Emerging Roles for Lipid Droplets in Immunity and Host-Pathogen Interactions

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Abstract

Lipid droplets (LDs) are neutral lipid storage organelles ubiquitous to eukaryotic cells. It is increasingly recognized that LDs interact extensively with other organelles and that they perform functions beyond passive lipid storage and lipid homeostasis. One emerging function for LDs is the coordination of immune responses, as these organelles participate in the generation of prostaglandins and leukotrienes, which are important inflammation mediators. Similarly, LDs are also beginning to be recognized as playing a role in interferon responses and in antigen cross presentation. Not surprisingly, there is emerging evidence that many pathogens, including hepatitis C and Dengue viruses, *Chlamydia*, and *Mycobacterium*, target LDs during infection either for nutritional purposes or as part of an anti-immunity strategy. We here review recent findings that link LDs to the regulation and execution of immune responses in the context of host-pathogen interactions.

Contents

INTRODUCTION TO LIPID DROPLET BIOLOGY

Lipid droplets (LDs) are neutral lipid-rich intracellular organelles present in all eukaryotic cells (Murphy 2001, Reue 2011, Service 2009, Zhang et al. 2010) and in bacteria such as *Mycobacterium*, *Rhodococcus*, *Nocardia*, *Streptomyces*, and *Acinetobacter* (Alvarez et al. 1996, Daniel et al. 2004, Kalscheuer & Steinbuchel 2003, Waltermann et al. 2005). Although traditionally viewed as just passive depots of excess fat, the discovery of several LD-associated proteins with functions beyond lipid synthesis and storage has sparked interest among cell biologists as to what additional function(s) these organelles may play (Beckman 2006). Many recent outstanding reviews have captured the excitement in the cell biology community as to how these organelles are generated and how proteins and lipids are trafficked to LDs (Brasaemle 2007; Brasaemle et al. 2009; Fujimoto & Parton 2011; Fujimoto et al. 2008; Farese & Walther 2009; Goodman 2008, 2009; Greenberg & Coleman 2011; Greenberg et al. 2011; Guo et al. 2009; Kalantari et al. 2010; Thiele & Spandl 2008). However, the functions LDs play beyond neutral lipid storage are not well understood. This problem is compounded by the observation that these are not homogeneous organelles (Murphy et al. 2009, Thiele & Spandl 2008); distinct populations of LDs likely exist, which may represent different stages in a maturation process or perhaps are distinct entities that communicate with different cellular compartments. In addition, LDs also may perform different functions in different cell types (Murphy et al. 2009). In this review, we explore recent unexpected findings linking these organelles to the regulation and execution of immune responses.

Background and Evolving Views of the Mammalian Lipid Droplet

LDs are spherical structures consisting mostly of triacylglycerols (TAGs) and sterol esters surrounded by a phospholipid monolayer (Tauchi-Sato et al. 2002) (**Figure 1**). In adipocytes, a large LD (~200-μm diameter) can occupy most of the cell's cytoplasm under conditions of excess lipids. In most other cell types, multiple LDs, ranging from 0.1- to approximately 5-μm diameter, are

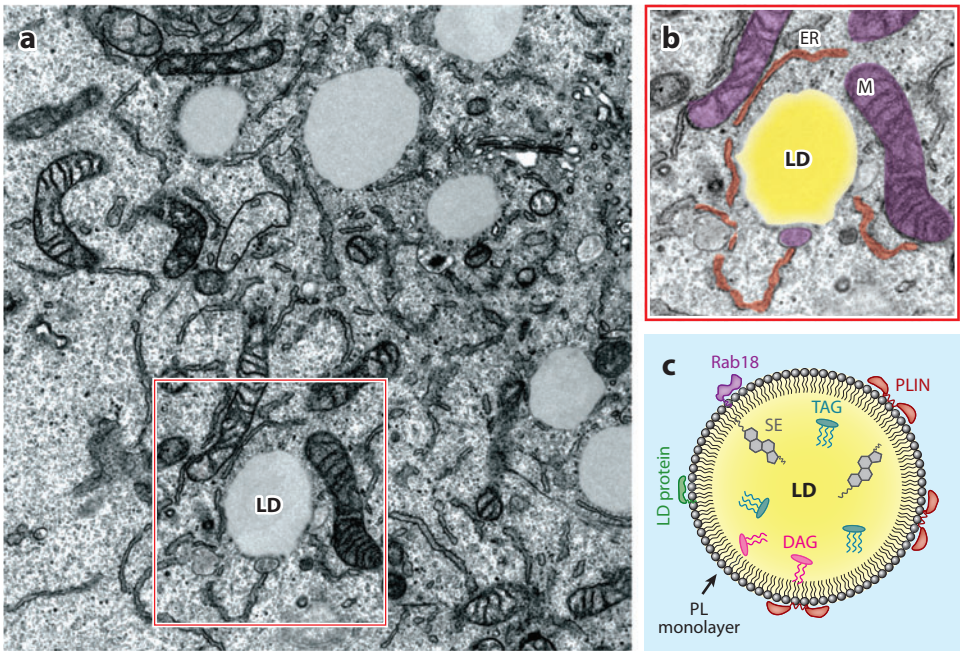

Figure 1

Lipid droplets (LDs) are neutral lipid-rich organelles surrounded by a phospholipid monolayer. (*a*,*b*) Transmission electron microscopy of HeLa cells treated with oleic acid. LDs are spherical, translucent structures that are often in close apposition to mitochondria (M) and endoplasmic reticulum (ER) membranes. (*c*) Schematic representation of a generic mammalian LD: a core of neutral lipids, mainly triacylglycerols (TAGs), sterol esters (SEs), and minor amounts of diacylglycerols (DAGs), is surrounded by a monolayer of phospholipids (PLs) and associated proteins. Among LD-associated proteins, the perilipin family (PLIN) of structural LDs and the Rab GTPase Rab18 are prominent. The number of LD-associated proteins may exceed 200, including lipid metabolic enzymes, SNARE proteins, ER-associated degradation components, and Rab proteins.

evenly distributed throughout the cytoplasm. Because LDs lack a phospholipid bilayer, these organelles are unlikely to follow classical vesicle-mediated membrane transport pathways to receive or deliver protein and lipid components. The neutral lipids that compose the core of LDs also vary depending on the cell type. For instance, in white adipocytes, TAGs are largely dominant, whereas in macrophage foam cells, sterol esters are more abundant, and in yeast, TAGs and sterol esters contribute equally (Bartz et al. 2007a, Leber et al. 1994, Tauchi-Sato et al. 2002). Other lipids found in the core of LDs include monoalk(en)yl diacylglycerols and free cholesterol in low levels (Bartz et al. 2007a, Hevonoja et al. 2000). Phospholipids found in the LD monolayer primarily consist of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol; minor levels of lyso-PC, lyso-PE, and cholesterol are also present (Bartz et al. 2007a, Prattes et al. 2000, Tauchi-Sato et al. 2002). By mass spectrometry, more than 160 lipid species have been identified in LDs. Notably, sphingomyelin, phosphatidylserine, and phosphatidic acid are relatively absent in these organelles (Bartz et al. 2007a).

LDs are intimately linked to energy and lipid homeostasis: they expand in size and number when excess lipids are present, which prevents lipotoxicity (Listenberger et al. 2003), and are rapidly consumed when carbon sources

are depleted and additional energy supplies are required (Masuda et al. 2006). Peroxisomes and mitochondria can directly access the core lipids of LDs for β-oxidation of released fatty acids or to provide precursors for protein lipidation, phospholipid biosynthesis, and membrane biogenesis (Binns et al. 2006, Murphy 2001, van Meer et al. 2008). Excess fatty acids acquired from lipid transporters in the plasma membrane, endocytosis of lipoprotein particles, or *de novo* synthesis in the cytoplasm can be esterified and incorporated into growing LDs (Brown et al. 1979, McArthur et al. 1999, Stremmel et al. 2001). This exchange of lipids likely occurs at structures analogous to membrane contact sites that have been described between the endoplasmic reticulum (ER) and mitochondria (Levine 2004, Levine & Loewen 2006). Indeed, LDs are found in close apposition to other intracellular organelles including the ER, endosomes, lysosomes, peroxisomes, mitochondria, and the plasma membrane (Binns et al. 2006, Dvorak et al. 1983, Liu et al. 2007, Ozeki et al. 2005, Pol et al. 2004, Stemberger et al. 1984). These observations are consistent with an organelle that is dynamic, fully engaged with the biology of the cell, and actively involved in a diversity of cellular processes including (but not limited to) lipid homeostasis, signal transduction, and membrane trafficking (Liu et al. 2008).

