Critical Review

The Role of Lipids in Host–Pathogen Interactions

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Abstract

Innate immunity relies on the effective recognition and elimination of pathogenic microorganisms. This entails sequestration of pathogens into phagosomes that promptly acquire microbicidal and degradative properties. This complex series of events, which involve cytoskeletal reorganization, membrane remodeling and the activation of multiple enzymes, is orchestrated by lipid signaling. To overcome this immune response, intracellular pathogens acquired mechanisms to subvert phosphoinositide-mediated signaling and use host lipids, notably cholesterol, as nutrients. We present brief overviews of the role of phosphoinositides in phagosome formation and maturation as well as of cholesterol handling by host cells, and selected Salmonella, Shigella, Chlamydia and Mycobacterium tuberculosis to exemplify the mechanisms whereby intracellular pathogens co-opt lipid metabolism in host cells.

Keywords: phagocytosis; phosphoinositides; cholesterol; lipid droplets; macrophage; bacteria; membrane trafficking; host-pathogen interactions

Abbreviations:

ACAT, acyl-CoA cholesterol acyltransferase; CE, cholesterol-ester; DAG, diacylglycerol; EEA1, early endosome antigen-1; EE, early endosomes; EB, elementary body; ER, Endoplasmic Reticulum; ESCRT, endosomal sorting complexes required for transport; GAPs, GTPase-activating proteins; Incs, inclusion proteins; ILVs, intraluminal vesicles; LE, late endosomes; LDs, lipid droplets; ManLAM, lipoarabinomannan; LDL, low-density lipoprotein; LAL, lysosomal acid lipase; MCP, M. tuberculosis-containing phagosome; MVBs, multivesicular bodies; NPC, Niemann-Pick type C protein; ORP, oxysterol-binding protein-related proteins; PIMs, phosphatidylinositol mannosides; PM, plasma membrane; PLC c, phospholipase C; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; RB, replicative reticulate; RB, reticulate body; SCV, Salmonella-containing vacuole; SapM, secretory acid phosphatase; StART, steroidogenic acute regulatory protein-related lipid transfer; TAG, triacylglycerol; TB, Tuberculosis; TTSS, type three secretion systems

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INTRODUCTION

Cells of the innate immune system have the ability to engulf pathogens, trapping them within a microbicidal vacuole, the phagosome. Particle engulfment, known as phagocytosis, is a complex process involving receptor engagement and activation, signal transduction, cytoskeletal rearrangement, membrane traffic and, ultimately, gene transcription and translation. Phagocytosis can coarsely be divided into two major events: phagosome formation and phagosome maturation. Formation refers to the trapping of the target within a sealed phagosomal vacuole. In addition to the entrapped target, the vacuole contains a small volume of extracellular medium and is lined by a membrane derived from the plasmalemma; as such, the nascent phagosome is not effectively microbicidal. After sealing, the phagosome gradually acquires a hostile, lytic environment capable of destroying and digesting pathogens, thereby generating antigens for presentation. This process, which requires extensive remodeling of the vacuolar enclosure and contents, is known as phagosome maturation. Remodeling is the result of staged fusion and fission events with early endosomes (EE), late endosomes (LE) and lysosomes that render the phagosome acidic, degradative and generally hostile to the ingested microorganisms.
In addition to functioning as signaling molecules, lipids are also exploited by pathogens, which harness host lipids as nutrients. The traffic and use of cholesterol by intracellular pathogens is dealt with in the Redirection of Lipids for the Pathogen’s Benefit section of this review (Fig. 3).

**ROLE OF PIS IN THE PHAGOSOME**

**Phagosome Formation**

To form a phagosome, the plasmalemma extends pseudopods around phagocytic targets; the tips of the pseudopods eventually fuse, generating a sealed vacuole (Figs. 2a and 2b). The extending plasma membrane (PM) contains PI(4,5)P2, PI(4)P and low levels of PI. The plasmalemmal pool of PI(4)P is maintained by PI4KA, which phosphorylates PI (4). PI(4,5)P2 is generated by type I phosphatidylinositol 4-phosphate 5-kinases (PIP5K). PI(4,5)P2 contributes to the structure and mobility of the cell membrane, which is also regulated by PI(4,5)P2.

