Ceramide 1-Phosphate, a Mediator of Phagocytosis*

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The agonist-stimulated metabolism of membrane lipids produces potent second messengers that regulate phagocytosis. We studied whether human ceramide kinase (hCERK) activity and ceramide 1-phosphate formation could lead to enhanced phagocytosis through a mechanism involving modulation of the membrane-structural order parameter. hCERK was stably transfected into COS-1 cells that were stably transfected with the FcγRIIA receptor. hCERK-transfected cells displayed a significant increase in phagocytic index in association with increased ceramide kinase activation and translocation to lipid rafts after activation with opsonized erythrocytes. When challenged with opsonized erythrocytes, hCERK-transfected cells increased phagocytosis by 1.5-fold compared with vector control and simultaneously increased ceramide 1-phosphate levels 2-fold compared with vector and unstimulated control cells. Control and hCERK-transfected cells were subjected to cellular fractionation. Utilizing an antibody against hCERK, we observed that CERK translocates during activation from the cytosol to a lipid raft fraction. The plasma membrane-structural order parameter of the transfectedants was measured by labeling cells with Laurdan. Cells transfected with hCERK showed a higher liquid crystalline order than control cells with stimulation, conditions that are favorable for the promotion of membrane fusion at the sites of phagocytosis. The change in the structural order parameter of the lipid rafts probably contributes to phagocytosis by promoting phosphoglycogen.

The second messengers produced by membrane lipids through agonist stimulation include not only glycerolipids but also sphingolipids. Sphingolipids are comprised of lipids that contain a long chain sphingoid base. Sphingolipids, in addition to being structural components of membranes, regulate cell-cell and cell-substrate interactions, proliferation, and differentiation. Members of this diverse group of lipids have emerged as a novel class of signaling molecules that also regulate phagocytosis. The mechanisms by which sphingolipids exert these effects remain incompletely defined. More than a decade ago, it was found that ceramide can be phosphorylated to ceramide 1-phosphate (C1P).1–3 C1P is found in brain synaptic vesicles, and it is thought to play a role in regulating the secretion of neurotransmitters by promoting the fusion of vesicle membranes (4). Ceramide kinase activity exists in HL-60 cells where C1P is derived from ceramide released from sphingomyelin (5). Human ceramide kinase (hCERK) was recently cloned based on the homology to two isoforms of mice and human sphingosine kinase (6). The expressed kinase displayed specific ceramide phosphorylating activity. BLAST search analyses using the hCERK sequence revealed that a series of putative CERKs exist in a variety of cellular organisms, including plants, nematodes, insects, and vertebrates. CERKs represent a new class of lipid kinases that are clearly distinct from sphingosine and diacylglycerol kinases (6, 7).

C1P shares structural homology with phosphatidic acid, a lipid shown to be highly fusogenic (8). In an earlier study, the generation of C1P was also observed to occur through the activation of a ceramide kinase in polymorphonuclear leukocytes (7). In this model, C1P was found to be a potent fusogen. The addition of C1P to liposomes shifted the rate and extent of calcium-dependent fusion (7). Thus C1P was postulated to play a role in phagosome formation. Subsequently, it was reported that the CERK mediates Ca2+-dependent degranulation in mast cells (9). Others have also found that A2A9 cells transfected with the hCERK can induce arachidonate release and prostanoitid synthesis (10).

Neutrophils are difficult to maintain in vitro for prolonged periods of time and are not amenable to transfection. Therefore, other model systems are often employed to probe the mechanism of phagocytosis and phagosome formation. COS-1 cells are one such model system. COS-1 cells acquire a phagocytic phenotype when transfected with the FcγRIIA receptor. In the present study, we evaluated the effects of transfecting COS-1 cells expressing FcγRIIA with the cDNA for hCERK on phagocytosis. Using the fluorescent probe Laurdan, we employed high-speed microspectrophotometry to examine the mechanism by which C1P enhances phagocytosis. We found that C1P increased during phagocytosis and that a distinct gel-like order lipid phase (Lo) was formed within the cell membrane at sites of C1P formation in the hCERK-transfected cells.

