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Intracellular lipid flux and membrane microdomains as organizing principles in inflammatory cell signaling[1]

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Abstract

Lipid rafts and caveolae play a pivotal role in organization of signaling by Toll-like Receptor (TLR)4 and several other immune receptors. Beyond the simple cataloguing of signaling events compartmentalized by these membrane microdomains, recent studies have revealed the surprisingly central importance of dynamic remodeling of membrane lipid domains to immune signaling. Simple interventions upon membrane lipid, such as changes in cholesterol loading or crosslinking of raft lipids, are sufficient to induce micron-scale reordering of membranes and their protein cargo with consequent signal transduction. In this review, using TLR signaling in the macrophage as a central focus, we discuss emerging evidence that environmental and genetic perturbations of membrane lipid regulate protein signaling, illustrate how homeostatic flow of cholesterol and other lipids through rafts regulates the innate immune response, and highlight recent attempts to harness these insights towards therapeutic development.

Keywords

Lipid raft; caveolae; macrophage; Toll like Receptor; cholesterol

Since the inception of the lipid raft hypothesis in 1997 (1), a profusion of studies have reported roles for these cholesterol-enriched membrane microdomains in organization of cell signaling. As a crossroads for immunology, biophysics, and lipid science, the raft field has suffered growing pains in terminology, technique, and interpretation. Progressively refined imaging techniques continue to support the existence of lateral protein/lipid heterogeneities in biological membranes (2, 3), but the precise nature, size, and malleability of these microdomains remain a matter of debate. A burgeoning field that has catalogued an increasing number of signaling events within rafts at the same time finds itself at risk of losing sight of the implications of this localization. In this review, rather than focus on definitions of rafts/caveolae (for this the reader is referred to recent scholarly reviews (2, 4, 5)), the objective will be to synthesize and interpret emerging insights on how genetic and environmental modification of raft lipid plays a fundamental role in determining immune signaling and disease. The case will be made that dynamic remodeling of raft lipid is not only necessary in many signaling cascades, but that primary perturbations of raft lipid (e.g., cholesterol loading or unloading, raft coalescence) can also be sufficient initiating events to

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trigger protein signaling. Using the macrophage, and, in particular, TLR signaling in macrophages as a primary case in point, the dependence of inflammatory signaling upon cholesterol-loading conditions and on the regulatory proteins that control homeostatic intracellular trafficking of cholesterol through rafts will be highlighted.

Lipid Rafts and Caveolae

Lipid rafts are thought to be highly dynamic, nanoscale (i.e., <200 nm), cholesterol- and sphingolipid-enriched membrane microdomains, likely present in all eukaryotic cells, that compartmentalize select signaling and functional events. While it is difficult to place a lower limit on their size in the resting state, and evidence indeed exists for 'lipid shells' surrounding individual proteins in biological membranes (2), rafts can also be driven to coalesce into more stable, micron-range domains through lipid-lipid, protein-lipid, and protein-protein interactions. The mechanism(s) underlying raft 'coalescence' or 'clustering,' however, in many cases remain elusive. It is generally thought that the saturated acyl chains of raft sphingolipids and phospholipids exhibit tight packing in a manner analogous to the liquid-ordered (Lo)2 domains observed in model membranes, and that this may account for their resistance to solubilization by cold nonionic detergents (e.g., Triton-X-100). However, as detergent can itself induce the formation of domains in membranes (6), rafts should not be equated with 'detergent-resistant membranes' (DRMs); nor can identification of a protein in DRMs be taken as sufficient evidence for assigning raft localization *in vivo*. While good evidence supports the co-existence within cell membranes of heterogeneous populations of lipid rafts, isolation of DRMs of discrete composition with the use of different detergents should not be considered as evidence for discrete raft domains *in vivo*.

