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Review

Lipids in host–pathogen interactions: Pathogens exploit the complexity of the host cell lipidome

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ABSTRACT

Lipids were long believed to have a structural role in biomembranes and a role in energy storage utilizing cellular lipid droplets and plasma lipoproteins. Research over the last decades has identified an additional role of lipids in cellular signaling, membrane microdomain organization and dynamics, and membrane trafficking. These properties make lipids an attractive target for pathogens to modulate host cell processes in order to allow their survival and replication. In this review we will summarize the often ingenious strategies of pathogens to modify the lipid homeostasis of host cells, allowing them to divert cellular processes. To this end pathogens take full advantage of the complexity of the lipidome. The examples are categorized in generalized and emerging principles describing the involvement of lipids in host–pathogen interactions. Several pathogens are described that simultaneously induce multiple changes in the host cell signaling and trafficking mechanisms. Elucidation of these pathogen-induced changes may have important implications for drug development. The emergence of high-throughput lipidomic techniques will allow the description of changes of the host cell lipidome at the level of individual molecular lipid species and the identification of lipid biomarkers.

Contents

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1. Introduction

Lipids are loosely defined as biological molecules with hydrophobic or amphipathic properties that render them soluble in organic solvents [1]. Despite the fact that this definition includes thousands of different chemical structures, a more defined classification seemed unnecessary for a long period of time. Lipids were believed to have two general functions: a structural role in biomembranes and a role in energy storage in cells (lipid droplets) and body fluids (lipoproteins). During the last two decades, however, we have come to realize that lipids have a multitude of different and essential functions in the cell. First indications for the involvement of specific lipids in biological processes came from the identification of platelet-activating factor as a lipid molecule [2]. The groundbreaking work of Irvine and Berridge in the 1980s showed the involvement of lipids in intracellular cellular signaling by the generation of second messengers from phosphoinositides [3]. Almost concomitantly the discovery was made that diacylglycerol and phosphatidic acid are biologically active lipids, as well as lipidic breakdown products of cellular sphingolipids [4–7]. Since the identification of the phosphatidylinositol transfer protein as an essential factor for protein trafficking from the trans-Golgi network (TGN) in yeast in the early 1990s [8], an overwhelming body of evidence showed the involvement of lipids in the regulation of membrane traffic [9]. Lipids have also been implicated in the generation of lateral heterogeneity in biological membranes, creating membrane domains that are often referred to as lipid rafts [10]. By specific recruitment of proteins and lipids to these domains while excluding others, many biological processes such as cell migration, the immune system, and the cell cycle are affected or regulated.

Thus, lipids have a multitude of functions in many biological processes and we are beginning to understand the long-time mysterious reason why nature synthesizes thousands of different lipids. This multitude of functions makes lipids also an attractive target for pathogens. In the innate immune system, microbes can be internalized by a phagocytosis-like process in order to be degraded by the acidic environment in subsequent phagolysosomal compartments. Some pathogens, however, escape the immune system by interacting with the cellular machinery at any one of these steps, allowing their survival and multiplication. Today there are many indications for an important role of lipids in various stages of host–pathogen interactions. One of the first examples for such involvement comes from the bacterium *Vibrio cholerae* that secretes the enterotoxin cholera toxin. The receptor of the toxin is a lipid termed GM1 [11]. This protein-lipid interaction results in the formation of a membrane pore, ultimately causing severe diarrhea.

In this review we will summarize the often ingenious strategies that pathogens utilize to divert cellular processes by modifying cellular lipid homeostasis. To this end pathogens take full advantage of the complexity of the lipidome (Fig. 1). The examples are categorized in generalized and emerging principles describing the involvement of lipids in host–pathogen interactions.

Many lipid-derived (signaling) molecules that classify as lipids themselves, and that might have a role in host–pathogen interaction, like prostaglandins, leukotrienes, quinones, vitamins, and tocopherols will not be discussed here. This still leaves us with thousands of different lipid species in the (phospho)lipid and sterol classes.

Until recently, only limited possibilities for lipid analysis were available, often only capable of lipid identification into different classes. Now the field of lipidology enters a new era, with mass spectrometry-based techniques providing novel tools to analyze samples at the single lipid species level and at the same time at the whole lipidome level [12–14]. We already see preliminary examples of and the urgent need for lipidomic approaches in various fields, including that of host–pathogen interaction, to unravel the involvement of lipids in infectious diseases [15]. This review aims to give an overview of the current status of our knowledge on the involvement of lipids in host–pathogen interactions, indicating that lipids from both host and pathogen play key roles in infection processes.

2. Modulation of the fatty acid moiety of host cell lipids

2.1. Fatty acid modulation of host cell lipids upon infection

During development in the erythrocyte, the malaria causing parasites, *Plasmodium* spp. modify the protein and lipid composition and various properties of the plasma membrane of their host cell [16–18]. In the membrane of erythrocytes parasitized by *Plasmodium falciparum* the composition of individual phospholipid classes, as well as that of their fatty acid constituents is altered [17–19]. Fatty acid alterations are not observed in all lipid classes.