During particle engagement for phagocytosis, PI(4,5)P2 increases transiently in the pseudopods (5) (Fig. 2a). This contributes to actin polymerization and drives pseudopod expansion around the target. However, PI(4,5)P2 accumulation is transient; as phagosome formation progresses, the inositol is hydrolyzed to diacylglycerol (DAG) and inositol trisphosphate by phospholipase C (PLC). The hydrolysis products can in turn regulate phagocytosis by stimulating protein kinase C (PKC) and other DAG-binding proteins, and by changing cytosolic calcium (5).

To enable the progression of a phagocytic cup into a phagosome, actin must be depolymerized from the base of the cup. PIs also play a role in depolymerization. Class I phosphoinositide 3-kinase (PI3K) is recruited to the base of the phagocytic cup, where PI(4,5)P2 is converted to PI(3,4,5)P3. When PI(3,4,5)P3 is formed at the base of the phagocytic cup, it binds and activates Rho-family GTPases to regulate cytoskeleton remodeling both directly and indirectly, as well as membrane traffic, which is also regulated by PI(4,5)P2.

The disappearance of PI(4,5)P2 coincides with an increase in PI(4)P. PI(4,5)P2 around the nascent phagosome is dephosphorylated to PI(4)P by 5'-phosphatases such as Oculocerebrorenal syndrome of Lowe-1 (OCRL-1) and possibly the synaptojanins. About a minute after the phagosome seals, PI(3,4,5)P3 disappears completely (5), through dephosphorylation into PI(3,4)P2 by SHIP and/or to PI(4,5)P2 by PTEN. PI(4)P is present throughout phagosome formation, closure and sealing, but then disappears and remains undetectable for about 10 min. During endosome formation, PI(4)P is hydrolyzed into PI or by PLC into inositol-
bisphosphate and DAG (6); similar processes seemingly account for its disappearance from early phagosomes (7). It is noteworthy that during Fcγ receptor-mediated phagocytosis, PI(4)P is not converted back into PI(4,5)P2, and PI(3,4,5)P3 is not produced again on the maturing phagosome. However, this does not apply for other types of phagocytosis. During complement-mediated phagocytosis, phagosomes undergo de novo formation of PI(4,5)P2, PI(3,4,5)P3 and/or PI(3,4)P2 (8).

**Early Phagosome**

The early phagosome is characterized by the presence of PI(3)P, which coincides with the disappearance of PI(4)P after phagosome formation. Production of PI(3)P is a hallmark of phagosome maturation. Before PI(3)P can be acquired, the class III PI3K Vps34 must be recruited by Rab5 to the cytosolic side of the maturing phagosome, where it is activated to phosphorylate PI into PI(3)P. Pharmacological inhibition of Vps34 arrests maturation. An important effector is early endosome antigen-1 (EEA1), which binds PI(3)P through its FYVE domain and simultaneously recognizes the active form of Rab5 (Fig. 2). EEA1 is crucial since it interacts with SNAREs such as Syntaxin-6 and Syntaxin-13, which catalyze endosome–phagosome membrane fusion. Syntaxin-6 is present on Golgi-derived vesicles, and EEA1 mediates their fusion with the phagosome. PI(3)P also recruits a component of the NADPH oxidase complex-2. The oxidase generates microbicidal reactive oxygen species within the phagosome. Furthermore, PI(3)P controls membrane remodeling of the early phagosome by recruiting the endosomal sorting complexes required for transport (ESCRT). ESCRT consists of four subunits (ESCRT-0 to ESCRT-3), where ESCRT-0 contains a FYVE domain that interacts with PI(3)P.