Our understanding of LD biology has significantly expanded as the compendium of LD-associated proteins has begun to be defined. Proteins associate with the LD surface through amphipathic α-helices, lipid anchors, and/or hydrophobic protein motifs with hairpin-like topologies (Boulant et al. 2006, Bussell & Eliezer 2003, Ostermeyer et al. 2004, Subramanian et al. 2004), but a universal motif present in all LD-associated proteins has not been identified. In addition, some proteins can be found within the hydrophobic environment of the LD (Robenek et al. 2005, 2009), but how these proteins access the core is unclear. Prominent among LD-associated proteins is the perilipin, also known as "PAT," family of proteins [perilipin, adipophilin (ADFP) or adipose differentiation-related protein (ADRP), and tail-interacting protein 47 (TIP47)], which groups the more abundant and best-characterized LD proteins (Brasaemle 2007). Members of this family (PLIN1 to 5) (Kimmel et al. 2010) share a conserved PAT domain and 11-mer repeats predicted to form amphipathic helices; the extreme 100 N-terminal amino acids are the most conserved (Bussell & Eliezer 2003, Lu et al. 2001).

Perilipin (PLIN1) is primarily expressed in adipocytes and steroideogenic cells (Londos et al. 1995) and was originally identified as a target for protein kinase A–mediated phosphorylation in lipolytically stimulated adipocytes (Greenberg et al. 1991). This was the first indication that a protein coating the surface of LDs regulated neutral lipid metabolism. PLIN1 is encoded in a single gene and exists in three different isoforms (A–C) derived from alternative splicing (Lu et al. 2001); perilipin A is the most abundantly expressed and the best-characterized isoform (Greenberg et al. 1993). Adipophilin (PLIN2, also termed ADRP or ADFP) was originally identified as a protein linked to adipocyte differentiation (Jiang & Serrero 1992). PLIN2 is induced during the differentiation of preadipocytes in cell culture, but the protein is soon degraded and is not detected in mature adipocytes (Brasaemle et al. 1997, Xu et al. 2005). In contrast with PLIN1, PLIN2 is ubiquitously expressed, and its overexpression results in increased formation of LDs (Brasaemle et al. 1997, Imamura et al. 2002). Thus, PLIN2 seems to play a role in the assembly of LDs. PLIN3 (or TIP47) was originally identified in a yeast two-hybrid screen for proteins that interacted with the C-terminal end of the mannose 6-phosphate receptor (M6PR) (Diaz & Pfeffer 1998), and it has a role in the transport of M6PR from endosomes to the *trans*-Golgi network both in vitro and in vivo. This protein was also identified in placental tissues and reported to have close homology to PLIN2 (ADRP) (Than et al. 1998). Further studies indicated that PLIN3 (TIP47) also localizes to LDs and plays an important role in the biogenesis of these organelles (Bulankina et al. 2009, Miura et al. 2002, Wolins et al. 2001).

The other two members of the perilipin family are PLIN4 (S3-12) and PLIN5 (PAT1), which is also known as LSDP5/OXPAT/MLDP (Dalen et al. 2007; Scherer et al. 1998; Wolins et al. 2003, 2006b; Yamaguchi et al. 2006). S3-12 expression occurs primarily in white adipose tissue (Wolins et al. 2003), and LSDP5 is preferentially expressed in tissues with increased rates of β-oxidation such as liver, muscle, and brown adipose tissue (Dalen et al. 2007, Scherer et al. 1998, Wolins et al. 2006b).

The number of proteins reported to associate with LDs has increased dramatically in recent years as sensitive mass spectrometry methods have been applied to purified organelles. Proteomics studies of LDs and their impact on our rapidly evolving understanding of the LD proteome have been reviewed in detail (Hodges & Wu 2010). More than 200 mammalian LD-associated proteins have been identified, including an extensive list of lipid metabolism enzymes, Rab GTPases, SNARE proteins, ARF-related proteins, and coatomer components (Hodges & Wu 2010 and references therein). These findings are consistent with the notion that LDs participate in membrane and protein trafficking and communicate with other organelles. Although contamination with other cellular compartments may explain the presence of some proteins in LD fractions (Digel et al. 2010), the unexpected diversity of these organelles' proteome underscores their potential roles in cellular functions beyond lipid storage and metabolism.

Current Models for Lipid Droplet Biogenesis

Several observations indicate that the ER is the source for LDs. Many of the enzymes required for the biosynthesis of TAGs and phospholipids as well as for the esterification of sterols reside in the ER (Murphy 2001). The lipid composition of the LD membrane monolayer closely mimics that of the ER, and LDs are found in close proximity and possibly connected to ER membranes (Bartz et al. 2007a, Ozeki et al. 2005, Robenek et al. 2006, Tauchi-Sato et al. 2002). The prevalent model of LD biogenesis is that neutral lipids accumulate within the membrane lipid bilayer in specialized regions of the smooth ER to form a lens-like structure that becomes the seed for the nascent droplet (Kalantari et al. 2010). The curvature imposed by the deformed bilayer grows as neutral lipids accumulate to eventually release a freestanding, fully formed droplet into the cytoplasm that contains the cytosolic membrane leaflet of the ER (**Figure 2*a***). In a variation of this model, LDs remain physically attached to the ER through a continuous ER cytosolic leaflet (also known as the stalk) (**Figure 2*b***) and thus represent specialized domains of the ER (Blanchette-Mackie et al. 1995, Fujimoto & Parton 2011, Goodman 2008, Zehmer et al. 2009b). In such a model, proteins could diffuse on the membrane surface between the ER and the droplet.

An alternative model proposes that LD biogenesis involves the transient formation of bicellar structures through fusion of the cytoplasmic and luminal leaflets of the ER membrane (Ploegh 2007). In this hatching model, the generation of a transient pore excises LDs from the ER membrane (**Figure 2*c***). Ploegh (2007) proposed that such a pore could mediate the extrusion of proteins destined for ER-associated degradation (ERAD) by the proteasome. However, this is not an essential process for ERAD, as yeast strains unable to produce LDs still degrade proteins through ERAD (Olzmann & Kopito 2011). Walther & Farese (2009) proposed a different model involving vesicular budding. In this model, LDs are generated from small bilayer vesicles at specific regions of the ER where neutral lipid synthesis occurs (**Figure 2*d***). In this context, neutral lipids accumulate in the intermembrane space of the nascent vesicles while they are still in close apposition to the ER membrane. At the end of the process, the remaining vesicle lumen surrounded by the original luminal leaflet would be very small compared with the neutral lipid content and could either be eliminated through fusion with the outer leaflet or remain inside. This model, as opposed to the previous ones, is compatible with the finding that