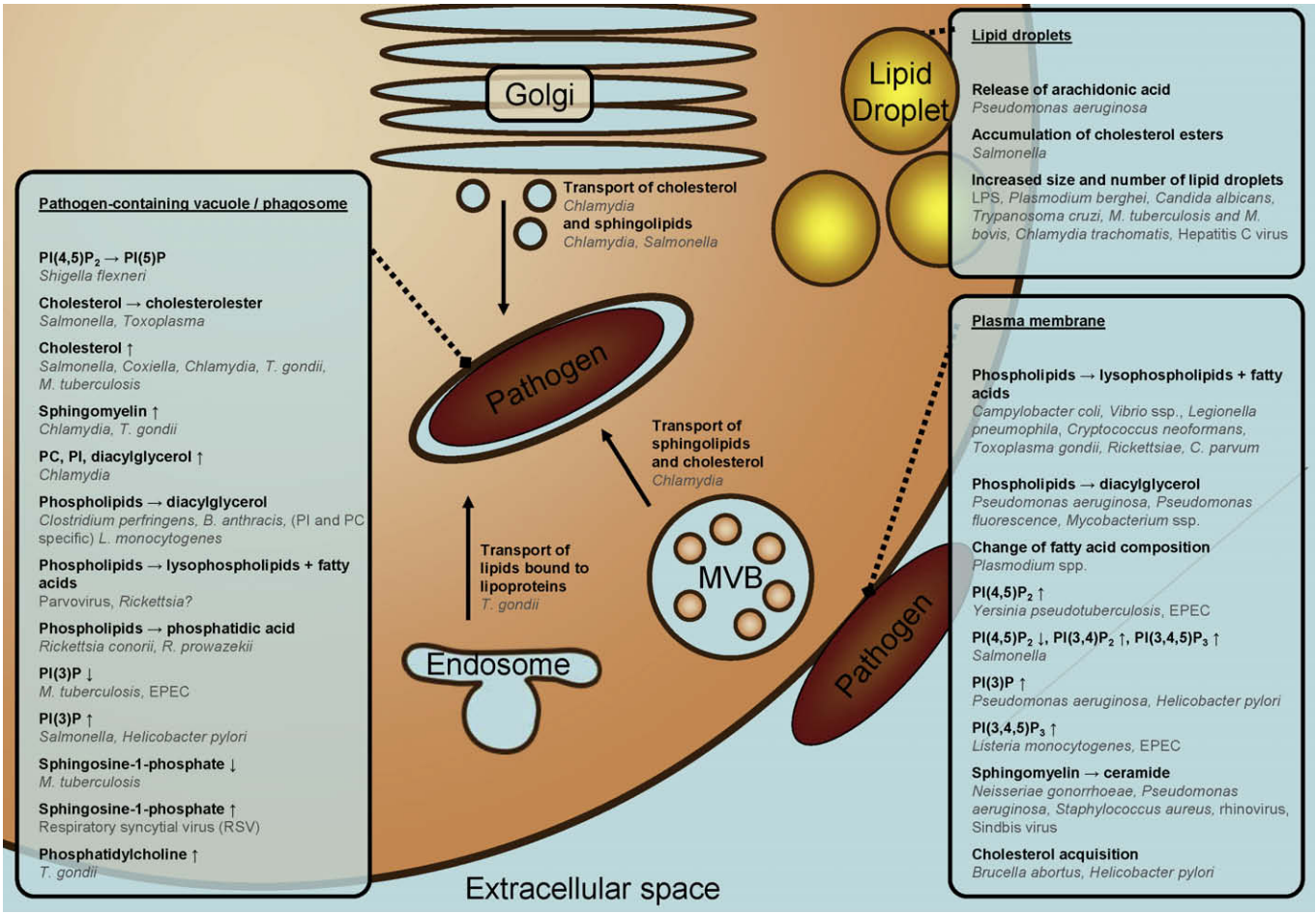

Fig. 1. Overview of modifications of the host cell lipidome by various pathogens. MVB: multivesicular body; EPEC: Enteropathogenic *E. coli*; PC: phosphatidylcholine; PI: phosphatidylinositol; PI(3)P: phosphatidylinositol-3-phosphate; PI(5)P: phosphatidylinositol-5-phosphate; PI(3,4)P_2: phosphatidylinositol-3,4-bisphosphate; PI(4,5)P_2: phosphatidylinositol-4,5-bisphosphate; PI(3,4,5)P_3: phosphatidylinositol-3,4,5-trisphosphate.

Alterations in the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine of erythrocyte membranes were observed for *Plasmodium knowlezi* [20]. Intraerythrocytic development of *Plasmodium chabaudi* is also associated with alterations in the overall fatty acid composition of the plasma membrane of the host cell [21], but not in that of the host cell's plasma membrane phosphatidylcholine [22]. At this moment it is not clear whether the observed alterations in fatty acid composition are due to enzymatic modifications *in situ* of the phospholipids in the plasma membrane of the erythrocyte, or to selective uptake of phospholipid molecular species from the erythrocyte plasma membrane by the parasite. Alterations in fatty acid composition of host cell lipids are not limited to parasitic infections. Changes in phospholipid fatty acid composition were also observed in mouse tissues after infection with Bacillus Calmette-Guérin, an attenuated mutant of *Mycobacterium bovis* [23], while human immunodeficiency virus-1 (HIV-1) infection of cultured lymphocytes was found to result in alterations of the fatty acid composition of the membrane lipids of the lymphocytes [24].