PI(3)P is cleared at the end of the early phagosome stage by three possible mechanisms: 1-phosphatidylinositol 3-phosphate 5-kinase (PIKfyve)-mediated phosphorylation to PI(3,5)P2, dephosphorylation by myotubularin-1 (MTM1) to PI, or hydrolysis by phospholipases. The disappearance of PI(3)P marks the transition from early to late phagosome, which coincides with the loss of Rab5 and EEA1.

**Late Phagosome and Phagolysosome**

As maturation progresses, Rab7 is acquired and PI(4)P reappears in the phagosome. Reappearance of PI(4)P is a key step in the transition from early to late phagosomes. PI(4)P is synthesized by phosphatidylinositol 4-kinase 2 (PIKfyve)-mediated phosphorylation to PI(3,5)P2, dephosphorylation by myotubularin-1 (MTM1) to PI, or hydrolysis by phospholipases. PI(3,5)P2 production delays phagosome maturation; both

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**FIG 2**

Phs in phagosome formation and maturation. After binding (a), pseudopods extend around the pathogen (b). At this stage, PI(4,5)P2 is enriched in pseudopods, aiding actin polymerization. As the incipient phagosome develops, actin is depolymerized at the base of the cup due in part to loss of PI(4,5)P2 and gain of PI(3,4,5)P3, which activates Rho-family GAPs. Phagosome maturation is initiated upon closure. Rab5 and PI(3)P are acquired, which together with EEA1 induce fusion with endocytic vesicles (c). PI(3)P is replaced by PI(4)P at late stages of maturation (d). The phagosome gradually acidifies and becomes a phagolysosome as a result of fusion with lysosomes, which deliver V-ATPases. Acidification leads to autophagy of proteases and degradation of the pathogen (e). The phagolysosome contains PI(3,5)P2, which functions in lysosome fusion.
PI(3)P disappearance as well as the acquisition of lysosome markers are delayed, and the degradative capacity of the phagosome is reduced (9). PI(3,5)P2 may act by regulating lysosomal calcium channels (10). Later, PI(3,5)P2 can be hydrolyzed to PI(5)P by myotubularins. Whether PI(5)P accumulates and plays a role in phagosome maturation remains undefined.

SUBVERSION OF PHOSPHOINOSITIDE METABOLISM BY PATHOGENS

*Mycobacterium tuberculosis*

Tuberculosis (TB) is a severe pulmonary disease caused by *M. tuberculosis*. Despite the availability of antibiotics, TB is the ninth cause of death worldwide and the number one caused by an infectious agent (11). Following phagocytosis, *M. tuberculosis* can persist and replicate within host alveolar macrophages for decades in a dormant state, without manifesting as clinical TB. An estimated 85–95% of the 1.7 billion total infections worldwide remain in this latent state (11). Following primary infection of the lung, the hallmark of latent TB is the infiltration of inflammatory cells, which form a granuloma, yet fail to eradicate the pathogen. *M. tuberculosis* is ingested by macrophages; its entry can occur via several different phagocytic receptors such as complement, Fc or mannose receptors and Dendritic Cell-specific ICAM 3-Grabbing Nonintegrin (DCSIGN). Remarkably, the outcome differs in each instance. For example, intracellular survival is enhanced if *M. tuberculosis* is internalized via complement receptors (12), while Fcγ receptor-mediated uptake can kill the pathogen. When it survives, *M. tuberculosis* proliferates within phagosomes that fail to mature into phagolysosomes (13).