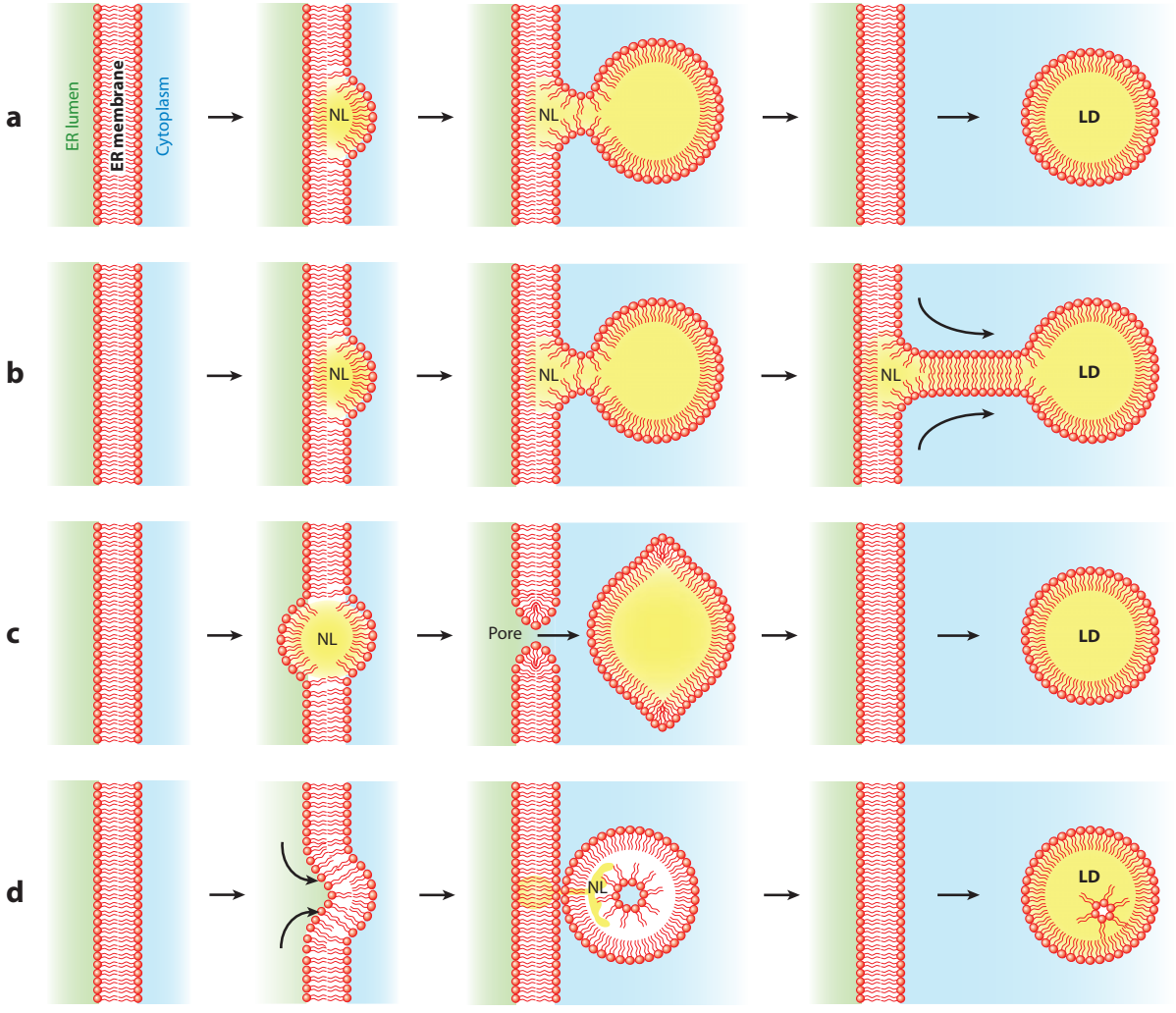

Figure 2

Current models of lipid droplet (LD) biogenesis. (*a*) Neutral lipids (NLs, *yellow*) accumulate in the space between the cytosolic and luminal leaflets of endoplasmic reticulum (ER) membranes. This imposes a curvature in the ER membrane, which pushes the cytosolic leaflet to eventually release a fully formed LD. In this model, the LD phospholipid monolayer originates from the cytosolic membrane leaflet of the ER. (*b*) According to this model, LDs are formed as in panel *a*, except that they stay physically connected to the ER to generate a NL-enriched lipid ER domain. In this model, proteins associated to the cytosolic leaflet of the ER can readily diffuse between the ER and the LD. (*c*) In the hatch model, after NLs accumulate within the luminal and cytosolic membrane leaflets of the ER, the LD is excised from the ER membrane (hatching), to form a transient pore. (*d*) An alternative model posits that LDs originate from bilayer vesicles found at specific regions of the ER where NL synthesis occurs. NLs accumulate in the intermembrane space of these vesicles, which stay in close apposition to the ER membrane. At the end of LD generation, the original luminal leaflet would be relatively small and remain inside or be eliminated through fusion with the outer leaflet.

hydrophilic proteins can be found in the lumen of LDs (Robenek et al. 2005). As appealing as these models are, experimental data to fully support them are still lacking. Ultrastructural analysis of LDs by 3D reconstruction of freeze-fracture electron microscopy failed to provide evidence for lens structures within the ER membrane (Robenek et al. 2009).

Instead, LDs are often found nestled in tight apposition to ER membranes. Given the biophysical predictions that nascent LDs are ~12 nm (Zanghellini et al. 2010), perhaps these structures cannot be visualized with current sample fixation and imaging technologies.

Growth of LDs is linked to functions performed by the ER. The final steps in TAG and sterol ester synthesis are mediated by the ER proteins diacylglycerol acyltransferases (DGAT1 and 2) and acyl-coenzyme A (CoA):cholesterol acyltranserases (ACAT1 and 2), respectively (Chang et al. 2009, Yen et al. 2008). If LDs and ER membrane leaflets remain connected, newly synthesized lipids could potentially diffuse into the LD core. But if the LD is detached from the ER, local synthesis on LDs or lipid transport from the ER would be required to support LD expansion (Kuerschner et al. 2008, Levine & Loewen 2006). Using fluorescent lipid analogs, Thielle and colleagues demonstrated that TAG and its precursor diacylglycerol accumulate on LDs and that LDs acquire TAG at different rates, which suggests unequal access to the biosynthetic machinery (Levine & Loewen 2006). To track lipid incorporation into LDs, Cheng et al. (2009) used the high reactivity of unsaturated fatty acids to osmium tetroxide to track the incorporation of newly added unsaturated fatty acids into LDs by quantitative electron microscopy. In fibroblasts, preformed LDs uniformly incorporate newly synthesized triglycerides, even after microtubule depolymerization, which suggests that triglycerides are synthesized in the local vicinity of individual LDs. In contrast, in adipocytes TAG incorporation into LDs is uneven (Cheng et al. 2009). Nonetheless, TAG biosynthesis likely takes place in the immediate vicinity of the LD, which is consistent with the observation that TAG biosynthetic enzymes are present in LDs and that purified LDs have TAG biosynthetic activity (Fujimoto et al. 2007, Kuerschner et al. 2008). Bostrom et al. (2005) also proposed that LDs can form from the fusion of smaller droplets. Although the SNARE proteins SNAP23, syntaxin-5, and VAMP4 can mediate LD fusion (Bostrom et al. 2007), that LD fusion events occur at low frequency in live cells strongly suggests that this is not the major mechanism of LD growth (Wolins et al. 2005). Finally, autophagy is linked to LD biogenesis, as processed forms of MAP1-LC3 (LC3-II) localize to LDs in starved hepatocytes and cardiac myocytes, and LD formation is impaired in hepatocytes unable to undergo autophagy (Shibata et al. 2009).

Factors that influence LD abundance and morphology have been identified through genome-wide loss-of-function screens in yeast and *Drosophila* cells (Fei et al. 2008, Guo et al. 2008, Szymanski et al. 2007). No single factor appears to be sufficient to abolish LD formation except for the enzymes required for neutral lipid biosynthesis (Harris et al. 2011b, Olzmann & Kopito 2011, Sorger et al. 2004). Factors that modulate the size and abundance of LDs include vesicle trafficking regulators such as the ADP-ribosylation factor (ARF)/coatomer complex (D'Souza-Schorey & Chavrier 2006, Guo et al. 2008). This is most likely because of their role in the transport of adipose triglyceride lipase (ATGL) and PLIN2 (ADRP) to LDs (Soni et al. 2009). As such, the turnover of LDs increased in the absence of coatomer-mediated vesicle transport. Additional GTPases such as ARF-related protein 1 (ARFRP1) are also required for proper LD formation, as adipocytes derived from ARFRP1-deficient mice are defective in the transfer of newly formed lipid particles to larger droplets (Hommel et al. 2010). The role of ARF in LD biogenesis was first revealed through the use of brefeldin A (BFA), an ARF GEF (guanine nucleotide exchange factor) inhibitor (Nakamura et al. 2004), but BFA can also induce alterations in lipid metabolism that influence LD formation independently of its inhibitory activities on ARF. For instance, BFA-induced mono-ADP-ribosylation of the transcriptional repressor CtBP1/BARS leads to the activation of genes that regulate neutral lipid storage, and small interfering RNA (siRNA) knockdown of CtBP1/BARS recapitulates the effect of BFA (Bartz et al. 2007b).