Pathogens can modify the fatty acid composition of host cell lipids in several ways. One way is the modulation of host cell fatty acid uptake from the environment. Enteropathogenic *Escherichia coli* (EPEC) inhibits the uptake of short-chain fatty acids, as illustrated by inhibition of butyrate uptake into Caco-2 cells during infection [25]. Genome-wide RNAi screens in *Drosophila* cells infected by *Listeria monocytogenes* or *Mycobacterium fortuitum* indicated the involvement of a member of the CD36 family of scavenger receptors, which are presumed fatty acid translocases [26,27]. A second way to modify the host cell fatty acid composition is the modulation of the activity or expression of enzymes involved in fatty acid synthesis. RNAi screening in *Drosophila* SL2 cells indicated that infection by *M. fortuitum* or *L. monocytogenes* requires sufficient expression of acetyl-CoA carboxylase, fatty acid synthase and stearoyl-CoA 9-desaturase [26]. Likewise, inhibition of acetyl-CoA carboxylase or fatty acid synthase in the host cells inhibits Hepatitis C virus (HCV) RNA replication [28,29]. Moreover, during infection HCV induces an upregulation of the expression of mRNAs encoding enzymes of fatty acid synthesis [28,29]. It is not clear whether alterations in expression levels of enzymes involved in fatty acid biosynthesis also result in altered fatty acid profiles of membrane lipids. In the next sub-sections we will focus on a third way for pathogens to modulate the fatty acid composition of host cell lipids which involves phospholipase activities, lipase activities, and/or cholesterol acyltransferase activities to efficiently modify of the fatty acid composition of host cell lipids by fatty acid exchange.

2.2. Pathogen and host cell phospholipases

Phospholipases, enzymes that cleave phospholipids, are involved in modification of membrane composition, in cell signaling pathways and in inflammatory cascades. Based on the position within the phospholipid at which the cleavage takes place, they are classified into four major groups: phospholipase A, B, C and D (PLA, PLB, PLC and PLD, respectively). Phospholipases from bacteria,

viruses and parasites act as virulence factors by modifying host cell lipids to their advantage [30,31]. They exert their action by being involved in a variety of aspects of host–pathogen interaction, such as causing membrane damage (hemolysis in the case of erythrocytes), entry into the host, and exit from vacuoles (Fig. 2). Phospholipases of pathogens may also be involved in acquisition of fatty acids for nutritional purposes (see Section 8), and activation of host signaling pathways to regulate the immune response (see Section 3). Lastly, pathogens can also trigger the activity of host cell phospholipases. Distinct isoforms of PLA_2 were found to mediate the ability of *Salmonella typhimurium* and *Shigella flexneri* to induce the transepithelial migration of neutrophils [32].

Pseudomonas aeruginosa injects an effector protein ExoU with PLA_2 activity directly into host cells by means of a type 3 secretion system [33–35]. This PLA_2 requires Cu^{2+}, Zn^{2+}-superoxide dismutase (SOD1) from the host cell as an activating factor [36]. Recently, ExoU was also found to have lysophospholipase A activity [37]. ExoU injected into the host cell is cytotoxic. By lysing cell membranes it may contribute to the ability of *Pseudomonas* to disseminate rapidly from lung tissue into the bloodstream. However, the cellular effects of ExoU are not limited to localised cell death and tissue destruction. This protein triggers an arachidonic acid-dependent inflammatory cascade *in vivo* [38]. Recent data indicate that ExoU liberates the arachidonic acid from intracellular lipid droplets [39]. In addition, it activates several transcription factors that control proliferative responses and proinflammatory cytokine production (for review, see [30]. *S. typhimurium* injects an effector protein SseJ into the host cell cytoplasm from within a vacuole in which the bacterium resides after its uptake into the host cell. SseJ exhibits both PLA and glycerophospholipid:cholesterol acyltransferase activity [40,41]. The exact role of this enzyme, which is activated by as yet unidentified proteinaceous host cell factor(s), is not yet clear [40]. SseJ is not cytotoxic [42].

2.3. Phospholipases and host cell plasma membrane damage

Many pathogenic bacteria secrete or contain in their outer membrane a phospholipase activity, which can destabilize the erythrocyte plasma membrane. This hemolytic activity has been characterized for several pathogens: In the case of *Campylobacter coli* [43], *Vibrio* ssp. [44–46] and *Legionella pneumophila* [47] it is a PLA, while in the case of *P. aeruginosa* [48,49] and *Pseudomonas fluorescence* [50] it is a PLC. The observation of a positive correlation between the presence of PLC activity and the hemolytic activ-

