, Inc., Arvada, CO) and an argon ion laser (100 mW; 488 nm; Laser Technologies, Inc., Arvada, CO) as the light sources with a HeNe laser for the small TO-PC/SM (2/1) emulsions (137 mW; 633 nm; Laser Technologies, Inc., Arvada, CO) and an argon ion laser (100 mW; 488 nm; Laser Technologies, Inc., Arvada, CO) as the light sources with a HeNe laser.

**Assay for SM Hydrolysis—** Lipid emulsions (250 μM TO) were incubated with 10 milliunits/ml SMase at 37 °C for 2 h in the absence or presence of 10 milliunits/ml SMase. For experiments with apoE, PMC oleate-labeled emulsions were preincubated with apoE at 37 °C for 30 min, allowing sufficient time for equilibrium binding (28). After incubation, the cells were chilled on ice and washed twice with cold Hepes buffer containing 0.2% BSA and then washed twice with cold Hepes buffer alone. Cells were then dissolved in 0.2% Triton X-100. Fluorescence intensity of PMC oleate (excitation 342 nm, emission 377 nm) was measured with a Hitachi F-4500 spectrofluorometer and protein concentration determined by the method of Lowry (29) were determined to calculate particle uptake. By this method, uptake means binding and internalization by the cells.

**Confocal Fluorescence Microscopy—** Cholesteryl-BODIPY-labeled TO-PC/SM (2/1) emulsions (250 μM TO) were added to J774 macrophages grown on coverglasses, and incubation was carried out at 37 °C for 2 h in the presence or absence of 10 milliunits/ml SMase and 5 mg/ml lactoferrin. After incubation, the cells were washed twice with cold Hepes buffer containing 0.2% BSA and with cold Hepes buffer. The cells were then fixed with 4% paraformaldehyde in PBS for 10 min on ice and washed three times with Hepes buffer. The plasma membrane glycoproteins were labeled by incubation with 20 μg/ml rhodamine-concanavalin A (ConA) at room temperature for 5 min. The labeled cells were washed three times with Hepes buffer to remove excess reagent. After removal of excess reagent, the cells were mounted in 2.5% triethylenediamine and 50% glycerol in PBS. The plasma membrane fluorescence was observed with a confocal microscope (MRC-1024; Bio-Rad) at 488 and 568 nm excitation with emission filters of 522DF35 for cholesteryl-BODIPY and HQ598DF40 for rhodamine-ConA. The objective specifications were 40× oil immersion and numerical aperture 1.0.

**Statistical Analysis—** The statistical significance of differences between mean values was analyzed using the non-paired t-test. Multiple comparisons were performed using the Sheffe test following analysis of variance. Differences and correlation were considered significant at p < 0.05. Unless indicated otherwise, results are given as mean ± S.E. (n = 3).

**RESULTS**

**SM Hydrolysis and Aggregation of Emulsion Particles by SMase—** To study the effect of SM hydrolysis on the aggregation of emulsion particles, TO-PC, TO-PC/SM (4/1), and TO-PC/SM (2/1) emulsions (250 μM TO) were treated with 10 milliunits/ml *B. cereus* SMase at 37 °C. Bacterial SMases are a reasonable model for mammalian SMases in terms of enzymatic activity but not necessarily in terms of their structure. The degrees of hydrolysis by SMase were determined from the amounts of released phosphorylcholine. Under the above conditions, almost all of the SM molecules on TO-PC/SM (4/1) and TO-PC/SM (2/1) emulsions were hydrolyzed by SMase after incubation for 30 min, whereas TO-PC emulsions were not hydrolyzed (Fig. 1A). The aggregation of the emulsion particles was determined from the mean diameter of emulsion particles measured by DLS. As shown in Fig. 1B, SMase induced aggregation of TO-PC/SM (4/1) and TO-PC/SM (2/1) emulsions following a rapid enzymatic action. After 120-min incubation with SMase, the mean diameters of TO-PC/SM (4/1) and TO-PC/SM (2/1) emulsions increased 1.6- and 2.1-fold, respectively, but TO-PC emulsions were not aggregated. The degree of aggregation was consistent with the degree of SM hydrolysis in each type of emulsions. When EDTA was added at the beginning of the reaction to inhibit the enzymatic activity of *B. cereus* SMase, an Mg²⁺-dependent enzyme (30), the mean diameters
of three types of emulsions did not change (data not shown). These results suggest the aggregation of SM-containing emulsions is due to the enzymatic activity but not the structural action of B. cereus SMase.