Caveolae are ~60–80 nm cholesterol-enriched membrane invaginations whose flask-shaped morphology derives from caveolin proteins, expression of which suffices to confer caveolar morphology (7). Caveolae are thought to represent a discrete, specialized subpopulation of membrane microdomains, and thus should not be simply equated with 'lipid rafts'. The caveolin proteins, through direct regulatory interactions with other proteins (e.g., TLR4 (8)), are in particular thought to play a central role in signal regulation within caveolae. Of interest, while caveolae are well-studied in certain cell types (e.g., endothelial cells, fibroblasts) and thought to be absent in others (e.g., lymphocytes), their presence in macrophages is less well-defined and indeed controversial, varying by macrophage type (reviewed in (9)).

While rafts and/or caveolae promote immune receptor signaling in several pathways by serving as platforms for dynamic assembly of signaling complexes, in other cases, raft-localization suppresses signaling (e.g., TGFβ and epidermal growth factor receptors) (10-12). Moreover, in addition to concentrating signaling proteins, the lipid micro-environment of rafts may itself alter protein function (13), in some cases shaping signaling much more selectively than as just a simple binary switch. Thus, localization of the TNF receptor to raft vs. non-raft domains determines responses to TNFα, including cell fate as well as signaling events (14).

Protein localization to rafts, in many cases determined by GPI linkage or palmitoylation, is also thought to be responsive to raft cholesterol levels. Indeed, lipid-induced changes in the

[2]Abbreviations used in this paper: ABC, ATP Binding Cassette; ADAM, a disintegrin and metalloproteinase domain-containing protein; Apo, apolipoprotein; DPPC, dipalmitoyl-phosphatidylcholine; DPPE, dipalmitoyl-phosphatidylethanolamine; DRM, detergent resistant membrane; HDL, high density lipoprotein; mβCD, methyl-betacyclodextrin; MyD88, myeloid differentiation primary response gene 88; NPC1, Niemann Pick C1; oxLDL, oxidized low density lipoprotein; PUFA, polyunsaturated fatty acid; SR, scavenger receptor; TRIF, Toll-Interleukin-1 receptor-domain-containing adapter-inducing interferon-β; TRAM, TRIF-related adaptor molecule.

raft proteome likely explain reports, discussed below, that acute or chronic changes in raft cholesterol may determine protein signaling. Conversely, some proteins (e.g., NAP-22) and peptides (apolipoprotein A-I mimetic 4F) may themselves induce phase separation of cholesterol-rich and –poor domains (15), or induce raft signaling by deforming membrane lipids (16). It is also important to note that proteins, through scaffolding and other interactions, have been shown in some contexts to play dominant roles in determining membrane domains in immune cells (17, 18). Raft coalescence induced in dendritic cell membranes by physical contact of uric acid crystals (19), or in RAW 264.7 membranes by altered topography of the cell substratum (20), can also activate signaling proteins including Syk and NF-κB. Taken together, these findings suggest that protein and lipid remodeling of the membrane interact to shape domains and cell signaling, and that raft signaling may be profoundly influenced or indeed induced by 'ligand independent' interventions upon plasma membrane lipid.

Rafts as poised signaling units: signal initiation by microdomain coalescence

Interestingly, recent work indicates that the resting plasma membrane may be poised at the edge of a phase boundary such that simple membrane perturbations can drive large-scale phase separation of discrete protein/lipid macrodomains, thereby inducing signaling. Thus, crosslinking of the raft glycosphingolipid GM1 with cholera toxin B subunit induces cholesterol-dependent coalescence of micron-scale GM1 domains that recruit lipid-anchored raft proteins but exclude the non-raft transferrin receptor (21). Cholesterol depletion with methyl-β-cyclodextrin (mβCD) also induces micron-scale phase separation of the plasma membrane into fluid and ordered domains in living CHO cells (22), GM1-rich domains that concentrate Lck and LAT and signal to ERK activation in T cells (23), and GM1- and CD11b-rich domains in neutrophils (24). Imaging techniques with higher resolution than fluorescence microscopy will almost certainly be required to properly characterize raft co-localization and coalescence. Nonetheless, together, these findings confirm that membrane lipid remodeling is sufficient to drive cell signaling by reorganizing protein cargo, and also demonstrate, perhaps paradoxically, that cholesterol depletion can increase membrane order and coalescence of raft-like domains, a topic to which we will return below.