Lipid and protein transport to LDs share some features common to canonical vesicular traffic. For example, a subset of Rab GTPases, which are central regulators of membrane traffic, copurify with LDs. In particular, Rab18 associates with the surface of LDs in a manner that is likely regulated by the metabolic state of individual LDs (Ozeki et al. 2005). Cytoplasmic lipid transporters and sensors also regulate lipid transport. ORP2, a member of the oxysterol binding protein family that was previously shown to bind 25-hydroxycholesterol and is implicated in cellular cholesterol metabolism, localizes to LDs in a sterol-dependent manner (Hynynen et al. 2009). RNA interference (RNAi)-mediated silencing of ORP2 expression dampens cellular TAG hydrolysis and leads to an increased accumulation of cholesteryl esters (Hynynen et al. 2009). The phospholipid content of the LD also plays an important role in regulating the dynamics of the organelle because these membrane lipids are required to cover the surface area of the growing LD. Krahmer et al. (2011) provided important mechanistic clues as to how phospholipids are incorporated into the LD surface during the expansion of these organelles. These investigators demonstrated that CTP:phosphocholine cytidylyltransferase is recruited to the LD surface during LD expansion to provide in situ phosphatidylcholine synthesis, which in turn is important for LD stability.

The development of cell-free systems for LD generation and fusion will help delineate the biochemical requirements for distinct steps in LD biogenesis. Early attempts with cell-free systems identified phospholipase D and vimentin as central components that permit the release of LDs into a form that could be collected over density gradients (Marchesan et al. 2003). More recently, a novel type of membrane vesicles, whose generation was enhanced by addition of the lipid biosynthetic substrates glycerol-3-phosphate and oleoyl-CoA, was obtained in vitro from yeast microsomes (Takeda & Nakano 2008). The formation of these vesicles was COPII independent, and Dpm1p, an enzyme involved in dolichol-sugar synthesis, was identified as a potential cargo. These Dpm1p-containing vesicles consisted of small vesicular/saccular structures of approximately 40 to 50 nm in diameter. Dpm1p localizes to LDs and the ER, and Dpm1p-green fluorescent protein is present in subregions of isolated LDs, which raises the intriguing possibility that these vesicles constitute ER intermediates in LD formation (Takeda & Nakano 2008). Such a model would also suggest that ER membrane proteins can migrate in the plane of the membrane to sites of nascent LD formation. This is consistent with the observed behavior of two ER proteins, AAM-B and UBXD8 (Zehmer et al. 2009a), that migrate to LDs even when expression of dominant-negative forms of the small GTPase Sar1 blocks the exit of proteins from the ER through COPII-coated vesicles. Surprisingly, these proteins return to the ER as the level of neutral lipid declines, which suggests that some integral LD proteins that originally come from the ER can regress independently of canonical secretory pathway–mediated traffic. Overall, these observations suggest that vesicular and nonvesicular pathways likely exist to deliver lipids and proteins to LDs.

The plasma membrane, specifically at caveolae, can also contribute to protein and possibly lipid transport to LDs (Le Lay et al. 2006). LDs isolated from adipocytes of caveolin-1-deficient mice were largely devoid of cholesterol. Moreover, addition of exogenous cholesterol induces the translocation of caveolin to LDs in a dynamin- and protein kinase C–dependent manner (Le Lay et al. 2006), which suggests that LDs may be potential target organelles for caveolar endocytosis. Caveolin may even physically connect the plasma membrane and LDs with an LD-targeting signal consisting of a central hydrophobic domain followed by positively charged sequences (Ingelmo-Torres et al. 2009).

Finally, LD formation is also a target of regulation by classical signaling pathways. Gubern and collaborators investigated the signaling cascades activated during serum-induced LD biogenesis (Gubern et al. 2009a) and determined

that activation of cytoplasmic phospholipase A_2 ($cPLA_2$), a phospholipase that participates in LD generation (Gubern et al. 2008, Gubern et al. 2009b), was dependent on JNK-mediated phosphorylation. LD biogenesis was accompanied by increased synthesis of ceramide 1-phosphate, a reaction mediated by ceramide kinase. Overexpression of ceramide kinase increased JNK-mediated phosphorylation of $cPLA_2$ at Ser-505 and formation of LDs, whereas its downregulation (by siRNA knockdown) prevented both $cPLA_2$ phosphorylation and LD biogenesis (Gubern et al. 2009a).

Functional Diversity of Lipid Droplets

A consensus is emerging that LDs should be considered multifunctional organelles that are integrated with multiple cellular processes. Not surprisingly, LDs from different cell types or obtained under different stimuli or metabolic states can differ greatly in their protein and/or lipid compositions (Cheng et al. 2009, Hodges & Wu 2010, Soni et al. 2009). Even within the same cell, different subpopulations of LDs have been observed. For example, ectopically expressed Rab18 and $Cav3^{DMV}$ (a truncated version of caveolin-3 that inhibits LD catabolism and motility) labeled different populations of LDs within the same cell (Martin et al. 2005). Similarly, endogenous Rab18 and PLIN2 (ADRP) show reciprocal LD labeling (Ozeki et al. 2005). In adipocytes, nascent LDs are preferentially labeled with PLIN3 (TIP47) and PLIN4 (S3-12), whereas in established LDs, PLIN1 (perilipin) and PLIN2 (ADRP) are more dominant (Wolins et al. 2005, 2006a). These findings suggest that LDs, similar to secretory organelles, may undergo a process of maturation. Additionally, Beller et al. (2006) characterized the proteome of LDs isolated from *Drosophila* fat tissue and performed intracellular localization studies to find subsets of droplets with a distinct pattern of proteins within a given cell at the larva step. The heterogeneity of LDs is also evident through differences in lipid composition. For example, a study using label-free coherent anti-Stokes Raman scattering microscopy demonstrated that within a given cell, the lipid composition of individual droplets varied significantly and, even more surprisingly, that differences were observed within a single droplet (Rinia et al. 2008). As the diversity of proteins identified in LDs increases, largely through proteomics studies (reviewed in Hodges & Wu 2010), different protein markers for these organelles should become available, which will generate new opportunities to characterize the functional heterogeneity of LDs. These markers may help clarify LD interactions with the ER, mitochondria, peroxisomes, endosomes, lysosomes, and the plasma membrane (Binns et al. 2006, Dvorak et al. 1983, Liu et al. 2007, Ozeki et al. 2005, Pol et al. 2004, Stemberger et al. 1984).

Genome-Wide Approaches to Understand Lipid Droplet Function

Two morphological screens based on genome-wide RNAi gene silencing in *Drosophila* S2 cells (Beller et al. 2008, Guo et al. 2008) were the first to reveal that genes required for phospholipid biosynthesis and Arf1-COPI vesicular transport proteins influence LD size and number. These findings, which were corroborated in mammalian cells, revealed the COPI complex as an evolutionarily conserved regulator of lipid homeostasis. An RNAi screen performed in human cells focused on the analysis of 600 human kinases and a thin-layer chromatography-based assay for abnormal neutral lipid storage (Grimard et al. 2008) identified JNK2 as an evolutionarily conserved regulator of TAG homeostasis and LD lipolysis, which is consistent with data reported by Gubern et al. (2009a). A similar screen in yeast revealed additional regulators of LD formation, including the yeast homolog of BSCL2/seipin, a gene associated with Berardinelli-Seip Congenital Lipodystrophy Type 2 (Szymanski et al. 2007). The absence of yeast seipin results in few strikingly enlarged and irregular LDs, similar to what is seen in fibroblasts from BSCL2 patients. Wild-type human seipin, but not alleles that cause

lipodystrophy, can complement yeast seipin mutants. Although these screens focused on altered LD morphology, this should not necessarily be equated with LD function. As detailed below, these organelles perform functions that, although integrated with lipid homeostasis, may not be linked to LD morphology.

THE INTERSECTION OF LIPID DROPLETS AND PATHOGENS

One unexpected aspect of LD biology is its interaction with pathogens. Recently, many pathogens have been found to target LDs, including a range of viruses, intracellular bacteria, and protozoan pathogens. Although at first glance it would make sense that these organelles would be attractive targets for pathogens seeking lipid resources from their host cells, emerging evidence suggests that LDs may also be central mediators of immune responses. In the following section we discuss several examples of pathogen interactions with LDs, the consequences of these interactions in pathogen survival, and emerging themes in LD-mediated immune functions.