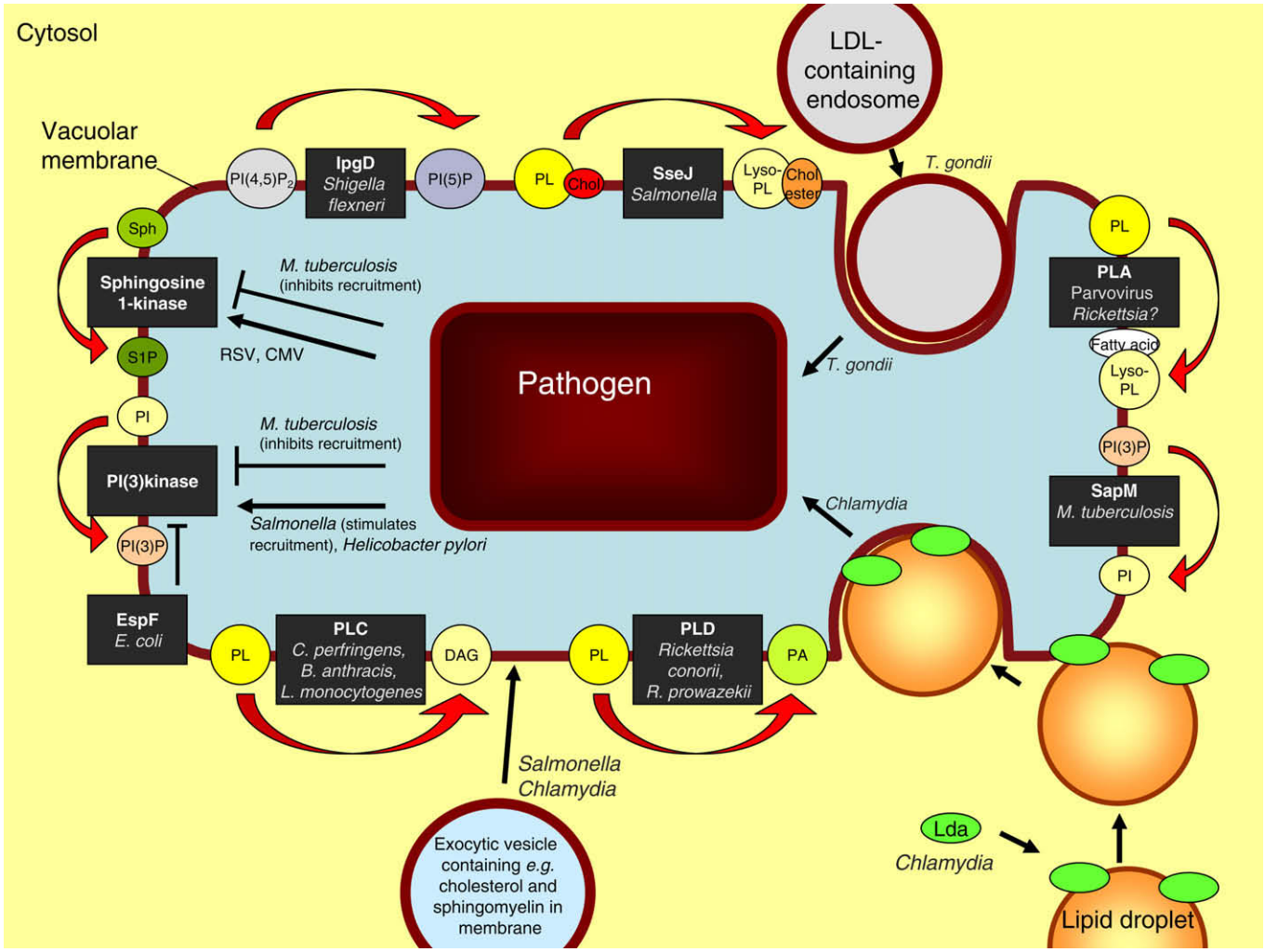

Fig. 2. Lipid metabolism at the pathogen-containing vacuole. The various effectors and enzymes are drawn in the vacuolar membrane. However, their exact location relative to the membrane is, in most cases, unknown. PLA: phospholipase A1 or A2; PLC: phospholipase C; PLD: phospholipase D; PL: phospholipid; PA: phosphatidic acid; DAG: diacylglycerol; Sph: sphingosine; S1P: sphingosine-1-phosphate; RSV: respiratory syncytial virus; CMV: cytomegalovirus; Chol: cholesterol; LDL: low-density lipoprotein; EspF: *Escherichia coli* effector molecule F; SapM: *M. tuberculosis* effector molecule; IpgD: *Shigella flexneri* effector molecule; SseJ: *Salmonella* SPI-2 effector molecule; Lda: Lipid droplet-associated *Chlamydial* effector proteins. For other abbreviations: see legend to Fig. 1.

ity in a range of *Mycobacterium* isolates indicates that also in *Mycobacterium* species a PLC is involved in hemolysis [51]. In some cases the bacteria may acquire iron from the lysed erythrocytes. PLA secreted by *L. pneumophila* may act as an important agent in causing lung disease by destruction of phospholipids of pulmonary surfactant and damage to cell membranes by the generated lysophosphatidylcholine [52].