Notably, antibody-mediated crosslinking of several GPI-linked proteins can also coalesce/remodel rafts and induce signaling by co-patching proteins within rafts. Crosslinking of external leaflet raft proteins induces co-patching and activation of inner leaflet raft proteins such as H-ras (25), whereas crosslinking of GM1 can interestingly induce its co-patching with TLR4 (26) and CD18 (27). As oligomeric cholesterol-binding cytolysins such as listeriolysin O both cluster CD14-rich rafts (28) and activate TLR4 (29), it seems plausible that some TLR4 agonists may activate this receptor through raft-mediated receptor clustering. In this light, it is important to remember that lipopolysaccharide (LPS), the canonical TLR4 ligand, is itself a polymeric molecule that induces receptor clustering.

The two faces of rafts: signal inhibition and activation by raft-perturbing agents

Perhaps the most widely used experimental tools used to 'disrupt' rafts are the β-cyclodextrins, mβCD and 2-hydroxyl-β-CD, cyclic oligosaccharides that remove cholesterol from membranes. While numerous papers have used mβCD to infer that cell signals, including those induced by LPS, are raft-dependent, some caution is warranted (reviewed in (30)). Thus, mβCD depletes cholesterol to varying degrees in different cell types, may under high concentrations (i.e., >10 mM) or prolonged incubations (>30 min) also remove extra-

raft cholesterol or even cause cell death, and may interact with non-sterol lipids or immobilize membrane proteins through effects on the cytoskeleton (30). Moreover, mβCD and other *in vitro* manipulations of cell membrane lipid may not necessarily be physiologically relevant. These concerns notwithstanding, good evidence suggests that low concentration/short incubation usage of mβCD may be selective for raft cholesterol (30–32). Moreover, multiple control strategies are available, including clamping of cell cholesterol with mβCD-cholesterol complexes, use of the structurally dissimilar cholesterol-sequestering agents filipin and nystatin, cholesterol depletion by lipoprotein-deficient serum, as well as additional raft-perturbing agents that have been described (Table I).

While raft isolation and perturbation strategies have been used to show the requirement for raft integrity in several signaling pathways, a perhaps more intriguing chain of literature has shown that acute cholesterol depletion can itself initiate signaling cascades in a cell type-dependent fashion. CDs activate ERK in Rat-1 and RAW 264.7 cells (31, 33), p38 and Cdc42 in human neutrophils (34), and tyrosine phosphorylation in RBL-2H3 cells (35). MβCD and filipin initiate ligand-independent activation of epidermal growth factor receptor (10, 36) and Fas (37) by a mechanism involving displacement of these receptors from rafts. Conversely, cholesterol depletion may also cause a disintegrin and metalloproteinase domain-containing protein (ADAM)10- and/or ADAM17-dependent cleavage of several receptors (IL-6R, CD44, CD30, TNFR1, TNFR2) by causing their displacement from rafts (38–41). MβCD also activates NF-κB in macrophages by a mechanism involving the adaptor myeloid differentiation primary response gene 88 (MyD88)(33). Consistent with these findings with CDs, we recently reported that the physiologic cholesterol acceptor apolipoprotein (apo)A-I activates a TLR2-, TLR4-, and MyD88-dependent pathway to NF-κB in macrophages (33). While the full significance of these assorted findings is not yet clear, and multiple underlying mechanisms are likely involved, taken together, these reports suggest that native microdomains of the cell membrane may serve to maintain signal quiescence by sequestering pathway components, and that their perturbation through cholesterol removal may induce pathways the nature of which is determined by the specific signaling proteins expressed in the cell under study.