The *M. tuberculosis*-containing phagosome (MCP) is arrested at an early stage that features accumulation of Rab5, but absence of Rab7 and paucity of vacuolar ATPases, LAMP-1 and bactericidal hydrolases. Despite having some characteristics of early phagosomes, the MCP is depleted of PI(3)P, which is thought to be critical to the maturation arrest. Bacterial proteins proposed to subvert PI signaling to arrest phagosome maturation include secretory acid phosphatase (SapM) and the phosphatase MptpB. SapM was shown to dephosphorylate PI(3)P, thereby preventing maturation of the phagosome (Fig. 1) (14). Deletion of SapM renders *M. tuberculosis* incapable of maturation arrest (15). While SapM is PI(3)P-specific, the secreted phosphatase MptpB displays phosphatase activity toward phosphotyrosine, phosphoserine/threonine as well as PIs (16). MptpB dephosphorylates all mono-phosphorylated PIs, as well as PI(3,5)P2, into PI (Fig. 1). Decreased phagosomal PI(3)P causes limited acquisition of key fusion-promoting molecules such as EEA1 and Hrs, resulting in maturation arrest.

Bacterial analogs of mammalian PIs also contribute to block phagosome maturation. These are glycolipids of the bacterial membrane and upper layers of the mycobacterial peptidoglycan. Mycobacterial PI analogs include phosphatidylinositol mannosides (PIMs) and lipoarabinomannan (ManLAM) (17,18). During infection, PIM is released from engulfed mycobacteria and inserts itself into the phagosome membrane, promoting continuous fusion with EEs in a PI3K-independent,
wortmannin-insensitive manner. ManLAM acts by blocking a cytosolic calcium increase claimed to occur during phagosome maturation, ostensibly preventing the calmodulin kinase II-dependent activation of Vps34 (19). Furthermore, ManLAM inhibits recruitment of EEA1 to the phagosome (20), which prevents EEA1-Rab5-Vps34 complex formation on the MCP, thus diminishing PI(3)P formation. Normal phagosomes also receive cargo from the trans-Golgi-network during maturation. ManLAM seemingly also interferes with the trans-Golgi-network-to-vacuole pathway, thus limiting delivery of V-ATPases and bactericidal hydrolases to the MCP.

**Salmonella enterica**

*Salmonella enterica* is a major cause of food-borne gastroenteritis and can cause typhoid fever (21). During *Salmonella* spp. infection, the bacterium induces its entry and survival in both professional phagocytes and non-phagocytic cells using type three secretion systems (TTSS) that inject bacterial effectors across the plasmalemma and *Salmonella*-containing vacuole (SCV) into the host cytosol (22).

The early SCV resembles the early phagosome as it acquires Rab5, active Vps34 and accumulates PI(3)P, which further recruits EEA1 (22). One of the key virulence factors of *Salmonella* is SopB, a TTSS effector that mediates invasion of *Salmonella* into mammalian cells and delays the maturation of the SCV (23). The enzymatic activity of SopB has been debated. *In vitro*, SopB was initially found to hydrolyze IP5 to IP4 (24). SopB was also shown to display a phosphatase activity resembling that of mammalian PI-5'-phosphatases, as it could hydrolyze PI(3,4,5)P3, PI(3,4)P2 and PI(3,5)P2 into PI(3)P (25). When heterologously expressed in mammalian cells, SopB caused extensive hydrolysis of PI(4,5)P2 (26). However, the natural substrate(s) and true enzymatic activity of SopB when translocated by bacteria *in vivo* remained unclear. Mallo et al. (27) subsequently showed that SopB was required for PI(3,4)P2 and PI(3,4,5)P3 to accumulate at invasion ruffles during invasion, and for PI(3)P formation by the nascent SCV. Rab5 was recruited to the early SCV in a SopB-dependent manner, inducing Vps34 activity and generation of PI(3)P from PI. Mallo et al. concluded that this accounted for the production of PI(3)P, rather than SopB directly dephosphorylating PI(3,4)P2 and PI(3,5)P2. This is supported by studies showing that class III PI3K is required for acquisition of PI(3)P by the SCV (28).