**EXPERIMENTAL PROCEDURES**

Materials—All of the phospholipids, imidazole, diethylenetriaminepentaaetic acid, N-octyl-β-D-glucopyranoside, and proteinase inhibitors pepstatin and leupeptin and monoclonal anti-c-Myc antibody were obtained from Sigma. Dithiothreitol was purchased from Calbiochem. Silica Gel 60 thin layer chromatography plates were purchased from VWR (Chicago, IL). [γ-32P]ATP was obtained from ICN Pharmaceuticals.

human ceramide kinase; CERK, ceramide kinase; ElgG, erythrocytes; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; mCD, methyl-β-cyclodextrin.
C1P Leads to Enhanced Phagocytosis

Stably transfected COS-1 cells were labeled with \( \nu \)-erythro-[\( ^3 \text{H} \)]sphingosine (2 \( \mu \text{Ci} \)/ml) for 24 h in Dulbecco’s modified Eagle’s medium at 37°C. After incubation, the cells were removed with trypsin EDTA. The reaction was terminated by the centrifugation of the COS-1 cells, removal of the supernatant, and the addition of 2 ml of methanol. After transferring the samples to a glass tube, methanol and chloroform were added and the samples were sonicated and centrifuged for 30 min at 3000 \( \times g \). The supernatant was saved, and the pellets were reextracted with chloroform/methanol (2:1, v/v). The supernatants were combined with those of the first extraction, and 1 ml NaCl was added. After centrifugation at 2000 \( \times g \), the lower layer was transferred to another tube and subjected to mild alkaline hydrolysis. The lipids were dried under nitrogen, dissolved in chloroform/methanol (2:1), applied to high performance TLC plates, and developed using the system described above. The corresponding spots for C1P were identified with a radiolabeled standard. Cells were labeled with \( \nu \)-erythro-[\( ^3 \text{H} \)]sphingosine for 4 h. Following phagocytosis, the medium was aspirated, the cells were washed twice with a buffered saline containing Ca\(^{2+}\) and Mg\(^{2+}\) and cells were challenged with erythrocytes (ElG). The extraction was performed as outlined for phosphate labeling.

**Lipid Raft Isolation—FcRRIIA, FcRRIIA/vector, and FcRRIIA/hCERK-transfected COS-1 cells were challenged with opsonized ElG and the ElG not ingested were lysed. COS-1 cells were harvested by sonication in a radioisotope buffer containing 3 mM Mg\(^{2+}\) acetate, 37°C, 5% CO\(_2\). To establish stable transfecants, cells were cultured with medium containing 200 \( \mu \)g/ml G-418 and 200 \( \mu \)g/ml Zeocin to select double transfecants.**

**Sheep Erythrocytes—Sheep red blood cells were sensitized with rabbit Ig anti-sheep erythrocyte antibody as previously described (12). Phagocytosis assays were conducted as previously described (13).**

**Construction of Catalytically Inactive hCERK—A search of the hCERK amino acid sequence for signaling domains using the SMART search tool revealed a similarity in residues 132–278 of hCERK to the proposed ATP-binding site of the DGK catalytic domain. This region in hCERK was excised at HindIII and XhoI (11). To create double expression transfectants with FcRRIIA, the open reading frame of the hCERK was excised at HindIII and XhoI and was ligated into the pCR4-TOPO vector (Invitrogen).**