Regulation of rafts and TLR signaling by intracellular cholesterol traffic

While it is now well recognized that pharmacologic raft-perturbing agents can modify raft-dependent signaling by delocalization of proteins, the effects of cholesterol loading on raft function under more physiologic settings are perhaps less widely appreciated. In primary murine macrophages, raft levels of TLR4 and TLR9, and cell responsiveness to TLR2, TLR4, TLR7, and TLR9 ligands, are all directly associated with exogenously manipulated raft cholesterol levels (42, 43). Moreover, hypercholesterolemia increases macrophage raft cholesterol in mouse and man *in vivo*, increasing cell responsiveness to LPS (44, 45). Perhaps more striking are reports that indicate that acute cholesterol loading of membranes may suffice to activate TLRs. Thus, cholesterol loading of the macrophage plasma membrane induces TLR4-dependent signaling, and loading of endosomal membranes induces TLR3- and TLR4-dependent responses (46). Conversely, it is also recognized that, in other contexts (e.g., modified lipoprotein treatment) cholesterol loading can also be associated with reduced macrophage inflammatory function (47, 48). This may in part reflect the propensity of conditions to load cytosolic cholesterol ester instead of membrane cholesterol, as well as to activate nuclear receptors (e.g., Liver X Receptors, Peroxisome Proliferator-activated Receptors).

Physiologically, raft/caveolar cholesterol content is regulated by homeostatic trafficking of cholesterol through the cell (Figure 1), a topic covered in depth by recent comprehensive reviews (49). In brief, following cholesterol synthesis in the endoplasmic reticulum (ER) or

endosomal recycling of internalized cholesterol to the ER/Golgi by Niemann Pick C1 (NPC1) protein, it is thought that caveolae are assembled in the Golgi and transported to the plasma membrane in a caveolin- and NPC1-dependent fashion (7, 50). Thus, NPC-deficient fibroblasts have reduced plasma membrane caveolar cholesterol (50) and late endosomal cholesterol overload with raft overcrowding (51). Raft/caveolar cholesterol is, in turn, regulated by transporter-mediated (ATP Binding Cassette [ABC]A1; ABCG1; and scavenger receptor [SR]-BI) efflux of plasma membrane cholesterol to extracellular acceptors including lipid-free apoA-I and HDL, as well as by aqueous diffusion. Overexpression of ABCA1 (52), and treatment with HDL or apoA-I (53, 54) all disrupt/deplete raft domains, inhibiting raft-dependent signaling. The effect of stimulated cholesterol efflux is quite complex, however, as apoA-I, like mβCD, can enhance responses to some stimuli such as platelet derived growth factor (55) by removing cholesterol from rafts (55, 56). Similarly, SR-BI-mediated cholesterol efflux to HDL activates eNOS (57). Moreover, apoA-I may increase caveolar cholesterol by stimulating its transfer from intracellular compartments faster than its efflux (58).

Building upon earlier reports that TLR4 signaling occurs in lipid rafts (34), an exciting chain of literature has recently demonstrated profound effects of cholesterol trafficking through rafts on TLR signaling in the macrophage. ABCA1-null macrophages have enlarged, cholesterol-laden lipid rafts (42, 59) (Figure 2) containing increased TLR4 (43), and are hyperresponsive to LPS (42, 59–61) as well as to TLR-2, -7, and -9 ligands (42, 61). ABCG1-null macrophages display a similar albeit perhaps more pronounced TLR-hyperresponsive phenotype (61, 62). NPC1-null macrophages display basal activation of TLR3, -7, -8, and -4 (46), the first three of which may reflect cholesterol overloading of endosomal rafts, and the last, TLR4 accumulation in endosomes due to blocked trafficking (63). Taken together, these reports indicate an intriguing degree of overlap between the pathway for trafficking of host lipids and that for recognition of microbial lipids, perhaps even suggesting common evolutionary roots between the two. Indeed, it was recently reported that, in addition to regulating efflux of cholesterol and phospholipid, ABCA1 also regulates efflux of LPS from the macrophage (64).