**Shigella flexneri**

*Shigella* spp. is transmitted via the fecal–oral route after ingestion of contaminated food or water. *Shigella* triggers its transcytosis through microfold cells of the intestine and infects underlying Peyer’s patches. After transcytosis, *Shigella* encounters macrophages in the lamina propria, which rapidly engulf the bacteria (29). In addition, like *Salmonella*, *Shigella* facilitates its entry into non-phagocytic cells using a TTSS, which secretes bacterial effectors into the host cell. A key effector is IpgD, a homolog of the *Salmonella* SopB. IpgD modulates PI metabolism by acting as a PI4 phosphatase (Fig. 1) (30); it dephosphorylates PI(4,5)P2 into PI(5)P, leading to a decreased ability of the membrane to tether to actin filaments (31), along with increased membrane ruffling and disappearance of actin stress fibers (30). Furthermore, the PI phosphatase activity of IpgD is sufficient to induce activation of class IA PI3K, which increases the levels of PI(3,4)P2 and PI(3,4,5)P3 (32).

**CHOLESTEROL AS A HOST METABOLITE**

Most lipid biosynthetic enzymes, including those that synthesize glycerolipids, sterols, glycerolipids, sterols, cardiolipin, and sphingolipid precursors cardiolipin and sphingolipid precursors, localize to the endoplasmic reticulum (ER) and mitochondrial matrix. These lipids need to be transported to the organelles where they are destined to reside for further processing and utilization (3,33). Cholesterol is no exception; it can be synthesized *de novo* in the ER. However, it can also be acquired from extracellular sources and can be stored in unique intracellular organelles called lipid droplets (LDs) (2).

Cholesterol is an essential component of eukaryotic membranes, serving as precursor for steroid hormones, bile acids and oxysterols. It regulates membrane rigidity, gene transcription, and protein function and degradation. Cholesterol is compartmentalized asymmetrically across cellular organelles, which requires specialized transport mechanisms; both vesicular and non-vesicular transport mechanisms have been described.

**Exogenous Cholesterol Uptake and Handling**

Cells acquire exogenous, largely esterified cholesterol mainly via clathrin-mediated endocytosis of low-density lipoprotein (LDL), very-low-density lipoprotein or chylomicron remnant particles (34). The LDL-receptor is recycled to the PM, while the cholesterol-ester (CE)-containing vesicles continue along the endocytic pathway to lysosomes, ultimately delivering free cholesterol throughout the cell. This is accomplished following hydrolysis of CEs by lysosomal acid lipase (LAL).

In resting conditions cholesterol accounts for 20–25% of the plasmalemmal lipid content and is believed to organize into microdomains with glycosphingolipids and sphingolipid lipids (33). It is also enriched in the Golgi apparatus; in contrast, the ER contains as little as 1% of the total cholesterol. In the endocytic pathway, cholesterol is distributed heterogeneously. Approximately 20% of endocytic cholesterol is in recycling tubulo-vesicles, while ≈60% is in internal membranes of multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) (35). Normally, lysosome membranes are cholesterol-poor, as they deliver the lipid to other organelles (2) (Fig. 3). These conditions favor the enzymatic digestion of endocytosed glycosphingolipids and sphingolipids in lysosomes (33).
The close apposition of endosomes/lysosomes and smooth ER tubules facilitates non-vesicular transport of cholesterol. The most prominent mechanisms proposed to transfer cholesterol between endosomes/lysosomes and the ER are VAP-interacting oxysterol-binding protein-related proteins (ORP), and steriodogenic acute regulatory protein-related lipid transfer (StART) proteins. VAP-A and -B are abundant ER integral membrane proteins, while ORP1L localizes to LEs/lysosomes via interaction with Rab7 (36); VAPs tether ORP1L to the ER (37). ORP1L was shown to transfer free cholesterol from the membrane of LE/Lysosomes to the ER for esterification (38).