Effects of SMase on Emulsion Uptake by J774 Macrophages—We examined the uptake of TO-PC, TO-PC/SM (4/1), and TO-PC/SM (2/1) emulsions by J774 macrophages in the presence of 10 milliunits/ml SMase without apolipoproteins (Fig. 2). In the absence of SMase, the uptake of TO-PC/SM (4/1) and TO-PC/SM (2/1) emulsions was slightly higher than TO-PC emulsions. SMase led to 2.3- and 3.0-fold enhancements of TO-PC/SM (4/1) and TO-PC/SM (2/1) emulsion uptake, respectively. In contrast, SMase increased the uptake of TO-PC emulsions only 1.3-fold, suggesting the enhanced uptake of SM-containing emulsions was caused primarily by the action of SMase on the emulsion particles rather than on the cell surface.

To determine which of the two consequences of SMase activity, generation of ceramide or particle aggregation, is responsible for the enhancement of the emulsion uptake, we next studied the relationship between cellular uptake and particle aggregation induced by the preincubation of TO-PC/SM (2/1) emulsions with SMase. As depicted in Fig. 3A, prolongation of the preincubation time led to enhanced degree of emulsion aggregation, characterized by increased mean diameter, as well as to decreased uptake into J774 macrophages. The fall in the emulsion uptake was significantly correlated with the increase in the mean particle diameter ($r = 0.983, p < 0.0001$) (Fig. 3B).

To examine the involvement of CER molecules in the emulsion uptake by J774 macrophages, we conducted cellular uptake studies using CER-containing emulsions with similar size to SM-containing emulsions. Fig. 4 shows that the uptake of TO-PC/CER (2/1) emulsions was 2.1-fold larger than that of TO-PC/SM (2/1). Although TO-PC/CER (2/1) emulsions were neither hydrolyzed nor aggregated in the presence of 10 milliunits/ml SMase for 2 h at 37 °C (data not shown), this emulsion uptake was increased 1.3-fold by SMase. Large TO-PC/SM (2/1) emulsion (about 230 nm) uptake was only 12% of TO-PC/SM (2/1) emulsion (about 120 nm) uptake, indicating the uptake of
the emulsions is largely dependent on their size. We conclude that CER formed in particles by SMase enhances their uptake into J774 macrophages, whereas the increase in size or particle aggregation decreases uptake.

Involvement of HSPGs and LRP in Emulsion Uptake—To assess if known lipoprotein receptors on J774 macrophages play roles in the interaction with emulsion particles, we conducted a series of experiments using inhibitors of lipoprotein receptors. Lipoproteins bind to cell surfaces through interactions with HSPGs. LRP has multiple ligands and mediates remnant lipoprotein uptake. HSPGs participate in the uptake, either associating with LRP or acting alone as a receptor (31). LRP has been reported to mediate the uptake of SMase-aggregated LDL by macrophages (32). We tested if cell surface HSPGs and LRP are responsible for the observed effect of SMase or CER on the emulsion uptake by incubating J774 macrophages with emulsions in the presence of heparin or lactoferrin (Fig. 5). At the current concentration (100 μg/ml), heparin selectively blocks ligand binding to heparan sulfate side chains of HSPGs but not lipoprotein binding to the members of the LDL receptor family or other lipoprotein receptors (33–36). Heparin significantly inhibited the uptake of TO-PC/SM emulsions in the presence and absence of SMase and that of TO-PC/CER emulsions. Lactoferrin interacts with HSPGs and LRP and has been suggested to inhibit remnant uptake by preventing their binding to HSPGs and subsequent transfer to LRP (37, 38). Lactoferrin also markedly reduced the uptake of TO-PC/SM emulsions in the presence and absence of SMase and that of TO-PC/CER emulsions. RAP is a potent inhibitor of all known ligand interactions of LRP (39). Inclusion of 40 μg/ml RAP in incubation with J774 macrophages resulted in significantly decreased uptake of TO-PC/SM (2/1) emulsions in the presence of SMase (17.2 ± 4.2% inhibition). However, the antibody (20 μg/ml) against either CD36 or class A scavenger receptor on J774 macrophages, known to block oxidized LDL or acetyl LDL uptake, respectively, had no effect on TO-PC/SM (2/1) emulsion uptake in the presence of SMase. We next explored the cell specificity of the enhancement of the emulsion uptake by SMase. As shown in Fig. 6, larger enhancement of SM containing emulsion uptake by SMase was observed in HepG2 cells, expressing HSPGs and LRP, as well as in J774 macrophages. Thus, the enhancement of emulsion uptake by ceramide appears to occur largely through HSPGs and LRP, non-phagocytic means.