In order to measure C1P, COS-1 cells, stably transfected with FcRRIIA, were labeled with D-[\( ^3 \text{H} \)]sphingosine for 24 h. Lipid Raft Isolation—FcRRIIA, FcRRIIA/vector, and FcRRIIA/hCERK-transfected COS-1 cells were challenged with opsonized ElG and the ElG not ingested were lysed. In this case, cells were harvested by sonication in a radioisotope buffer containing 3 mM Mg\(^{2+}\) acetate, 37°C, 5% CO\(_2\). To establish stable transfecants, cells were cultured with medium containing 200 \( \mu \)g/ml G-418 and 200 \( \mu \)g/ml Zeocin to select double transfecants. Sheep Erythrocytes—Sheep red blood cells were sensitized with rabbit Ig anti-sheep erythrocyte antibody as previously described (12). Phagocytosis assays were conducted as previously described (13). Construction of Catalytically Inactive hCERK—A search of the hCERK amino acid sequence for signaling domains using the SMART search tool revealed a similarity in residues 132–278 of hCERK to the proposed ATP-binding site of the DGK catalytic domain. This region in hCERK was excised at HindIII and XhoI (11). To create double expression transfectants with FcRRIIA, the open reading frame of the hCERK was excised at HindIII and XhoI and was ligated into the pCR4-TOPO vector (Invitrogen). To produce a catalytically inactive hCERK, the corresponding glycine (Gly\(^{158}\)) was mutated to aspartate in hCERK. A mutation was introduced in Myc-tagged hCERK in the pcDNA3 expression vector via mutagenic oligonucleotides using primers G189D-F (forward, 5′-CGGAGATGAATGTTACCCGACGAG-3′) and G189D-R (reverse, 5′-GCTGGAACATATCATCTGCCGACGAG-3′) by overlap extension methodology.

**Ceramide Kinase Activity Assay—Ceramide kinase activity was measured as described by Bajaleh et al. (1). Sonicated cells were incubated for 10 min at 30°C in a reaction mixture containing 5 \( \mu \)M ceramide (solubilized in 7.5% 2-mercaptoethanol, 5% glycerol, and 5% Triton X-100) with the addition of 5 \( \mu \)M 3H Ceramide.**

**Radiolabeling of COS-1 Cells—In order to measure C1P, COS-1 cells transfected with hCERK were labeled with \( \nu \)-erythro-[\( ^3 \text{H} \)]sphingosine.**
Peltier-cooled I-MAX-512 camera (approximately −20 °C) (Princeton Instruments) (19, 20). The camera was controlled by a high-speed Princeton ST-133 interface and a Stanford Research Systems (Sunnyvale, CA) DG-535 delay gate generator. Microspectrophotometry used a 355-nm bandpass discriminating filter for excitation and a 405-nm long-pass dichroic mirror. Winspec software (Princeton Instruments) was used to analyze the data.

RESULTS

hCERK distinct from that of diacylglycerol kinase was recently cloned (6). In a preliminary study following transient transfection of FcγRIIA-expressing COS-1 cells with hCERK plasmid, a 40% increase of FcγRIIA-mediated phagocytosis of E1gG compared with control cells and 20% compared with vector control cells was observed (data not shown). The efficiency of the transient transfection was between 40 and 50% as detected by cotransfection with a β-galactosidase expression construct. These results raised the possibility that C1P potentiates phagocytosis. To test this possibility, stable transfectants simultaneously carrying FcγRIIA, FcγRIIA/vector, FcγRIIA/hCERK, and FcγRIIA/G198DhCERK mutant and then challenged for 30 min with E1gG. A, photomicrographs at ×400 comparing FcγRIIA-transfected cells to cells transfected with FcγRIIA/hCERK. B, the phagocytic indices measured as a function of time following E1gG treatment. Values represent the mean ± S.D. of four experiments, * and *** denote p < 0.05 and p < 0.001, respectively, and indicate the difference among FcγRIIA, vector, G198DhCERK, and FcγRIIA/hCERK-transfected cells compared with the 5-min time point. C, percent of cells ingesting E1gG. COS-1 cells were stably transfected with FcγRIIA, FcγRIIA/vector, FcγRIIA/hCERK, and FcγRIIA/G198DhCERK mutant and then challenged for 30 min with E1gG. Values represent the mean ± S.D. of four experiments. *** denotes p < 0.001.
crease in ingested red cells compared with the FcRIIA control (Fig. 1A). Stably transfected cells containing hCERK displayed a 2.5-fold increase in their phagocytic index compared with control cells and a 1.5-fold increase compared with vector control cells at 30 min following challenge with ElgG (1 × 10⁹/ml). At the indicated time points, COS-1 cells were removed with trypsin. The ElgG not internalized were removed by lysis, and the ceramide kinase activity was measured as described under “Experimental Procedures.” The values represent the mean ± S.D. (n = 4). * denotes p < 0.05. B, the time course of ceramide generation during phagocytosis in COS-1 cells transfected with vector and FcRIIA/hCERK. COS-1 cells (3 × 10⁹/dish) transfected with hCERK were challenged with ElgG (1 × 10⁹/ml) at 37 °C. At the indicated time points, COS-1 cells were removed with trypsin. The ElgG not internalized were removed by lysis, and the ceramide generation was measured as described under “Experimental Procedures.” The values represent the mean ± S.D. (n = 6 for CERK, and n = 3 for vector control).