Modification of rafts and their signaling by non-sterol lipids

Complex effects upon raft remodeling and signaling in TLR and other pathways have also been described for sphingomyelin and the product of its breakdown by sphingomyelinase, ceramide. Sphingomyelin and cholesterol promote raft formation in the Golgi via strong physical interactions (65), and ceramide indeed stabilizes rafts more effectively than cholesterol (66). On the other hand, good evidence indicates that sphingomyelinase treatment and ceramide itself both displace cholesterol from rafts (67, 68) in a manner that could realistically occur *in vivo* during inflammation. Indeed, acid sphingomyelinase-induced remodeling of the plasma membrane into enlarged ceramide-rich rafts during *P. aeruginosa* infection is critical for bacterial internalization and for successful host defense (69). Interestingly, local acid sphingomyelinase-mediated ceramide production in rafts has also been reported to be required for LPS-induced recruitment of TLR4 to rafts (70), and ceramide itself elicits TLR4-dependent signaling (71). While the full implications of these findings are not yet clear, it appears plausible that ceramide and cholesterol may ‘compete’ to form somewhat distinct rafts, and that dynamic remodeling of raft lipid and protein composition by local ceramide induction may be a critical step in TLR signaling and perhaps other pathways.

Phospholipids have also been shown to modulate raft structure and function. Dipalmitoyl-phosphatidylethanolamine (DPPE) partitions into lipid rafts, inhibiting MHC peptide-induced raft recruitment of acylated proteins in $CD8^+$ T cells (72) and TNF-induced

recruitment of its receptor to rafts in HT1080 cells (14), without displaying overt effects on raft integrity. Similarly, the surfactant phospholipid dipalmitoyl-phosphatidylcholine (DPPC) and surfactant itself both attenuate LPS signaling by inhibiting TLR4 recruitment to rafts (73), perhaps suggesting that the lipid environment of the alveolus may dampen innate immune responses through effects on rafts. A role for phospholipid metabolism in cell-intrinsic TLR4 responses is also suggested by a report that lysophosphatidylcholine acyltransferase is required for LPS-induced translocation of TLR4 to rafts (74).

Oxidized lipids present during disease have been shown to modify rafts with associated effects on signaling. Thus, oxidized low density lipoprotein (oxLDL) reduces caveolar cholesterol, displacing eNOS and caveolin (75). The major oxysterol in oxLDL, 7-ketocholesterol, partitions into rafts, depleting them of cholesterol (56) and activating Src within them (76). Oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine, a major oxidized phospholipid present in oxLDL, has also been shown to disrupt rafts through a mechanism involving ceramide induction, thereby inhibiting LPS-induced translocation of TLR4 to rafts (77, 78). Reports that reactive oxygen species are not only necessary but also sufficient to induce assembly of TCR-associated proteins in T cell rafts (79) and TLR4 trafficking to rafts in macrophages (26), suggest that oxidation of membrane lipids may also serve as a critical step in innate and adaptive immune signaling through raft remodeling. Indeed, an antioxidant α-tocopherol derivative has been shown to attenuate LPS signaling by interfering with CD14 and TLR4 recruitment to rafts (80).

Emerging opportunities for targeting rafts in disease

Several strategies have shown early promise as potential therapeutic measures to modify raft signaling through intervening upon raft lipids. These include dietary polyunsaturated fatty acids (PUFAs), statins (i.e., HMG CoA reductase inhibitors), squalene synthase inhibitors, raft-targeting lipids, and edelfosine. PUFAs such as docosahexaenoic acid and eicosapentaenoic acid inhibit signaling in Jurkat T cells by incorporating into rafts and displacing acylated signaling proteins (Lck, Fyn, LAT) (81). Alternatively, it has been proposed that PUFAs do not incorporate into rafts due to their unsaturation, but rather form extra-raft domains that interfere indirectly with raft-dependent protein clustering (82, 83). Statins, increasingly studied for their anti-inflammatory actions, attenuate leukocyte function in part through membrane raft depletion (84). Inhibitors of squalene synthase, an enzyme downstream of HMG CoA reductase in the cholesterol biosynthetic pathway, selectively reduce raft cholesterol in cancer cells and induce cell death (85). In an additional raft-centric strategy for cancer therapy, adhesion and cell cycle progression of breast cancer cells was recently shown to be more effectively inhibited by targeting a Src family kinase inhibitor to rafts through palmitoylation (86). Finally, a recent study has shown that the phospholipid ether edelfosine may be an effective therapeutic for multiple myeloma by accumulating in myeloma cell rafts, thereby inducing apoptosis through co-clustering of rafts and death receptors (87). Taken together, these reports indicate the exciting potential to manipulate disease cells through several independent interventions that target raft lipids.