StARD3, attached to the limiting membrane of LEs via its N-terminal transmembrane domain, binds sterols using its cytoplasmic-facing START domain. StARD3 also contacts the ER by interacting with VAPs (39). Unlike ORP1L, StARD3 does not appear to extract cholesterol from endosomes for delivery to the ER. The opposite seems to be true: overexpression of StARD3 promotes accumulation of endosomal cholesterol, dependent on de novo synthesis of cholesterol (40).

From the endocytic pathway, cholesterol can also be routed to the Golgi complex or recycled to the PM. These pathways are detailed in ref. 41.

**Lipid Droplet Formation**

When intracellular free cholesterol is in excess, delivery to the ER allows esterification by acyl-CoA cholesterol acyltransferase (ACAT) and packaging of CEs into LDs (42). LDs are important for storage of excess neutral lipids and regulation of signal transduction, membrane transport and lipid homeostasis. LDs are composed of a CE and triacylglycerol (TAG) neutral lipid core and a surrounding phospholipid monolayer. They are generated within the ER, where the enzymes catalyzing neutral lipid synthesis reside (Fig. 3).

Cytosolic lipases allow turnover of LD-associated TAG and CE into free fatty acids, glycerol and free cholesterol—a process termed lipolysis. This process is metabolically regulated and coordinated by LD-associated proteins such as the perilipins and DFF45-like effector family members, which can shield the LD from cytosolic lipases (43,44).

**Cholesterol Efflux**

When free cholesterol flux from the endocytic pathway to the ER is high, synthesis of cholesterol is inhibited, and cholesterol efflux is activated. Three major proteins are coordinated to deliver cholesterol, phospholipids and other metabolites from cells to plasma, in the form of high-density lipoproteins. These are the extracellular apolipoprotein apoA-1 and two lipid transport proteins: the ATP-binding cassette transporters A1 and G1 (2). The process, known as reverse cholesterol transport, culminates in specialized tissues like the liver and adrenal cortex, where B1 type scavenger receptors bind the lipoprotein-ApoA-1 and extract the CEs for bile acid or steroid hormone biosynthesis.

**REDIRECTION OF LIPIDS FOR THE PATHOGEN’S BENEFIT**

Within a membrane-bound vacuole, bacteria are protected from the host’s defense responses, evading cytosolic surveillance (1). However, this comes at a cost, as the bacteria lose immediate access to the cytosol, where nutrients are plentiful. Bacteria use several methods to overcome this limitation: they can gain nutrients by transport across the vacuolar membrane, by extracting vacuolar membrane components or by translocation of the bacterium into an alternative nutrient-rich environment.

The importance of cholesterol and LDs for the growth and pathogenicity of numerous intracellular bacteria is now appreciated. This occurs despite the almost universal absence of sterols in bacteria, suggesting that these metabolites are host-derived. This section focuses on the acquisition of host cholesterol and LD-derived lipids by intracellular bacteria.

**Chlamydia trachomatis**

*C. trachomatis* is a gram-negative obligate intracellular bacterium with a biphasic life cycle, alternating between an infectious elementary body (EB) state and a replicative reticulate body (RB) state. Genital serovars of *Chlamydia* are the leading cause of sexually transmitted infections worldwide. If left untreated, genital serovars can lead to inflammatory disease, ectopic pregnancies and infertility, while trachoma serovar infections can lead to blindness (45).

*Chlamydia* uses a TTSS to enter host epithelial cells—mainly of the conjunctiva and urogenital tract. Spore-like, but metabolically inert, EBs deploy bacterial effectors that induce remodeling of the host actin cytoskeleton, leading to pathogen uptake into a PM-derived vacuole. Unlike normal phagosomes, the vacuole does not undergo canonical maturation via fusion with endosomes/lysosomes. Instead, EBs transform into metabolically active RBs, promoting homotypic fusion with other “like” vacuoles, yielding a large so-called inclusion (46). In the inclusion, bacteria replicate by binary fission. At later stages, RBs differentiate back to EB, which will eventually complete the infectious cycle upon release into the extracellular milieu (45).