Confocal Microscopic Images of Emulsion Uptake by J774 Macrophages—To investigate the distribution of emulsions in J774 macrophages, we observed the cells after incubation with TO-PC/SM (2/1) emulsions in the presence or absence of SMase, using confocal fluorescence microscopy. The emulsions were labeled by cholesteryl-BODIPY (green). Plasma membrane glycoproteins were stained by rhodamine-ConA (red), which selectively binds to α-1-mannosyl and α-1-glucosyl residues (40). Examination of the cell surface and inside the cells (Fig. 7A). As one can see, SMase enhanced both binding and internalization of the emulsions (Fig. 7B). Lactoferrin completely inhibited internalization of the emulsions in the presence of SMase, and only a slight amount of emulsion binding to the cell surface was.
Ceramide Enhances Particle Uptake by J774 Macrophages

SMase hydrolyzes SM molecules in emulsion particles, yielding CER molecules, which are retained in the particles, and water-soluble phosphorylcholine molecules, which are released (52). Previous studies have shown that treatment of LDL particles with SMase induces both aggregation and fusion of the particles resulting from the increase in CER content (18–20). The rapid enzymatic formation of CER is followed by much slower reorganization process, resulting in the formation of microdomains enriched in CER (52–54). Although PC can act only as acceptors of hydrogen bonds, SM and CER can act as both acceptors and donors through their hydroxyl and amide group. Microdomain formation arises due to the properties of CER headgroup, and the large hydration of the phosphorylcholine group may cause strong steric hindrance prohibiting hydrogen bonding in SM-SM interactions (53). The CER-enriched domains may act as non-polar spots at the particle surface and lead initially to particle aggregation through hydrophobic associations between the domains in different particles (13, 20). Thus, a driving force for particle aggregation is the formation of CER-enriched domains. Enzymatic generation of CER in SM-containing vesicles has been shown to lead extensive aggregation (55). In contrast, Holopainen et al. (53) ascertained that CER-containing vesicles formed by lipid mixing in organic solvent did not show any signs of aggregation for up to 24 h of incubation. In the present study, we showed that SMase promoted the aggregation and/or fusion of lipid emulsions depending on CER formation (Fig. 1), whereas TO-PC/CER (2/1) emulsions, in which CER incorporated into the lipid composition during emulsion preparation, were stable for several days. These may be related to the formation of CER-enriched domains around the SMase molecules.

Xu and Tabas (19) showed that in the presence of a very small amount of SMase (10 milliunits/ml), the endocytosis of LDL was enhanced and led to an increase in LDL degradation and cholesteryl ester accumulation in J774 and mouse peritoneal macrophages. SMase-treated LDL has been shown to be bound and degraded at higher rates than control LDL in a variety of cell lines, including HepG2 cells (58). LDL association with smooth muscle cells in the presence of SMase was greater than in the absence of SMase (57). Marathe et al. (21) reported that the uptake and degradation of lipoproteins from apoE-knockout mice by macrophages was dramatically increased by incubating the lipoproteins with SMase and suggested that neither LDL receptor nor scavenger receptors, CD36 or class A scavenger receptor, were involved in the process. However, J774 macrophages internalize and degrade both matrix-retained and non-retained SMase-aggregated LDL, which are mediated partially by LRP (32). The size of SMase-aggregated LDL (∼100 nm) would be too small to elicit a phagocytic response (19, 58). Örnö et al. observed that SMase-treated aggregated and fused LDL bound to human aortic proteoglycans more tightly in the affinity column than native LDL (20, 54). The data reported in this study demonstrate that the generation of CER by SMase in emulsion particles increases their ability to be taken up by J774 macrophages without apolipoproteins and indicate that HSPGs and LRP are crucial for this process. HSPGs act as potential receptors for atherogenic lipoproteins or facilitate the uptake by ligand transfer to LRP (31). LRP is present in macrophages and vascular smooth muscle cells from atherosclerotic lesions and normal vessels (59, 60). The ability of LRP to bind numerous structurally distinct ligands with high affinity arises from 31 ligand-binding sites with unique contour surface and charge distribution (61). LRP, contrary to LDL receptor, is not regulated by intracellular cholesterol. Therefore, the uptake of SMase-modified lipoproteins through LRP should play an important role in macrophage-lipid deposition in atherosclerotic lesions. Recent studies indicate that HSPGs facilitate aggregated LDL binding to human vascular smooth muscle cells and that LRP is essential to mediate the uptake of aggregated LDL (62, 63). Endocytosis of protein-free anionic liposomes in neurons is directly mediated by LRP but independent of HSPGs (64). Our current data demonstrate that the HSPG-LRP pathway can account for a large part of the emulsion uptake without apolipoproteins, which is modulated by lipid composition of the particles.