In addition to causing lysis of cells, membrane degradation by hydrolysis of phospholipids and (in the case of PLA activity) formation of lytic phospholipids may also facilitate entry of pathogens into host cells, while in the gastrointestinal tract phospholipases may also degrade the mucus layer overlying these cells. A PLA_2 of *Helicobacter pylori* plays a role in colonization of the gastric mucosa [53,54]. A similar role may be played by a PLA secreted by *Campylobacter pylori*, which was shown to degrade phospholipids of gastric mucus [55]. The *Plasmodium berghei* phospholipase Pb PL is involved in migration of sporozoites through cells to gain entry into the bloodstream [56]. Pb PL is most similar in sequence to LCAT, a member of the PLA_2 family of serine lipases. Like LCAT it contains the GxSxG motif found in many serine lipases, and the catalytic triad of serine, histidine and aspartate. The enzyme was shown to have PLA activity [56]. The penetration of host cells by *Rickettsia rickettsii, R. prowazekii and R. conorii* also appears to be mediated by a PLA_2 of rickettsial origin [57,58]. A PLA_2 may also be involved in host cell invasion by the protozoan parasites *Cryptosporidium parvum* [59] and *Toxoplasma gondii* [60,61]. PLB secreted by *Cryptococcus neoformans* is important in the binding of this fungus to human epithelial cells prior to its internalization, possibly due to the release of fatty acids from host cell plasma membranes or pulmonary surfactant [62].

2.4. Phospholipases and vacuolar membrane damage

After invasion into host cells or phagocytosis by macrophages, bacteria reside in a vacuole or phagosome. Some intracellular pathogens escape from this vacuole into the cytoplasm to successfully survive and replicate [63,64]. This vacuole escape involves the action of pore-forming proteins or phospholipases or both [64]. *Clostridium perfringens* escapes from its macrophage phagosome by use of its α-toxin (a PLC) in cooperation with its perfringolysin O, a cholesterol-dependent cytolysin [65]. The combined action of phospholipase and a pore-forming protein in escape from the vacuole is also seen for the well-studied *L. monocytogenes*, an intracellular pathogen that replicates in the cytosol of host cells [63,64]. After invasion into cells, *L. monocytogenes* escapes from its vacuole by the combined action of the pore-forming protein listeriolysin O (LLO) and a phosphatidylinositol-specific PLC (PI-PLC), both secreted by the bacterium [63,64,66,67]. It has been suggested that LLO forms holes into the vacuole membrane, which allow *L. monocytogenes* phospholipase to access exposed membrane leaflet [63]. However, in a liposome lysis assay membrane permeabilization by *L. monocytogenes* PI-PLC in cooperation with LLO was found to be independent of phospholipid hydrolysis [68]. In addition to directly invading a cell, *L. monocytogenes* can also spread from one cell to another without leaving the intracellular environment. After such cell-to-cell spread the bacterium resides in a secondary vacuole surrounded by a double membrane. The escape from this double-membrane vacuole requires the coordinated action of LLO, PI-PLC and PC-PLC (a PC-preferring PLC also secreted by *L. monocytogenes*) [67,69,70]. Recent data indicate that the PI-PLC and PC-PLC act specifically in the initial degradation of the inner membrane of the double-membrane vacuole, after which the outer membrane is disrupted by LLO [71]. PLCs may likewise be involved in phagosomal escape of *Bacillus anthracis*. Cooperation between the cholesterol-dependent cytolysin anthrolysin O and three PLCs in *B. anthracis* mediated macrophage-associated growth and survival of this pathogen, while ectopic expression of anthrolysin O from *B. anthracis* in *Bacillus subtilis* conferred limited phagosomal escape upon the recombinant bacterium [72]. However, at present it is unclear whether the mediation by the combined action of anthrolysin O and the PLCs of growth and survival of B. anthracis in macrophages is actually related to an effect on phagosomal escape. Also various *Rickettsia* species lyse their phagosomal compartment after entry into a host cell. The lysis of the vacuolar membrane has been attributed to the activity of rickettsia PLA_2 [57,58,73]. The failure to identify a rickettsia PLA_2 from the rickettsia genome, together with the identification of a PLD in *R. prowazekii* and *R. conorii* has led to the suggestion that a PLD is involved in the escape from the vacuole [74]. This is supported by the observation that expression of the *R. prowazekii* PLD gene in *S. typhimurium*, which normally does not escape from its vacuole, conferred on the *Salmonella* the ability of vacuole escape [75]. There are also viruses that use a phospholipase to escape from a host cell vesicle: parvovirus virions deploy a capsid-tethered PLA_2 to breach the endosomal membrane after entry into a cell [76].

2.5. Lipases

Lipases are enzymes hydrolyzing tri-, di- and monoacylglycerols and are in some cases involved in pathogenesis. *Burkholderia cepacia* secretes a lipase that increases its invasion into epithelial cells without affecting plasma membrane or tight junction integrity [77]. *P. aeruginosa* uses a secreted lipase together with a secreted PLC to induce inflammatory mediator release from human platelets and leukocytes. Among these inflammatory mediators are the lipidic 12-hydroxyeicosatetraenoic acid (12-HETE) and leukotriene B_4 [78,79]. A lipase secreted by *Staphylococcus aureus* decreases phagocytosis and intracellular killing by human granulocytes of staphylococci, but not the phagocytic killing of pneumococci or streptococci. The effect of the lipase was reported to be partly retained after heat inactivation, indicating that the effect of the lipase is not exerted by enzyme action alone [80]. Also *Candida parapsilosis* secretes a lipase which inhibits phagocytosis and intracellular killing by macrophages [81]. In addition to these functions, lipases secreted by pathogens may also be involved in generation of fatty acids from host cell lipids for the pathogens' energy production or complex lipid synthesis (see Section 8).