Ceramide kinase activity was measured to ascertain whether hCERK transfectants would exhibit enhanced activity during phagocytosis. Ceramide kinase activity increased to maximal levels by 7 min following challenge with ElgG (Fig. 2A). Ceramide kinase activity increased in the FcRIIA control cells from a resting value of 0.39 ± 0.1 to 0.86 ± 0.1 pmol/min/10⁶ cells. Ceramide kinase increased from 0.29 ± 0.1 to 0.82 ± 0.4 in the vector control cells. However, in hCERK-transfected cells, the CERK activity increased from a resting value of 0.7 ± 0.2 to 1.46 ± 0.24 pmol/min/10⁶ cells (p < 0.05) during the phagocytic challenge. Ceramide kinase activity (pmol/min/10⁶ cells) of the catalytically inactive mutant G198D was 0.12 ± 0.04 at rest, 0.16 ± 0.1 at 7 min, and 0.097 ± 0.07 at 30 min with a phagocytic challenge. These differences were not statistically significant. The CERK activity rapidly fell by 10 min to levels approaching the basal-inactivated state in both the FcRIIA and FcRIIA/hCERK-transfected cells, but the activity rose significantly by 30 min in the FcRIIA/hCERK-transfected cells (Fig. 2A).

Previous work has demonstrated the existence of phagocytosis-dependent ceramide formation in FcRIIA-transfected COS-1 cells (11). Ceramide mass was measured to determine whether the changes in ceramide kinase activity might second-
arily lower ceramide levels in hCERK transfectants. There were no significant differences in ceramide levels between vector and CERK-transfected cells (Fig. 2B). Because ceramide levels were no lower in the hCERK transfectants, these data support the view that changes in C1P and not ceramide accounted for differences in phagocytic response.
To ascertain whether C1P was also formed during phagocytosis, hCERK COS-1 cells were labeled with the ceramide precursor D-erythro-[3H]sphingosine. The lipids were then extracted, and the product profile was evaluated in several thin layer chromatography solvent systems by comparison to known standards. High performance thin layer plates were activated with 2% sodium borate for 1 hour at 100 °C. Using a solvent system consisting of chloroform/acetone/methanol/acetic acid/water (40/16/12/8/4, v/v), a labeled product comigrating with C1P (Rf 0.50) was detected. The product was resistant to alkaline hydrolysis and was clearly separable from sphingosine (Rf 0.56), phosphatidic acid (Rf 0.80), and lysophosphatidic acid (Rf 0.27).

C1P radioactivity increased by 3-fold in the hCERK-transfected cells compared with the vector control and non-transfected FcγRIIA cells (Fig. 3, A and B). Labeling stably transfected COS-1 cells with D-erythro-[3H]sphingosine and challenging them with ElgG for 30 min resulted in a further increase in the C1P formed. The phagocytic challenge increased C1P levels by 2-fold in the hCERK-transfected cells compared with FcγRIIA or FcγRIIA/vector control cells (Fig. 3B). D-Erythro-[3H]sphingosine-labeled hCERK-transfected COS-1 cells were also challenged with ElgG for different periods of time, and C1P formation was evaluated. C1P increased significantly at 7 min simultaneously with the increase of ceramide kinase activity and remaining elevated to a 30-min time point (Fig. 4). Assuming that the cell sphingolipids were labeled to equilibrium by 24 h, the change in C1P activity would represent an increase in C1P from 11.52 ± 0.9 to 20.67 ± 6 pmol/10⁶ cells at 7 min and to 21.1 ± 8 pmol/10⁶ cells at 30 min. There was a significant difference for C1P levels at the 7- and 30-min time points.