An important family of TTSS-secreted effectors, called inclusion proteins (Incns), localize to the inclusion membrane-host cytosol interface. Incns hijack host vesicular and non-vesicular transport of lipids to modify the inclusion.

**Cholesterol and Sphingomyelin Accumulation by Chlamydia**

*Chlamydia* can synthesize most phospholipids typically found in prokaryotes (47). By contrast, sphingomyelin and cholesterol must be acquired from the host, since *Chlamydia* lack machinery for their synthesis. Staining with filipin has revealed accumulation of cholesterol on the inclusion membrane, with increased concentration in microdomains where IncB, Inc101, Inc222 and Inc850 are found. Inclusion cholesterol is acquired from both biosynthetic and exogenous...
sources (48). Sphingomyelin has been visualized in segments of the inclusion membrane (49) and its biosynthesis is required for intracellular growth of Chlamydia (50). One source of both lipids appears to be the Golgi apparatus, where cholesterol and sphingomyelin normally intended for delivery to the PM are hijacked and rerouted to the inclusion (48,49). A second source is MVBs (Fig. 3); StARD3, CD63 and lysobisphosphatidic acid are found in the inclusion lumen. Pharmacological inhibition of MVV traffic or of cholesterol transport prevents delivery of both cholesterol and sphingomyelin to the inclusion (51,52). Third, the pathogen may also use the reverse cholesterol transport machinery for cholesterol delivery, considering that ABCA1, Apo-A1 and B1 type scavenger receptors localize to the inclusion membrane (53).

What are the consequences of sphingomyelin and cholesterol accumulation in the inclusion membrane? Cholesterol-rich microdomains of the inclusion recruit active Src-family kinases, which are required for displacement of the vacuole toward the microtubule-organizing centre. More importantly, acquisition of cholesterol and sphingomyelin-containing membranes is important for bacterial growth. Disruption of MVB biogenesis, traffic or ability to unload cholesterol disrupts inclusion maturation and the generation of infectious progeny (51). Finally, silencing ABCA1 or pharmacological inhibition of reverse cholesterol transport suffice to disrupt bacterial growth, presumably due to failed delivery of cholesterol to the inclusion (53).

Lipid Droplet Translocation into Chlamydia Inclusions

Fluorescence microscopy of Chlamydia-infected cells revealed the accumulation of LDs inside the inclusion membrane and the presence of neutral lipids within bacterial membranes (54). Follow-up work demonstrated direct docking of LDs on the inclusion membrane, entry into the lumen, and association with RBs (55) (Fig. 3).

Several studies support the role of LDs in bacterial pathogenesis. Pharmacological inhibition of LD biogenesis reduces chlamydial growth and development (54). Moreover, genetic ablation of TAG synthesis, and thereby LD synthesis, greatly reduced the development of Chlamydia (56). Finally, pharmacological inhibition of host ACAT interrupts C. trachomatis growth, an effect that can be bypassed by addition of cholesterol linoleate (57).

Several bacterial proteins are translocated across the inclusion membrane and accumulate on eukaryotic LDs surrounding the inclusion. Conversely, several LD proteins including perilipins, Rab18 and ATGL localize adjacent to the inclusion membrane (54). LD entry may occur at IncA microdomains on the inclusion membrane, as this protein cofractionates with LDs and, like the LDs, enters the lumen of the inclusion (55).

Accumulating LDs around and within the inclusion membrane may provide a direct source of non-vesicle-associated phospholipids, sterols and/or enzymes. Chlamydia possesses an enzyme with sterol esterase activity (58) that could release both free cholesterol and the associated fatty acid within the inclusion lumen. Alternatively, LD “organelle mimicry” may discourage lysosomal fusion, preventing degradation of the inclusion and its contents (54).