Conclusions

Many basic questions about the nature of rafts and caveolae remain unanswered, and many of these questions will almost certainly require high-resolution imaging techniques. Nonetheless, a convergence of independent approaches including biophysics, immunology, and lipid science has begun to indicate the fundamental importance of dynamic and chronic changes in membrane lipid to signaling in immune and other cells. Pathways for trafficking of cholesterol and other lipids through the macrophage, initially studied for their relevance to cell biology and metabolism, are now understood to be critical determinants of TLR

signaling. At a basic level, these studies have also challenged the traditional paradigm of hierarchical protein signaling, showing that lateral changes in lipid domain segregation in the plasma membrane are not only necessary, but also sufficient to initiate protein signaling. A challenge for the field as it looks now to apply these insights to better understand 'old' diseases and to develop new therapeutics, will be to avoid conceptual constraint by the raft hypothesis itself.

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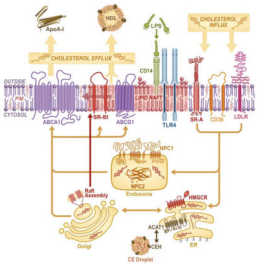

Figure 1. Intracellular cholesterol trafficking regulates macrophage rafts

Cholesterol synthesized in the endoplasmic reticulum (ER) by HMG CoA reductase (HMGCR) or internalized via scavenger receptors (CD36, SR-A) or low density lipoprotein receptor (LDLR), is assembled into nascent rafts in the Golgi apparatus for caveolin- and Niemann Pick C1 protein (NPC1)-dependent transfer to the plasma membrane. NPC1 together with NPC2 also regulates endosomal recycling of cholesterol to the plasma membrane. In turn, cholesterol is effluxed either by simple diffusion or via transporters (ATP Binding Cassette [ABC]A1, ABCG1, SR-BI) to extracellular acceptors (apolipoprotein [apo]A-I, high density lipoprotein [HDL]), and also likely equilibrates with non-raft regions. Cholesterol esterification is regulated in the cytosol by cholesterol ester hydrolase (CEH) and in the ER by acyl-coenzyme A:cholesterol acyltransferase 1 (ACAT1). Raft cholesterol/abundance and abundance of LPS recognition proteins (CD14, TLR4) are regulated by cholesterol flux through this pathway.