Lipid rafts are believed to be small membrane microdomains containing cholesterol and sphingolipids (21). Due to the high degree of saturated fatty acyl content of these sphingolipids, lipid rafts are thought to form a distinct gel-like ordered lipid phase within cell membranes. These domains have been reported to contain a variety of signaling molecules. Therefore, it was next confirmed that lipid rafts could be identified and isolated from COS-1 cells. Lipid rafts were subsequently identified by a caveolin-1 marker (Fig. 5A). Caveolin-1 was expressed in the lipid rafts but only weakly in non-membrane raft fractions obtained from FcγRIIA, vector control, and hCERK-transfected cells. Utilizing an antibody to c-Myc, c-Myc-tagged hCERK was identified in the appropriate raft fraction generated from Myc-tagged hCERK vector but not...
from cells generated with the control vector. An anti-hCERK antibody was employed to evaluate the subcellular distribution of hCERK. Immunoblots yielded bands consistent with the known molecular weight of hCERK (Fig. 5C). Using this antibody, the intracellular localization of CERK during activation was evaluated. COS-1 cells transfected with hCERK were stimulated with mβCD for different periods of time and then subjected to fractionation. Immunoblots revealed that, under resting conditions, CERK was distributed within the cytosol and only weakly in the raft fraction. With stimulation, CERK was translocated to the raft fraction and diminished in the cytosolic fraction (Fig. 5C). The ceramide kinase activity was higher in the cytosolic fraction at the zero time point (11.23 ± 0.05 pmol/min/mg protein). As time progressed, the activity in the raft fraction increased from 4.95 ± 0.1 to 8.76 ± 0.07 pmol/min/mg protein at 7 min and to 14.86 ± 0.2 pmol/min/mg protein at 30 min following stimulation (Fig. 5D). Correspondingly, ceramide kinase activity was enriched in the caveolar fractions generated from non-stimulated transfected cells. The activity in hCERK-transfected cells was 4.9 ± 0.1 pmol/min/mg protein compared with 1.0 ± 0.08 pmol/min/mg protein for FcγRIIA controls and 1 ± 0.04 pmol/min/mg protein from caveolae obtained from vector control-transfected cells.

To establish the critical role of the lipid rafts in phagocytosis, the lipid rafts were disrupted with 5 mM mβCD (17). After the metabolic labeling, the cells with mβCD and then challenged for 30 min with E1gG. The phagocytic index over time was determined as described under “Experimental Procedures.” Values represent the mean ± S.D. of three experiments. * denotes p < 0.05 and indicates the difference between FcγRIIA- and FcγRIIA/hCERK-transfected cells at the 30 min. ** denotes p < 0.01.
emission spectra of $-37$ nm ($<0.001$) at the site of phagocytosis (Fig. 7). However, no change in emission was noted at a lateral site distant from the point of contact with EIgG. These studies provide direct evidence in living COS-1 cells for the presence of $L_0$ phase lipid domains in sites engaged in phagocytosis that spatially correspond to sites where lipid rafts are present.

When Laurdan spectra were collected for 1 s with a 20-s interval between each collection, we observed that the spectra did not change at a region of the membrane distinct from the site of phagocytosis. In contrast, when spectra were obtained from the vicinity of phagocytosis, the Laurdan spectra shifted at the site of phagocytosis in the cells transfected with the FcRIIA/hCERK (Fig. 8). As noted in both Figs. 7 and 8, the spectra changed only slightly at the site of phagocytosis in FcRIIA control cells and in the vector control or the FcRIIA/G198DhCERK mutant.