2.6. Cholesterol acyltransferases

Several bacteria secrete enzymes with glycerophospholipid:cholesterol acyltransferase (GCAT) activity. These GCATs transfer an acyl chain from a phospholipid onto cholesterol, resulting in the formation of cholesterol esters and lysophospholipids. In addition, they possess phospholipase A activity. They are members of the GDSL lipase family, which is characterized by the presence of a conserved GDSL motif and a catalytic triad (S-D-H) [82,83]. Cholesterol is an important component of eukaryotic membranes, but is absent from the membranes of most prokaryotes. Acylation by a pathogen of host cell cholesterol might therefore be a good tool for the pathogen to specifically target host cells using an enzyme that in principle does not discriminate between host cell and pathogen lipids. Lowering the cholesterol level in the membranes of host cells may lead to increased fluidity of these membranes. In addition, GCAT/PLAs may influence signaling pathways in the host cell by generating lysophospholipids and fatty acids, or by affecting lipid raft composition through their effects on cholesterol. Here we will describe 3 examples of host–pathogen interactions involving GCAT activity that have been described in some detail.

In a survey of the distribution of a GCAT originally found in the culture supernatant of *Aeromonas hydrophila* [84] this enzyme was found to be present in the culture media of all *Aeromonas* species

tested, in that of *Vibrio anguillarum* and *V. parahaemolyticus*, and in that of *S. aureus* [85]. The enzyme has been purified from *Aeromonas salmonicida* and has a preference for phospholipids carrying short-chain or unsaturated fatty acids [86]. In addition to GCAT activity, the enzyme also possesses phospholipase A_2 and lysophospholipase A activity [87]. The enzyme is a major lethal toxin to fish, and lytic toward fish erythrocytes, especially when bound to lipopolysaccharide [88]. The AspA serine protease, another toxin secreted by *A. salmonicida*, is responsible for the proteolytic activation of pro-GCAT to GCAT after secretion of the former from the bacterium [89,90]. Despite their toxicity, however, neither the GCAT, nor the AspA appear to be essential for the virulence of *A. salmonicida* [91].

The second example comes from the intracellular pathogen *L. pneumophila* that secretes a GCAT activity termed PlaC. PlaC is probably secreted via a type 2 secretion system. It requires activation which is either directly or indirectly dependent on the type 2 secreted zinc metalloprotease ProA [92]. Beside GCAT activity, PlaC also possesses PLA and lysophospholipase A activity. As cholesterol is not present in the membrane of *Legionella*, PlaC may play a role in modification of host cell membranes. However, PlaC is not essential for infection of and replication within Acanthamoeba castellanii (*L. pneumophila*'s natural host) and U937 macrophages [92].

A third member of the GDSL lipase family is the *S. typhimurium* effector protein SseJ. This protein was recently found to have GCAT activity [40,41], PLA activity [40] and deacylase activity towards the artificial substrate *para*-nitrophenyl butyrate [93], but no lysophospholipase activity [40]. SseJ is translocated by a type 3 secretion system into the cytosol of a host cell from within the *Salmonella*-containing vacuole (SCV) in which the *Salmonella* resides after its entry into the host cell [94]. The GCAT and PLA activities of SseJ are both potentiated by proteinaceous factors from the host cell [40]. SseJ destabilizes the SCV membrane in the absence of another *Salmonella* effector SifA [42], and antagonizes the stimulatory effect of SifA on the formation of *Salmonella*-induced filaments (Sifs), which are tubular extensions of the SCVs [42,95]. SifA prevents the microtubule motor kinesin from being recruited to the SCV [96]. The GCAT activity of SseJ may lower the cholesterol level of the SCV membrane and lead to storage of the resulting cholesterol esters in lipid droplets [41]. The decreased cholesterol level in the SCV membrane may lead to increased fluidity of this membrane, thus facilitating kinesin-mediated rupture of the SCV around *Salmonella* lacking SifA [40]. Furthermore, it was suggested that by lowering the amount of cholesterol in the SCV membrane SseJ could lower the amount of SifA on the SCV, thereby decreasing the level of Sif formation [40]. Alternatively, the formation of lysophospholipid by SseJ may aid in giving the Sif membrane the right amount of curvature.

Another type of acyltransferase is produced by *S. aureus*. The elimination of *S. aureus* from staphylococcal abscesses is mediated by the generation of bactericidal fatty acids, possibly by the activity of leukocytes [97]. However, several species of *Staphylococcus* produce an enzyme, termed fatty acid modifying enzyme (FAME), which can inhibit the bactericidal activity of these fatty acids by esterifying them with an alcohol [98]. Cholesterol, which the bacterium may obtain from the host cell membranes, is a particularly good alcohol substrate for this enzyme. FAME has not yet been purified and characterized in detail. The enzyme does not require ATP for its activity and is probably not a cholesterol esterase acting in reverse [98].