Lipid Droplets as Assembly Platforms for Hepatitis C Virus and Other Viruses

The most extensively documented example of LD interactions with a pathogen is that of hepatitis C virus (HCV). A member of the *Flaviviridae*, HCV is a major human pathogen associated with chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Poynard et al. 2003). The HCV core protein was one of the first pathogen proteins reported to localize to LDs (Moradpour et al. 1996), although at the time it was unclear if this localization was an artifact of transient protein overexpression. Upon establishment of cell culture systems for the generation of infectious HCV, it became apparent that the virus uses LDs as a platform for assembly of nascent virions and that the HCV core protein is a main player in the manipulation of these organelles (Boulant et al. 2007, Miyanari et al. 2007). The HCV core protein recruits viral nonstructural proteins as well as replication complexes to LD-associated membranes, and this association is pivotal for viral replication (Miyanari et al. 2007). Indeed, if HCV core protein localization to LDs is disrupted, the levels of virus progeny generated are significantly decreased (Boulant et al. 2007). Interestingly, expression of the core protein increases the abundance of LDs both in cell culture and in transgenic mice, likely owing to decreased lipid turnover, thus linking this protein to HCV-induced steatosis (Harris et al. 2011a, Miyanari et al. 2007, Moriya et al. 1997). For the HCV core protein to associate with LDs, it must first undergo proteolytic processing, which involves removal of the signal peptide and intramembrane proteolysis by the signal peptide peptidase (McLauchlan et al. 2002). Mutational and structural studies determined that the C-terminal D2 domain of HCV core protein, which contains two amphipathic α-helices connected by a hydrophobic loop, is responsible for association with LD and ER membranes (Boulant et al. 2006, Hope & McLauchlan 2000). Palmitoylation of the HCV core protein is required for localization to smooth ER and to ER membranes as well as for efficient production of virus, but it did not affect localization to LDs (Majeau et al. 2009). Herker et al. (2010) assessed the relationship between HCV replication and the TAG-synthesizing enzymes DGAT1 and DGAT2, which are critical for LD biogenesis (Harris et al. 2011b). DGAT1, but not DGAT2, was required for efficient virion production. Strikingly, even though DGAT1 inhibition or knockdown did not alter the size or number of LDs (DGAT1 and DGAT2 have redundant functions in TAG synthesis), the HCV core protein no longer associated with LDs, and the yield of infectious HCV virions was greatly diminished. This observation indicates that the HCV core protein specifically targets DGAT1-generated LDs (Herker et al. 2010).

The ability of viruses to interact with LDs is not limited to HCV. A virus closely related to HCV, GB virus B (GBV-B) is associated

with acute hepatitis in experimentally infected tamarins (Stapleton et al. 2011). The GBV-B core protein also localizes to LDs through a region sharing homology with the HCV core protein D2 domain (Hope et al. 2002). Another member of Flaviviridae, the major human pathogen Dengue virus, also associates to LDs through its capsid protein, and this association is necessary for efficient viral replication (Samsa et al. 2009). Viral association with LDs is not limited to the Flaviviridae. Cheung et al. (2010) reported that rotaviruses, members of the Reoviridae and a global cause of acute gastroenteritis in children, also associate with LDs. The main components of rotavirus viroplasms (i.e., NSP5, NSP2, VP1, VP2, and VP6) colocalize with the LD structural proteins PLIN1 (perilipin) and PLIN2 (ADRP) on LDs, and inhibition of LD formation negatively affects viral replication. The orthoreoviruses (also members of the Reoviridae) encode an outer capsid protein μ1, which induces apoptosis and associates to LDs through two amphipathic α-helices located in the C-terminal region (Coffey et al. 2006). Interesingly, deletion of the amphipathic α-helices of μ1 abolished both association to LD and induction of cell death via apoptosis. Similarly, the agnoprotein of Polyomavirus BK [a nonenveloped double-stranded DNA virus associated with nephropathy and hemorrhagic cystitis in immunocompromised patients (Dropulic & Jones 2008, Ramos et al. 2009)] localizes to LDs. A predicted amphipathic α-helix in a region spanning from amino acid 20 to 42 of agnoprotein was essential for this localization (Unterstab et al. 2010). However, the biological relevance of this association remains to be determined. Hepatitis B virus expresses HBx (hepatitis B virus X protein), which induces the expression of the Liver X receptor leading to upregulation of the peroxisome proliferator-activated receptor γ and the lipogenic genes encoding sterol regulatory element binding protein-1c and fatty acid synthase, resulting in accumulation of LDs (Kim et al. 2007, Na et al. 2009). Finally, the human adenovirus 36, which has been linked to obesity (Trovato et al. 2009), was shown to decrease fatty acid oxidation and to induce the expression of Cidec/ Fat-specific protein FSP27, leading to lipogenesis and accumulation of lipid droplets in primary cultures of human muscle cells (Wang et al. 2010). Overall, these observations highlight that a range of viruses have evolved mechanisms to interact with LDs and possibly to subvert the function of these organelles to use them as a platform for assembly of viral particles.

Intersection Between Lipid Droplets and Bacterial Pathogens

Inflammatory signals triggered by many microbial pathogens induce the accumulation of LDs in immune cells such as neutrophils, eosinophils, and macrophages (Melo et al. 2011). As a result, macrophages adopt a foamy morphology and thus are termed foam cells. For instance, foam macrophages accumulate in granulomas during mycobacterial infections, a process that *Mycobacterium tuberculosis* mycolic acids can induce (Peyron et al. 2008). Interestingly, in foam macrophages, mycobacteria are present within phagosomes that are tightly apposed to LDs and transition to a dormant state (Peyron et al. 2008). Moreover, these investigators found that mycobacteria were ultimately delivered into the LDs, where they started to accumulate lipids. Recently, Daniel et al. (2011) reported that accumulation of lipids by *M. tuberculosis* within foam macrophages is primarily a result of incorporation of fatty acids derived from host TAG in a process largely mediated by a mycobacterial triacylglycerol synthase 1. These observations indicate that *M. tuberculosis* can use host LDs as a source of nutrients. Similarly, accumulation of LDs is induced in *M. leprae*–infected macrophages and Schwann cells (Mattos et al. 2010, 2011a). LDs are recruited to the *M. leprae*–containing phagosomes in Schwann cell microtubules in a PI3K signaling–dependent, Toll-like receptor 2 (TLR2)–independent manner (Mattos et al. 2011a). In contrast, TLR2-mediated signaling leads to LD accumulation in murine macrophages infected with *M. bovis* bacillus Calmette-Guérin (BCG) (D'Avila et al. 2006).

This induction of LD accumulation in *M. leprae*–infected macrophages was propagated to uninfected neighboring cells through a TLR2- and TLR6-dependent paracrine signal (Mattos et al. 2010).

Other bacteria, such as the respiratory pathogen *Chlamydia pneumoniae*, can infect macrophages and accelerate foam cell formation, which has been linked to an increased risk for the development of atherosclerosis (Boman & Hammerschlag 2002, Gaydos et al. 1996, Kalayoglu & Byrne 1998, Saikku et al. 1988). Macrophages and other antigen-presenting cells detect *C. pneumoniae* through TLRs (Bulut et al. 2002, Cao et al. 2007, Carratelli et al. 2009, Prebeck et al. 2001), and increased inflammation originated through TLR2- and TLR4-mediated signaling promotes atherosclerosis (Michelsen et al. 2004, Schoneveld et al. 2005). Furthermore, both live and UV-killed *C. pneumoniae* elicit foam cell formation in murine peritoneal macrophages in a TLR2-, TLR4-, MyD88-, TRIF-, and IRF3-dependent manner (Chen et al. 2008).