**DISCUSSION**

An emerging theme in the control of cellular signaling and metabolic regulation is the importance of the spatial localization of the molecules participating in cell activation. Recent advances in the study of cell membrane structure have led to the concept that microdomains exist within the fluid bilayer of the plasma membrane. One type of microdomain is the lipid raft, which is enriched with tightly packed sphingolipids and cholesterol (22). These rafts present in both excitable and nonexcitable cells localize a number of proteins, including multiple signal transduction molecules, while excluding others (23). Different types of rafts are likely to exist based on the specific marker proteins and ultrastructure (24). Caveolae represent a well studied subpopulation of lipid rafts having an invaginated morphology and containing caveolin that interacts directly with several other proteins (23, 25, 26). Caveolin forms a scaffold onto which many classes of signaling molecules can assemble to generate signaling complexes. Signaling components that are highly enriched in caveolae include low molecular weight and heterotrimeric G proteins, src family kinases, mitogen-activated protein kinase, protein kinase C, and the p85 subunit of phosphatidylinositol 3-kinase (27, 28). The FcRIIA receptor also localizes to caveolae upon cross-linking (29). Ceramide generation from sphingomyelin may occur primarily in lipid rafts (30) and may facilitate the clustering of FcRIIA and its association with rafts (31). These observations suggest that caveolae may provide the structure for assembling critical components of the signaling pathways that mediate the early phagocytic response.

The recent cloning of hCERK has made it possible to evaluate the physiological functions of ceramide kinase and of C1P. Because polymorphonuclear leukocytes are not readily amenable to transfection, an in vitro cell culture system was chosen to study the mechanisms of C1P-induced phagocytosis. We took advantage of the ability of COS-1 cells to express a phagocytic phenotype when transfected with the FcRIIA. In the present study, COS-1 cells stably transfected with FcRIIA were also stably transfected with hCERK. Using this model, two important observations were made. First, the increased expression of hCERK resulted in heightened FcRIIA-mediated phagocytosis. In concert with the increase in phagocytosis, we observed that, in both FcRIIA- and FcRIIA/hCERK-transfected cells, ceramide kinase activity and C1P generation increased.

Second, in labeling cells with Laurdan, we observed that...
FcγRIIA/hCERK transfectants exhibited a dramatic shift in emission during phagocytosis, corresponding to a distinct change in lipid-ordered structure. The change in Laurdan emission provides strong evidence for the existence of lipid raft-like Lo domain in COS-1 cells at the site of phagosome formation. Lo phase lipids have been observed in reconstituted lipid rafts using Laurdan (32). Their demonstration in living cells has been limited to neutrophils. In this study, optical microspectrophotometry of Laurdan-labeled polarized neutrophils revealed the presence of large lipid raft-like domains at the lamellipodium (33).

Phospholipid composition is known to play a significant role in membrane fusogenicity. The fusion of apposed membranes requires destabilizing of the membranes to render them susceptible to fusion. This can be the result of the Ca²⁺-induced phase separation of rigid crystalline domains of acidic phospholipids (e.g. phosphatidic acid) within mixed lipid membrane (34). Fusion can be initiated between closely opposed membranes at the boundaries between such crystalline and the surrounding non-crystalline domains. Such boundaries represent structurally unstable points and thus offer focal points for the mixing of molecules from opposed membranes. A calcium-dependent ceramide kinase was reported previously in synaptic vesicles, which led to the hypothesis that C1P may attenuate membrane charge, regulate vesicle transport, or mediate membrane fusion (1). This possibility was evaluated in neutrophils in a previous study in which we reported that C1P is formed during phagocytosis and that C1P promotes the fusion of complex liposomes (7). Recently, Igarashi and co-workers (9) found that Ca²⁺-dependent C1P formation was involved in the degranulation pathway of mast cells. The degranulation process includes the fusion of the plasma membrane with secretory granules (9). Their findings can potentially be explained by the alteration in membrane fusogenicity elicited by an increase of C1P. In combination with the present report, these data suggest that C1P may promote phagosome formation by enhancing the rapidity by which pseudopods engage and merge with each other in lipid rafts.