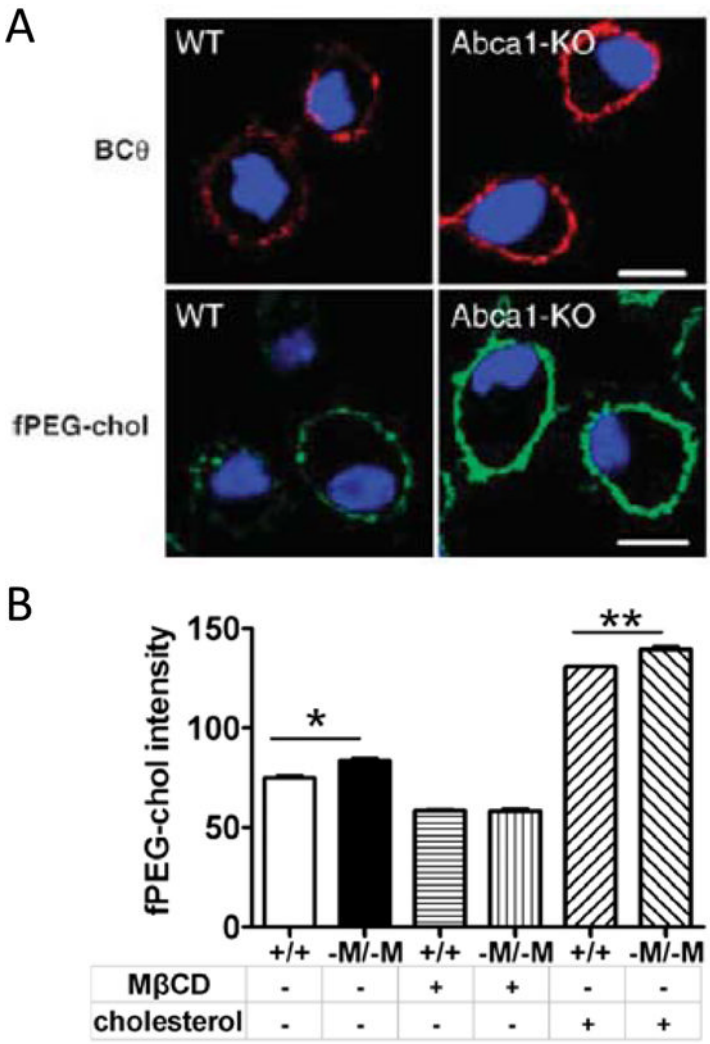

Figure 2. ATP Binding Cassette (ABC)A1-deficient macrophages have enlarged lipid rafts

(A) Rafts were imaged in peritoneal macrophages from WT and *Abca1* null mice with the use two raft cholesterol probes, BCθ toxin (red) and fPEG-chol (green). Nuclei were stained with 4'6-diaminophenylindole (blue). Reprinted from (59) with permission. **(B)** Peritoneal macrophages from wild type (+/+) or macrophage-specific *Abca1* null (-M/-M) mice were cholesterol-depleted with mβCD or cholesterol-loaded with mβCD-cholesterol, stained with fPEG-chol, and then quantified by flow cytometry. Data are mean +/− SEM. *, p<.05; **, p<.01. Reprinted from (43).

Table 1

Agents reported to 'disrupt' raft structure and/or function and their associated effects on the cell.*

Disruptor	Cell type	Effect	
PUFAs	EL4 cell	↓raft coalescence, MHC I mislocalization	(82)
HDL	monocyte	↓raft chol, ↓CD11b activation	(54)
4F	MDM	↓rafts, altered cell differentiation	(88)
LXR agonists	prostate Ca cell	↓raft size, ↓raft Akt phosphorylation	(89)
SQS inhibitor	prostate Ca cell	↓raft chol, ↓cell proliferation	(85)
Statins	NK cell	↓membrane chol, ↓NK cell cytotoxicity	(84)
OxLDL	endothelial	↓raft chol, ↓raft eNOS, ↓eNOS activation	(75)
OxPAPC	endothelial	↓LPS-induced raft TLR4, ↓LPS response	(77)
Ceramide	PBMC	↓raft Lck, ↓raft PLD1, ↑PLD1 activity	(90)
DPPE	CD8+ T cell	↓MHC-induced raft proteins, ↓CTL activation	(72)
DPPC, surfactant	A549	↓LPS-induced TLR4 translocation to rafts	(73)
High glucose	THP-1	↓number and size of caveolae	(91)
Ethanol	Mϕ	↓LPS-induced raft CD14 and TLR4	(92)
ESeroS-GS	Mϕ	↓LPS-induced raft CD14 and TLR4	(80)

*Select examples are shown for each agent. Measures of raft 'disruption' differ among reports. For some agents (e.g., ceramide), both raft stabilization and destabilization have been reported. For others, (e.g., ethanol), inhibitory and stimulatory effects have been reported on the TLR4 pathway. Ca, cancer; chol, cholesterol; CTL, cytotoxic T lymphocyte; DPPC, dipalmitoyl-phosphatidylcholine; DPPE, dipalmitoyl-phosphatidylethanolamine; eNOS, endothelial nitric oxide synthase; HDL, high density lipoprotein; LPS, lipopolysaccharide; LXR, Liver X Receptor; Mϕ, macrophage; MDM, monocyte-derived macrophage; oxLDL, oxidized low density lipoprotein; oxPAPC, oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine; PUFA, polyunsaturated fatty acid; SQS, squalene synthase; TLR4, Toll like Receptor 4; 4F, apolipoprotein mimetic peptide 4F.