As a final example of the involvement of cholesterol acyltransferase in host–pathogen interactions we mention the apicomplexan parasitic protozoa *T. gondii* that resides in a parasitophorous vacuole. In the vacuole the parasite depends on host cell cholesterol derived from endocytosed low-density lipoproteins (Fig. 3) (see Section 8 for further details). Replication of *T. gondii* in its vacuole is dependent on the conversion of cholesterol to cholesterol ester. The parasite expresses two isoforms of acyl-CoA:cholesterol acyltransferase (ACAT), which differ from mammalian ACAT in their substrate affinity and specificity, and in their mechanism of regulation [99]. The endogenous ACAT activities of *T. gondii*

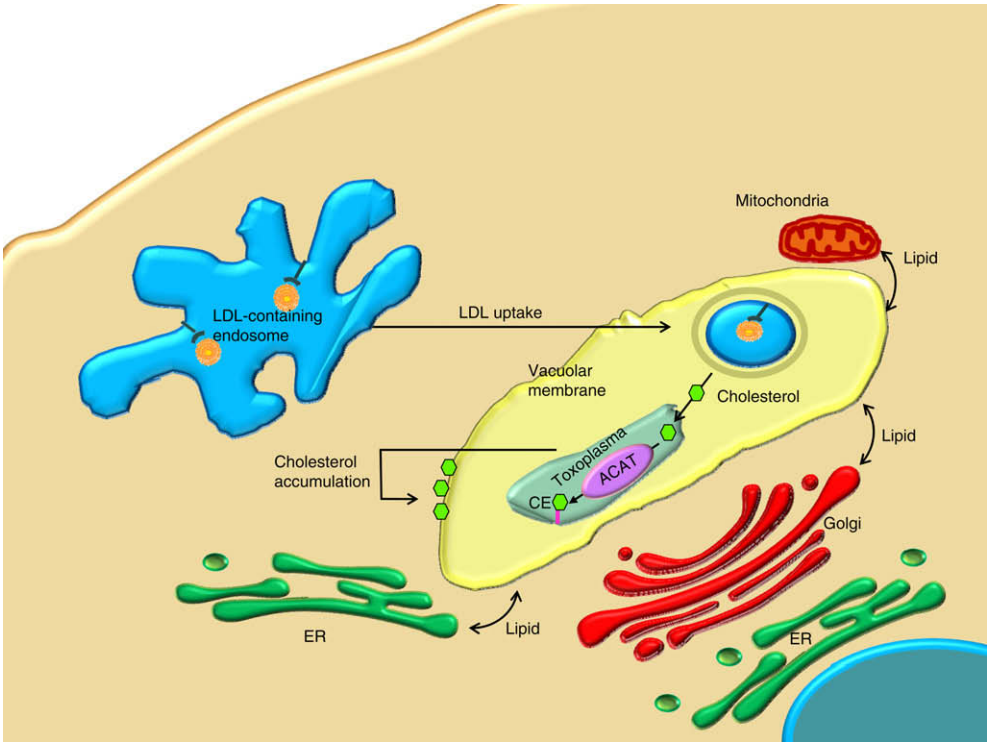

Fig. 3. Effects of *Toxoplasma gondii* on the host cell lipidome. ACAT: acyl-CoA:cholesterol acyltransferase; ER: endoplasmic reticulum; LDL: low-density lipoprotein; CE: cholesterolester.

are thought to be involved in the parasite's cholesterol ester synthesis and lipid droplet biogenesis [99,100] (Fig. 3).

Utilization of host cholesterol is also critical for the intraerythrocytic proliferation of a related apicomplexan parasite, *P. falciparum* [101]. The genome of this parasite was screened for genes that may be involved in acyl coenzyme A:DAG acyltransferase (DGAT) [102]. In addition to this gene, a sequence was identified which, based on its homology to the sequences for human and mouse lecithin:cholesterol acyltransferase (LCAT), was suggested to encode a plasmodial LCAT [102]. LCAT transfers an acyl chain from phosphatidylcholine (lecithin) to cholesterol, similarly to the GCATs described above. Despite the presence of the sequence for the putative LCAT in the *Plasmodium*, this parasite was found not to produce cholesterol esters, at least not while residing in erythrocytes [102].

3. Pathogen interference with lipid signaling in host cells

3.1. Phosphoinositide signaling during phagocytosis

Lipids play a major role in cellular signaling [103,104]. In general, signaling lipids have a rapid turnover and are present in minute amounts. This does not exclude the possibility that signaling lipids transiently appear at high concentrations in subdomains of the membrane. Phosphoinositides are an important class of signaling lipids and they are involved in numerous cellular signaling cascades. Phosphatidylinositol can be phosphorylated at its 3, 4 and 5 position in all possible combinations, leading to 7 different phosphoinositide species. Phosphorylation at other positions is also possible but less common. Phosphoinositides are involved in cellular signaling via two different mechanisms: (1) their hydrolysis yields second messengers that transmit downstream signals; and (2) they serve as a docking site for proteins with domains that recognize specific phosphoinositides [105]. In the latter case, phosphoinositides must be present in stoichiometric amounts and must be generated at (or targeted to) specific organelles or membrane domains. Therefore, phosphoinositides help define the identity of an organelle or of a domain by recruitment of specific proteins [106].