**Fig. 8. Kinetic study of lipid order membrane domains.** Each spectrum was collected for 1 s with a 20-s period between each collection. A–C, spectra were collected from a region of the membrane distant from the site of phagocytosis denoted by the dark rectangle. D–F, spectra obtained from the vicinity of phagocytosis as denoted by the dark rectangles. Panels A and B show cells before and after the collection of the spectra in panel C, respectively. Similarly, panels D and E show cells before and after collection of the spectra shown in panel F. As time passes, the Laurdan spectra far from the site of phagocytosis do not change (panel C). However, the emission spectra at the site of phagocytosis change over time (panel F).
Cellular fractionation of COS-1 cell transfectants with hCERK also demonstrated that ceramide kinase activity is localized in cytosol and transferred to lipid rafts during stimulation with IgG. Using an antibody raised against CERK, the cytosolic distribution of CERK was reported in mast cells (9). However, in another study, CERK was found in the membrane fraction in human embryonic kidney 293 cells (6). The observation of the translocation of CERK during activation in the present study may provide and explanation for these discrepant observations (30, 31).

Lipid rafts are also sites of signal transduction. Cross-linking of receptors may increase their affinity for rafts. Partitioning of receptors into rafts results in a new microenvironment where their phosphorylation state can be modified by local kinases and phosphatases modulating downstream signaling. The coalescence of several rafts may result in an amplification of a transduced signal.

One approach for studying the function of lipid rafts involves depleting cells of cholesterol. Lipid rafts are held together to a great extent via interactions between cholesterol and sphingolipids. COS-1 transfectants were treated with methyl-β-cyclo-dextrin. Treatment with d-erythro-[3H]sphingosine and erythro-[3H]sphingosine and then treated with methyl-β-cyclo-dextrin presumably disturbed the lipid-ordered structure of COS-1 transfectants and reduced phagocytosis.

Lipid and protein interactions play an important role in keeping the plasma membrane intact and functionally active. Many membrane-bound enzymes require a special conformation in order to display their optimal activity. There are a large number of lipids present with the potential to form rafts in the plasma membrane including C1P (35). Because of the presence of C1P, a liquid-order domain with higher rigidity than the plasma membrane including C1P (35). Because of the presence of C1P, a liquid-order domain with higher rigidity than the plasma membrane including C1P (35). Because of the presence of C1P, a liquid-order domain with higher rigidity than the plasma membrane including C1P (35). Because of the presence of C1P, a liquid-order domain with higher rigidity than the plasma membrane including C1P (35). Because of the presence of C1P, a liquid-order domain with higher rigidity than the plasma membrane including C1P (35). Because of the presence of C1P, a liquid-order domain with higher rigidity than the plasma membrane including C1P (35). Because of the presence of C1P, a liquid-order domain with higher rigidity than the plasma membrane including C1P (35).