LD proliferation in response to pathogens is not restricted to phagocytic cells. Infection of epithelial cells with *Chlamydia trachomatis* also leads to the proliferation of LDs in the vicinity of the pathogen-containing vacuole (**Figure 3**), and a cohort of secreted chlamydial proteins can associate with these organelles (Kumar et al. 2006). Importantly, blocking the proliferation of LDs with triacsin C impairs chlamydial replication, which suggests that the pathogen takes advantage of these organelles (Kumar et al. 2006). The interaction between *C. trachomatis* and LDs is rather unique, as this bacterium translocates the entire LD from the host cytoplasm into the lumen of the pathogenic vacuole in a process that resembles endocytosis (Cocchiaro et al. 2008) (**Figure 3**). In a mouse model of intracervical infection with *C. muridarum*, LDs have also been observed in an inclusion formed in vivo (Rank et al. 2011). The chlamydial protein Lda3 may participate in the co-option of these organelles by linking cytoplasmic LDs to inclusion membranes and promoting the removal of PLIN2 (ADRP) from

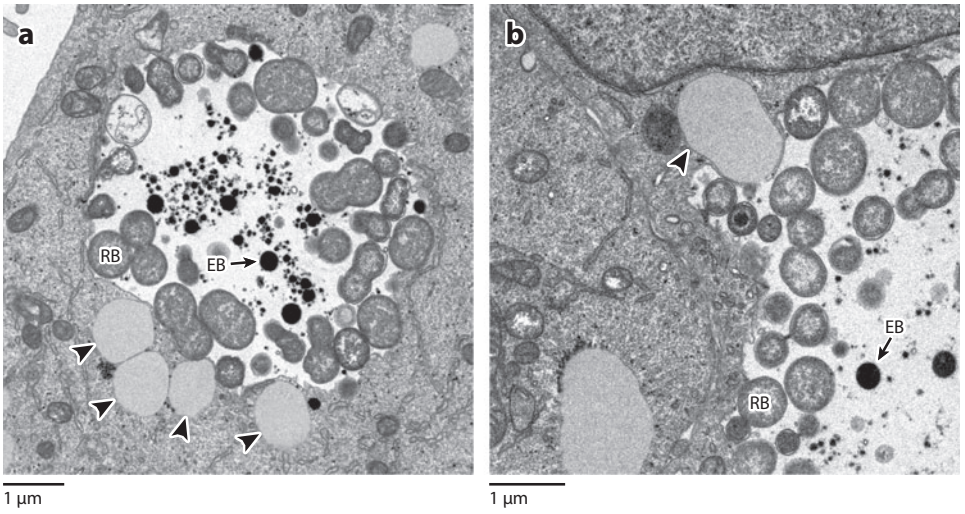

Figure 3

Lipid droplets (LDs) are recruited to and transported into the *Chlamydia*-containing vacuole. Transmission electron micrographs of lipid-loaded HeLa cells infected with *Chlamydia trachomatis*. (*a*) *C. trachomatis* replicates inside a vacuole, known as an inclusion, containing the two developmental forms of this bacterium: the infectious elementary body (EB) and the replicative reticulate body (RB). Arrowheads indicate LDs in intimate contact with the inclusion membrane. (*b*) The arrowhead highlights an LD in the process of internalization into the chlamydial inclusion.

the surface of LDs (Cocchiaro et al. 2008). The mechanism underlying the wholesale transport of intact LDs into the *Chlamydia*-containing vacuole remains to be elucidated.

The signal underlying LD proliferation during *Chlamydia* infection of epithelial cells may be linked to activation of TLR receptors. However, extracellular signal-regulated kinase 2 (ERK2) is also known to participate in basal LD formation (Andersson et al. 2006), and *Chlamydia* infections lead to a robust activation of ERK (Su et al. 2004). In addition, ERK2-dependent activation of dynein and its association with LDs may contribute to enhanced LD motility and amplification (Andersson et al. 2006). Multiple mechanisms could be redundantly involved in triggering LD accumulation during *Chlamydia* infection.

As researchers begin to focus on the roles played by lipids during infection, more pathogens have been found to exploit LDs or impact LD function. For instance, the facultative intracellular pathogen *Salmonella* Typhimurium secretes the effector protein SseJ into the cytoplasm of host cells to mediate the esterification of cholesterol in cell membranes (Nawabi et al. 2008). Both epithelial and macrophage cells infected with a wild-type *Salmonella* strain but not with a *sseJ* mutant, as well as cells ectopically expressing SseJ, display an increased number of LDs (Nawabi et al. 2008). Additionally, absence of the effector SseL, a putative deubiquitinase, leads to the hyperaccumulation of LDs in gallbladder epithelial cells during systemic infections in a mouse model system (Arena et al. 2011). This phenotype required the deubiquitinating activity, which suggests a role for ubiquitin-dependent modifications in host lipid metabolism in these cells.

Lipid Droplet Interactions with the Protozoan Pathogen *Trypanosoma cruzi*

The intracellular protozoan pathogen *Trypanosoma cruzi*, the causative agent of Chagas disease, also induces the accumulation of LDs in macrophages (Melo et al. 2003). As with *Chlamydia*, LDs are recruited to and delivered into the *Trypanosoma*-containing vacuole (Melo et al. 2006). Interestingly, morphological differences point to distinct subpopulations of LDs in *T. cruzi*-infected macrophages (Melo et al. 2003). These apparent subpopulations were characterized by electron microscopy and classified into three main categories: light, electron dense, and strongly electron dense (Melo et al. 2006). Differences in electron density between LDs may correlate with distinct lipid composition, i.e., phospholipid/neutral lipid ratio, and may suggest that LDs modify their lipid composition in response to infection.

LIPID DROPLETS AS MODULATORS OF IMMUNE RESPONSES

The accumulation of LDs within leukocytes during inflammatory conditions such as infection, cancer, and allergy has been extensively documented (Bozza et al. 2009 and references therein). In this manner, LDs compartmentalize several proteins and lipids involved in the control of the biosynthesis and secretion of inflammatory mediators (Bozza et al. 1997, Pacheco et al. 2002).

Lipid Droplets as Sites of Eicosanoid and Inflammatory Lipid Biosynthesis

Eicosanoids, which originate from the oxidation of Ω-3 or Ω-6 20-carbon polyunsaturated essential fatty acids, are signaling molecules with a range of biological functions including inflammation, immunity, and tissue homeostasis. These highly active molecules have a short half-life (seconds to minutes) and can elicit potent physiological and pathological changes in inflamed tissues (Wymann & Schneiter 2008). As such, their production is tightly regulated and activated only in response to the proper stimuli, which include the presence of cytokines, growth factors, mechanical trauma, microbial peptides, or allergens. Eicosanoids bind to specific G-protein binding receptors or

nuclear receptors, which results in activation of complex and interconnected signaling pathways (Wymann & Schneiter 2008).

There are four families of eicosanoids: prostaglandins, prostacyclins, thromboxanes, and leukotrienes. Arachidonic acid (AA), an Ω-6 polyunsaturated fatty acid with four *cis*-double bonds, is a main substrate for biosynthesis of eicosanoids including prostaglandins and leukotrienes (Wang & Dubois 2010). AA is not stored free in the cell but in an esterified form in phospholipids and neutral lipids. When a cell senses the proper stimulus, AA is released from phospholipids or diacylglycerols in a reaction mediated by $cPLA_2$. The free AA is metabolized into eicosanoids by the cyclooxygenase, the lipoxygenase, and the cytochrome P450 monooxygenase pathways (Wang & Dubois 2010). LDs are rich deposits of esterified AA in leukocytes and epithelial cells (Dvorak et al. 1983, Plotkowski et al. 2008, Weller et al. 1991). Additionally, active $cPLA_2$ localizes to the LD surface, where it can induce the release of AA (Moreira et al. 2009). Moreira et al. (2009) showed that in intestinal epithelial cells, stimulation with either oleic acid or AA induced LD biogenesis as well as translocation of $cPLA_2$ to LDs and that these events promote local synthesis of the prostaglandin E2 (PGE_2).

PGE_2 is one of the most studied metabolites of AA, as it exerts a wide range of biological functions in pathological conditions such as inflammation, cancer, fever, and stress (Furuyashiki & Narumiya 2011). In human colon adenocarcinoma cell lines, sites of PGE_2 synthesis colocalize with LDs (Accioly et al. 2008). In *M. leprae*-infected Schwann cells, TLR6-dependent increased LD biogenesis correlates with increased production of PGE_2 and interleukin-10, which links these organelles to the production of innate immunity modulators in the context of bacterial infection (Mattos et al. 2011b). In a murine model of pleural tuberculosis by *M. bovis* BCG, apoptotic neutrophils at the site of infection are engulfed by macrophages, which accumulated LDs and produced PGE_2 (D'Avila et al. 2008). In this work, the investigators showed that preventing neutrophil apoptosis led to decreased accumulation of LDs in macrophages and that blocking *M. bovis*-induced LD accumulation correlated with decreased amounts of PGE_2.