Our results show that particle size is negatively correlated with emulsion uptake (Fig. 3), which is mainly mediated by

**DISCUSSION**

**Effects of ApoE and LPL on Emulsion Uptake by J774 Macrophages**—ApoE is a ligand for LDL receptor, LRP and VLDL receptor, and binds to cell surface HSPGs (31, 42). Within atherosclerotic lesions, lipoproteins have been found to contain apoE in addition to apoB-100 (43, 44). β-VLDL with apoE are avidly taken up by and markedly stimulate cholesteryl ester formation in macrophages (45). ApoE directly modifies macrophage-mediated immune responses that contribute to atherosclerosis (46). Increased LPL activity in the arterial wall is correlated with increased areas of lipid deposition and increased atherosclerotic lesion formation (47). LPL activity in mouse macrophages is correlated with their propensity to develop atherosclerosis (48). LPL contains binding sites for lipoproteins and for specific cell-surface receptors, including HSPGs and LRP (49, 50). Both apoE and LPL promote the internalization of lipoproteins and lipid emulsions by cells (22, 51). Lastly, we assessed the effects of apoE and LPL on the uptake of TO-PC/SM (2/1) and TO-PC/CER (2/1) emulsions by J774 macrophages (Fig. 8). The effect of apoE was remarkable on TO-PC/CER (2/1) emulsions. The uptake of TO-PC/CER (2/1) emulsions further increased up to 2.5-fold compared with an up to 1.3-fold increase in TO-PC/SM (2/1) emulsion uptake in the presence of apoE. In the presence of LPL, TO-PC/CER (2/1) emulsions showed significantly higher uptake than TO-PC/SM (2/1) emulsions. However, LPL similarly enhanced both TO-PC/SM (2/1) and TO-PC/CER (2/1) emulsion uptake, in contrast to apoE.

**Fig. 6. Effect of SMase on the uptake of emulsion particles by HepG2 cells.** HepG2 cells were incubated for 2 h at 37 °C with TO-PC (open bars), TO-PC/SM (4/1) (cross-hatched bars), and TO-PC/SM (2/1) (filled bars) emulsions (250 µg TO) in the absence or presence of 10 milliunits/ml SMase. The mean particle diameter of emulsions was about 120 nm. Each bar represents the mean ± S.E. of three measurements. *, p < 0.05, significant difference between TO-PC and TO-PC/SM (4:1) emulsions. #, p < 0.05, significant difference between TO-PC and TO-PC/SM (2:1) emulsions. $, p < 0.05, significant difference between TO-PC/SM (4:1) and TO-PC/SM (2:1) emulsions.
The mean particle diameter of the emulsions was about 120 nm. The cell surface was visualized with rhodamine-ConA (red). Bar, 10 μm.

Because J774 macrophages do not synthesize apoE (68), the enhanced emulsion uptake by SMase or CER does not require apoE as shown in Figs. 2–5 and 7. We have shown in this study that apoE further enhances the uptake of TO-PC/CER emulsions more effectively than TO-PC/SM emulsions (Fig. 8), presumably by increased binding of apoE to CER-containing particles. Our previous study showed that incorporation of SM into the emulsion surface reduced the binding amount of apoE and emulsion uptake by HepG2 cells (22). ApoE has seven amphipathic helical segments that are responsible for lipid binding (69). The apolipoprotein helices are predicted to insert deeply into the hydrophobic interior of PL monolayers (70). By binding to the hydrophobic surface areas, apoE can inhibit phospholipase C-induced aggregation and fusion of LDL particles (71). It is conceivable that apoE prefers a more hydrophobic surface. In contrast, LPL is suggested to interact with the head group region rather than with the hydrophobic interior of the surface monolayers (72). We also demonstrated in the previous report that the stimulatory effect of LPL on emulsion uptake was decreased by emulsion surface SM (22). In the present study, LPL had similar effects on the uptake of TO-PC/CER emulsions and TO-PC/SM emulsions (Fig. 8).

In conclusion, the generation of CER in lipoproteins by SMase may stimulate HSPG and LRP-mediated uptake by macrophages, which would be intensified by apoE, and play a crucial role in foam cell formation. Furthermore, large amounts of CER ingested together with lipoproteins by macrophages possibly induce apoptosis. Therefore, elevation in plasma SM levels may result in the accumulation of CER in the arterial intima and would be related to the development of atherosclerosis.
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