References

1. Bajjalieh, S. M., Martin, T. F., and Floor, E. (1989) J. Biol. Chem. 264, 14354–14360.
2. Martin, T. W. (1988) Biochim. Biophys. Acta 962, 292–296.
3. Kolesnick, R. N., and Hemer, M. R. (1990) J. Biol. Chem. 265, 18803–18808.
4. Shinghal, R., Scheller, R. H., and Bajjalieh, S. M. (1993) J. Neurochem. 61, 2279–2285.
5. Dressler, K. A., and Kolesnick, R. N. (1990) J. Biol. Chem. 265, 14917–14921.
6. Sugiuura, K., Kono, K., Liu, H., Shizukuda, T., Minekura, H., Spiegel, S., and Kohama, T. (2002) J. Biol. Chem. 277, 23294–23300.
7. Hinkovska-Galcheva, V. T., Boxer, L. A., Mansfield, P. J., Harsh, D., Blackwood, A., and Shayman, J. A. (1998) J. Biol. Chem. 273, 33203–33209.
8. Blackwood, R. A., Transeau, A. T., Harsh, D., Brower, R. C., Zacharek, S. J., Smolen, J. R., and Hessler, R. J. (1996) J. Leukocyte Biol. 59, 663–670.
9. Mitsutake, S., Kim, T. J., Inagaki, Y., Kato, M., Yasuhata, T., and Igarashi, Y. (2004) J. Biol. Chem. 279, 17579–17587.
10. Petrus, B. J., Bieaweska, A., Spiegel, S., Roddy, P., Hannun, Y. A., and Chal-fant, C. E. (2003) J. Biol. Chem. 278, 38306–38321.
11. Hinkovska-Galcheva, V., Boxer, L., Mansfield, P. J., Schreiber, A. D., and Shayman, J. A. (2003) J. Biol. Chem. 278, 974–982.
12. Suchard, S. J., Hinkovska-Galcheva, V., Mansfield, P. J., Boxer, L. A., and Shayman, J. A. (1997) Blood 89, 2139–2147.
13. Hinkovska-Galcheva, V., Kjoldsen, L., Mansfield, P. J., Boxer, L. A., Shayman, J. A., and Suchard, S. J. (1996) Blood 91, 4761–4769.
14. Shu, L., Lee, L., Chang, Y., Holzman, L. B., Edwards, C. A., Shelden, E., and Shayman, J. A. (2000) Arch. Biochem. Biophys. 383, 89–90.
15. Van Veldhoven, P. P., Bishop, W. R., Yurivich, D. A., and Bell, R. M. (1995) Biochem. Mol. Biol. Int. 36, 21–39.
16. Preiss, J. E., Loomis, C. R., Bell, R. M., and Niedel, J. E. (1987) Methods Enzymol. 141, 294–300.
17. Ottico, E., Prinetti, A., Prioni, S., Giannotta, C., Basso, L., Chigorno, V., and Sonnino, S. (2003) J. Lipid Res. 44, 2142–2151.
18. Parassassi, T., Gratton, E., Yu, W. M., Wilson, P., and Levi, M. (1997) Biophys. J. 72, 2413–2429.
19. Kindezelski, A. L., and Petty, H. R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 9207–9212.
20. Worth, R. G., Kim, M. K., Kindzelislski, A. L., Petty, H. R., and Schreiber, A. D. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 4533–4538.
21. Simons, K., and Toomre, D. (2000) Nat. Rev. Mol. Cell. Biol. 1, 31–39.
22. Simons, K., and Ikonen, E. (1997) Nature 387, 569–572.
23. Harder, T., and Simons, K. (1997) Curr. Opin. Cell Biol. 9, 534–542.
24. Volonte, D., Galbiati, F., Li, S., Nishiyama, K., Okamoto, T., and Lisanti, M. P. (1999) J. Biol. Chem. 274, 12702–12709.
25. Okamoto, T., Schlegel, A., Scherer, P. E., and Lisanti, M. P. (1998) J. Biol. Chem. 273, 5419–5422.
26. Hooper, N. M. (1999) Mol. Membr. Biol. 16, 145–156.
27. Oka, N., Yamamoto, M., Schwenke, C., Kawabe, J., Ebina, T., Ohno, S., Cour, J., Lisanti, M. P., and Ishikawa, Y. (1997) J. Biol. Chem. 272, 33416–33421.
28. Pike, L. J., and Casey, L. (1996) J. Biol. Chem. 271, 26453–26456.
29. Kwiatkowska, K., Frey, J., and Sobota, A. (2003) J. Cell Sci. 116, 537–550.
30. Liu, P., and Anderson, R. G. (1995) J. Biol. Chem. 270, 27179–27185.
31. Abdel Shaker, A. B., Kwiatkowska, K., and Sobota, A. (2004) J. Biol. Chem. 279, 36778–36787.
32. Dietrich, C., Bagatolli, L. A., Volovry, Z. N., Thompson, N. L., Levi, M., Jacobson, K., and Gratton, E. (2001) Biophys. J. 80, 1417–1428.
33. Kindzelislski, A. L., Sitrin, R. G., and Petty, H. R. (2004) J. Immunol. 172, 4681–4685.
34. Papahadjopoulos, D., Vail, W. J., Newton, C., Nir, S., Jacobson, K., Poste, G., and Lazo, R. (1977) Biophys. Chem. Acta 465, 579–598.
35. Mayr, S., and Maxfield, F. R. (1998) Mol. Biol. Cell 9, 929–944.
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