The role of LDs in the production of inflammatory mediators is not restricted to PGE_2. Various reports indicate that LDs also are sites of leukotriene synthesis (Maya-Monteiro et al. 2008, Pacheco et al. 2007, Silva et al. 2009). Overall, these findings provide evidence that LDs are compartments competent for AA mobilization and production of inflammatory mediators in response to a variety of inflammatory stimuli.

Lipid Droplets as Sites of Assembly of Effectors of Interferon Responses

Type I and II interferons (IFNs) are effector molecules critically involved in the immune response to viruses and intracellular pathogens (Platanias 2005, Trinchieri 2010, and references therein). To exert their antimicrobial activity, IFNs modulate the expression of hundreds of IFN-stimulated genes (Katze et al. 2002). One such gene, encoding viperin, is evolutionarily conserved and found to be highly upregulated in response to bacterial lipopolysaccharide (LPS), double-stranded DNA and RNA analogs, and infection with various viruses (Boudinot et al. 2000, Chan et al. 2008, Helbig et al. 2005, Olofsson et al. 2005, Severa et al. 2006). Viperin possesses antiviral activity against a range of viruses including HCV, influenza virus, human immunodeficiency virus, Dengue virus, human cytomegalovirus, and alphaviruses (Fitzgerald 2011 and references therein). Interestingly, viperin localizes to the cytoplasmic leaflet of the ER and to LDs via an N-terminal amphipathic α-helix (Hinson & Cresswell 2009a,b). Given that LDs are sites of viral assembly, perhaps it should not be surprising that an antiviral protein would target these organelles to interfere with viral assembly.

Another group of IFN-induced proteins that has attracted attention is the immunity-related GTPases (IRGs). These proteins

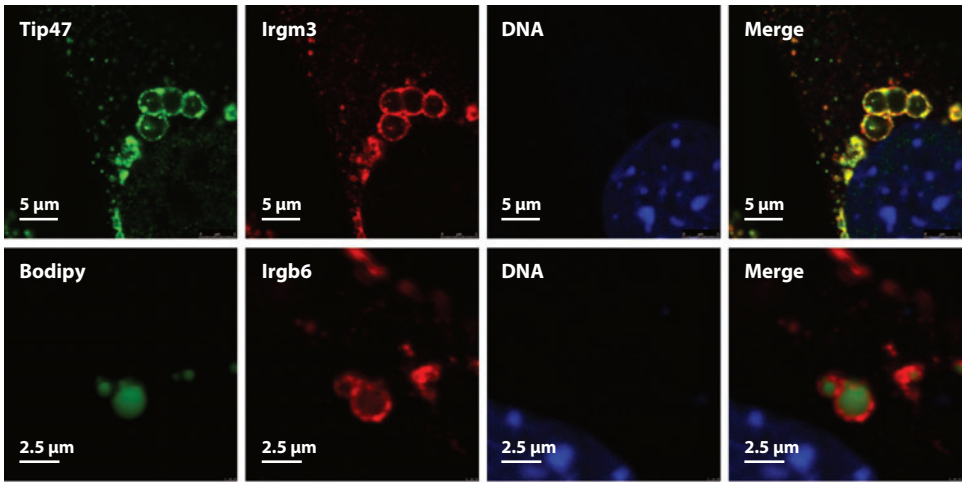

Figure 4

The interferon (IFN)-related GTPases Irgm3 and Irgb6 associate with lipid droplets (LDs). (*Top panels*) IFNγ-stimulated mouse embryo fibroblast (MEF) cells were immunostained with anti-Tip47 and -Irgm3 antibodies to visualize LDs and Irgm3, respectively. Both Tip47 and Irgm3 colocalize extensively, which indicates that Irgm3 is associated with LDs. (*Bottom panels*) Similarly, IFNγ-stimulated MEF cells were immunostained for Irgb6, and LDs were stained with Bodipy 493/503. Irgb6 was observed closely apposed to Bodipy-positive round structures (see merge), which highlights its association with LDs. Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole).

exert a role in mammalian cell-autonomous resistance to a wide range of intracellular pathogens (reviewed in Hunn et al. 2011, Martens & Howard 2006). The mechanisms of IRG-mediated pathogen killing are still not fully understood (Howard 2008). In mice, the IRG protein Irgm3 is required for resistance to *Toxoplasma gondii*, *Leishmania major*, *C. trachomatis*, and *Chlamydia psittaci* (Hunn et al. 2011 and references therein). Irgm3 localizes to the ER membrane (Taylor et al. 1997). However, in IFNγ-stimulated mouse dendritic cells (mDCs) Irgm3 localizes to the surface of LDs (Bougneres et al. 2009). Interestingly, LDs accumulated in IFNγ-treated wild-type but not Irgm3 knockout mDCs, which indicates a role for Irgm3 in LD generation during IFN treatment (Bougneres et al. 2009). We have found that another IRG protein, Irgb6, also associates with LDs in IFNγ-stimulated mouse embryo fibroblasts, which suggests a broader role for LDs in IFN responses (**Figure 4**) (H.A. Saka & R. Valdivia, unpublished observation). Intriguingly, JNK-mediated activation of $cPLA_2$ is required for LD biogenesis (Gubern et al. 2009a), and type I IFN responses during *Chlamydia* infection also require $cPLA_2$ activation (Vignola et al. 2010). Whether LDs can perform functions related to detection of pathogen-derived compounds and the initiation (or amplification) of IFN responses remains to be determined.

An Emerging Role for Lipid Droplets in Antigen Cross Presentation

DCs can present phagocytosed exogenous antigens in the context of class I major histocompatibility complex (MHC-I) molecules to CD8+ T lymphocytes in a process termed cross presentation (Cresswell et al. 2005). Presentation of peptides by MHC-I involves proteasome-mediated proteolytic processing of antigens followed by peptide import into the phagosome lumen or the ER by the transporters associated with antigen processing (TAP1 and TAP2) (Cresswell et al. 2005). To investigate the poorly defined transport

pathways involved in antigen cross presentation, Bougneres et al. (2009) tested the role played by IRGs, as these proteins regulate vesicular trafficking and exert immune functions, and unexpectedly observed that Irgm3 localized to LDs. DCs isolated from Irgm3 knockout mice showed impaired capacity to stimulate ovalbumin-specific CD8+ T lymphocytes upon phagocytosis of latex beads coated with ovalbumin. In addition, DCs from wild-type but not from Irgm3 knockout mice accumulated more LDs upon IFNγ treatment. Moreover, DCs with an increased number of LDs were more competent for antigen cross presentation, whereas the opposite was found upon chemical inhibition of LD biogenesis. The role for Irgm3 in cross presentation is linked to functions performed by LDs, as DCs from PLIN2 (ADRP) knockout mice, which have impaired LD generation, had similar defects in antigen cross presentation. Furthermore, Irgm3 interacts with PLIN2 (ADRP), as GST-Irgm3 specifically coprecipitated with Myc-tagged PLIN2 (ADRP). Therefore, LDs play an important role in regulating cross presentation of exogenous antigens to CD8+ T lymphocytes in DCs. Furthermore, increased levels of some forms of saturated free fatty acids (e.g., palmitic acid) can reduce MHC-I surface expression and the rate of antigen-presenting cell–T lymphocyte conjugation (Shaikh et al. 2008). Thus, LDs accumulating fatty acids into TAG may also play an indirect role in regulating MHC-I expression.

Lipid Droplets and Their Role in Autophagy

Autophagy, an intracellular pathway pivotal for recycling damaged organelles and nutrients from cytoplasmic pools, also exerts important functions in innate and adaptive immunity against intracellular pathogens (reviewed in Deretic 2011). Recent observations point to a role for autophagy in LD homeostasis (Singh et al. 2009). Inhibition of autophagy leads to increased levels of TAG and LDs, and pharmacological induction of autophagy leads to decreased levels of LDs (Singh et al. 2009). Under these conditions, the autophagy marker LC3 was associated with LDs, as LD proteins and lipids were present in autophagic compartments. Although the mechanism for this process is not clear, the occurrence of LD-containing autophagosomes could indicate that small LDs or limited regions of large LDs are delivered into autophagosomes for eventual degradation in lysosomes (Singh et al. 2009). Thus, this lipophagy pathway may control the size and number of LDs under basal conditions but during stress may constitute a survival mechanism that provides the cell with an energy source. This raises new questions concerning the relationship between lipophagy, canonical autophagy, and cytosolic lipolysis as well as how these are regulated by known regulators of lipolysis such as insulin or tumor necrosis factor, a key player in systemic inflammation (Sethi & Hotamisligil 1999). In contrast, Shibata et al. (2009) proposed that LC3 activation plays a role in the biogenesis of these organelles because LD formation is decreased in autophagy-deficient cells. This apparent contradiction in the role of autophagy in LD homeostasis still needs to be resolved.