Cholesterol as a Nutrient for M. tuberculosis

M. tuberculosis can metabolize cholesterol as a carbon source and likely acquires this nutrient while within the phagosome (59). To this end, M. tuberculosis has a dedicated cholesterol uptake pathway (60). During chronic mouse infection, this uptake pathway and catabolism of cholesterol are necessary for mycobacterial growth and survival. Growth on cholesterol greatly alters the abundance of several bacterial metabolites, including intermediates of the methylcitrate cycle that catabolizes propionyl-CoA. Propionyl-CoA, liberated largely from the side-chain of cholesterol, is toxic to M. tuberculosis unless cleared (61). For this reason, induction of methylcitrate cycle enzymes is essential for growth under cholesterol-rich conditions as well as for growth of the bacteria inside macrophages (62), ostensibly by relieving propionyl-CoA toxicity.

Sterol Accumulation by M. tuberculosis

The phagosomal membrane surrounding the bacterium is one likely source of cholesterol. Filipin stains the perinuclear region of interferon-γ stimulated macrophages infected with M. tuberculosis, where the bacteria are found (60). It has also been suggested that EEs can deliver cholesterol esters from LDL to the MCP (59). Another possibility is that the ongoing fusion of recycling endosomes with the MCP (63) provides a continuous source of LDL (Fig. 3). Finally, M. tuberculosis may acquire cholesterol and fatty acids from oxidized LDL following uptake by scavenger receptors. However, the manner whereby the bacterium extracts cholesterol or CE from lipoproteins remains unclear. Processing by LAL would in principle grant the bacterium access to free cholesterol (59). However, this model does not take into account that LAL has little activity above pH 4.5, and that the pH of the MCP was determined to be ≥6.4 (59). Interestingly, M. tuberculosis possesses several putative lipases that could perform this function.

A second source of cholesterol appears to be the interaction of LDs with the MCP (Fig. 3). Macrophages infected by M. tuberculosis become “foamy,” that is, filled with LDs, which have been hypothesized to serve as a nutrient reservoir for the bacterium (64). Recent work suggests mycolic acids, hydrophobic lipids of the bacterial envelope produced only by virulent strains, are responsible for LD and foamy macrophage formation. Specifically, keto-oxygenated mycolic acids induce cholesterol esterification and the formation of abundant intracellular LDs (65). Of note, a number of proteins important for LD biogenesis and processing are upregulated in human TB granulomas (66), suggesting global transcriptional changes during infection.

In macrophages located within granulomas generated in vitro, about 20% of the MCP membranes lie in close proximity to LDs, often apposed in a zipper-like fashion (64). This may
facilitate transfer of lipid nutrients such as sterols, to the mycobacteria.

**DISCUSSION**

Although this review focused primarily on PIs and cholesterol, other lipids have been implicated in host-pathogen interactions, notably cardiolipin. This mitochondrial lipid binds to the Nlrp3 inflammasome and is required for its activation (67). Cardiolipin also accumulates in *Klebsiella*-infected lungs, accentuating inflammation (68). Of note, cardiolipin is also produced by bacteria and is involved in their pathogenicity: it is required for optimal virulence of *Shigella* and *Salmonella* (69,70). Cardiolipin is thus important to the biology of both host and pathogen.

In summary, PIs and their metabolites are now appreciated to provide key signals directing the formation of phagosomes and marking the transition between distinct maturation stages. By establishing microdomains and membrane asymmetry, cholesterol and sphingolipids are also appreciated to be essential determinants of phagocytosis. Remarkably, the dynamics and distribution of cholesterol and phosphoinositides are closely intertwined; alterations of one species can potentially alter the other.

Pathogenic bacteria “appreciated” these subtleties long before researchers did and took advantage of lipids to secure their own survival and proliferation. The processes whereby bacteria hijack lipids are attractive targets for therapy and should be studied in greater depth, as they seem essential for their infectivity. Interference with such pathogen-specific pathways promises to yield effective antimicrobial drugs.

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