Work by Ohsaki et al. (2006, 2008) has hinted at further links between LDs and autophagy. When the proteasome or autophagy is inhibited, lipidated apolipoprotein B accumulates in hepatocyte LDs, which suggests that these organelles may be intermediates for proteasome- and autophagy-mediated protein degradation (Ohsaki et al. 2006). Similarly, the Parkinson's disease protein α-synuclein, which is degraded both through autophagy and the proteasome, also accumulates on the surface of LDs in lipid-loading conditions (Cole et al. 2002, Webb et al. 2003).

A link between LDs and autophagy in the context of infection was recently reported; Heaton & Randall (2010) found that to favor replication, Dengue virus induces lipophagy, which leads to a decrease in LD volume and cellular levels of TAGs, with a concomitant increase in free fatty acids. These free fatty acids undergo β-oxidation to generate ATP, which

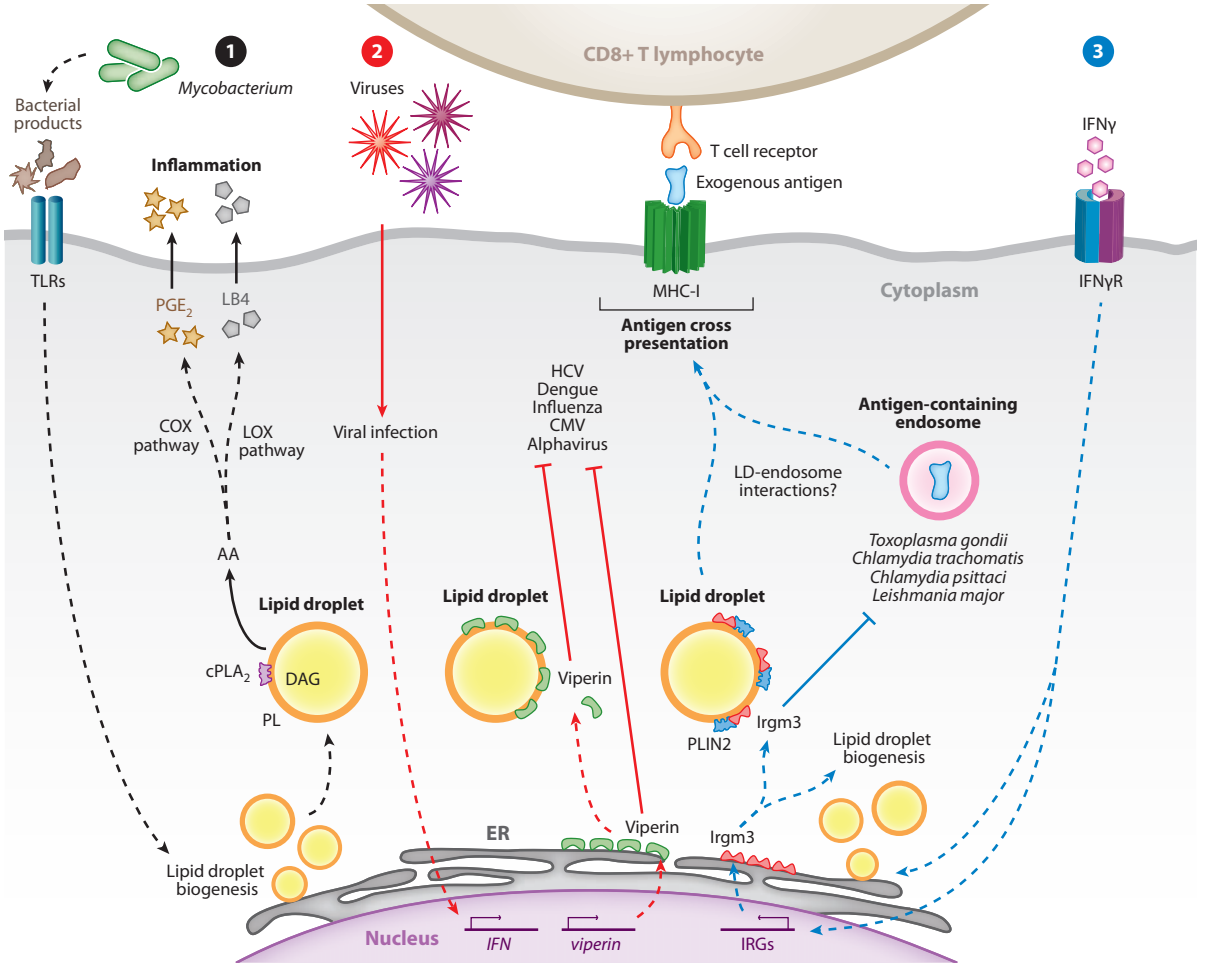

Figure 5

Model of the emerging role of lipid droplets (LDs) in immunity and host-pathogen interactions. ① LDs participate in the biosynthesis and secretion of inflammatory mediators (*black pathways*). For instance, in response to mycobacterial infection, Toll-like receptor (TLR)-mediated signaling can trigger accumulation of LDs. Cytosolic phospholipase A2 ($cPLA_2$) localized on LDs can release arachidonic acid (AA), which is stored esterified in diacylglycerols (DAGs) and phospholipids (PLs). AA then enters the cyclooxygenase (COX) or lipoxygenase (LOX) pathway to generate the eicosanoids prostaglandin E2 (PGE_2) or leukotriene B4 (LB4), respectively. These eicosanoids are potent inflammation mediators and immunity modulators. ② Intersection between interferon (IFN) responses and LDs in viral infections (*red pathways*). Upon infection with viruses, cells respond by producing IFN, which induces the expression of IFN-stimulated factors. One such factor, viperin, localizes to the endoplasmic reticulum (ER) and to LDs. Viperin exerts antiviral activity against a range of viruses including hepatitis C (HCV), Dengue, influenza, cytomegalovirus (CMV), and alphaviruses. ③ LD intersection with effectors of interferon responses and antigen cross presentation (*blue pathways*). A family of IFN-related GTPases (IRGs) plays a role in cell-autonomous resistance to a wide range of intracellular pathogens. Upon IFNγ stimulation, the IRG proteins are induced. Irgm3, a member of this family of proteins, localizes to the ER and to LDs where it interacts with PLIN2 (ADRP). Irgm3 is required for IFNγ-induced LD biogenesis and for efficient presentation of exogenous antigens to CD8+ T lymphocytes in the context of class I major histocompatibility complex (MHC-I), a process known as cross presentation of antigens. How LDs participate in antigen cross presentation is not clear, but it could involve cross talk with the endocytic pathway.

presumably sustains robust viral replication. Inhibition of autophagy negatively affects Dengue virus replication. However, this inhibition in viral replication can be reversed if exogenous free fatty acids are added, which supports the idea that Dengue virus may induce selective autophagy of LDs. The rerouting of autophagosomes to LDs may also serve as an immune evasion strategy, as autophagosomes participate in the processing of antigens for antigen presentation (Heaton & Randall 2011) and possibly in the orchestration of IFN responses. However, any turnover of LDs must be selective, as Dengue virus also uses these organelles as platforms for replication (Samsa et al. 2009).

PERSPECTIVES

Clearly, we are just beginning to scratch the surface of the multitude of functions performed by LDs, and these functions will multiply as we appreciate the diversity of these organelles within a cell and among different cell types. In **Figure 5**, we present a model summarizing evidence that supports an emerging role for LDs in immunity and host-pathogen interactions. The function(s) these organelles play in regulating inflammation, IFN responses, and antigen presentation should lead to further scrutiny by researchers in the area of host-pathogen interactions. In particular, as LDs are uniquely responsive to changes in lipid homeostasis within a cell, it would not be surprising if they act as central sentinels for pathogen-mediated lipid imbalances and as initiators of innate immune responses. As such, many central molecules important in generating and regulating LDs may also end up playing unique roles during immune responses. This as an area ripe for investigation and one that should lead to new paradigms at the crossroads of cell biology and immunity.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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