Phosphoinositide metabolism plays a central role in the regulation of receptor-mediated endocytosis and phagocytosis [107,108]. It contributes to the dynamics of the entire phagocytic maturation process, starting with the formation of a phagocytic cup at the plasma membrane and ending with the maturation process transforming a phagosome into a phagolysosome. During these processes, lipid kinases and phosphatases act in concert to regulate the extensive interconversion of various phosphoinositides. In brief: at the phagocytic cup, rapid interconversions between PI(4)P, PI(3,4)P_2, PI(4,5)P_2, and PI(3,4,5)P_3 take place [109,110]. Some of the phosphoinositides are involved in the recruitment of cytoskeletal elements, necessary for the formation of a phagocytic cup [111]. During the subsequent maturation process, formation of PI(3)P is essential, as well as the action of PI(3) kinases. Multiple waves of PI(3)P attract effector proteins such as EEA1 and Hrs to the phagosome, which is important for downstream signaling events [112]. In later stages of phagosome maturation, PI(3,5)P_2 is generated through phosphorylation of PI(3)P by PIKfyve [113]. As a result, PI(3)P effector proteins are released from the membrane and PI(3,5)P_2 effector proteins are recruited to the phagosomes.

3.2. Modulation of phosphoinositide signaling by pathogens

Pathogens have evolved ingenious strategies to subvert phosphoinositide metabolism by interference with many of the interconversion steps (for excellent reviews on this topic, see [114,115]). Here we will discuss some general principles, updated with recent examples. By interference with phosphoinositide metabolism, pathogens can affect either the uptake process (A) or the phagosomal maturation process (B).

(A) *L. monocytogenes* is a Gram-positive pathogen that secretes an effector named InlB via its type 3 secretion system. The *Listeria* surface protein InlB promotes bacterial internalization into host cells by activation of type I phosphatidylinositol 3-kinase (PI3KI) [116], resulting in the generation of PI(3,4,5)P_3. The effector protein In1B also results in the co-recruitment of type II phosphatidylinositol 4-kinases (PI4KII) to phagosomes [117]. The mechanism of action PI4KII remains to be established, as knockdown of PI4KII did not affect the levels of phosphoinositides but did affect the uptake process.

Yersinia pseudotuberculosis activates PIP(5)KIα to form PI(4,5)P_2 and causes activation of the small GTPase Rac1, involved in membrane ruffling during the uptake in epithelial cells [118].

An ingenious example of modulation of host cell phosphoinositide signaling is provided by *P. aeruginosa* that can only enter cells via basolateral membranes. To enable its uptake at the apical membrane, *P. aeruginosa* stimulates a PI(3) kinase at the apical membrane to increase local phosphoinositide levels that are phosphorylated at the 3 position, including PI(3,4,5)P_3 [119]. Formation of PI(3,4,5)P_3 leads to the generation of domains in the apical membrane resembling a basolateral membrane [120]. This transformation makes the apical membrane accessible for *P. aeruginosa* entry [121].

S. typhimurium secretes SigD (also known as SopB) via a type 3 secretion system. SigD is a phosphatase that, at least *in vitro*, is capable of dephosphorylating inositol phosphates and phosphoinositides at various positions [122]. This effector influences phosphoinositide metabolism in several ways, affecting both the uptake process and the maturation process (see below). By elimination of host cell PI(4,5)P_2, SigD promotes membrane fission at the plasma membrane during *Salmonella* invasion [123,124]. At the same time, SigD phosphatase activity leads to an accumulation of PI(3,4)P_2 and PI(3,4,5)P_3 on invasion ruffles, suggesting the concurrent recruitment to or activation at the plasma membrane of PI(3)-kinase [125].

Enteropathogenic *E. coli* (EPEC) also induces a transient PI(4,5)P_2 accumulation at bacterial infection sites, resulting in local actin accumulation and induction of PI(3,4,5)P_3 clustering [126].

(B) After uptake, pathogens disrupt phosphoinositide signaling by secretion of lipid modifying enzymes, allowing them to interfere with the maturation process. As these enzymes lead to propagation of the infection, they are often regarded as virulence factors [127]. As mentioned before, *Salmonella* secretes SigD which affects phosphoinositide metabolism at the plasma membrane of the host cell. The same enzyme activity of SigD is, however, also active at the Salmonella-containing vacuole (SCV). After entry of the Salmonella, SigD is delivered from the plasma membrane to the SCV via a process controlled by SigD ubiquitination [128]. At the SCV, SigD promotes formation of PI(3)P [125]. In this process SigD does not dephosphorylate phosphoinositides to produce PI(3)P, as suggested earlier [129], but promotes recruitment of Rab5 and its effector the PI(3) kinase Vps34, which in turn results in the formation of PI(3)P [125]. The mechanism described for the effector protein SigD is reminiscent of the effector IpgD that is secreted by *S. flexneri.* IpgD is a phosphatase that dephosphorylates PI(4,5)P_2 to PI(5)P. PI(4,5)P_2 is a key regulator of the actin cytoskeleton and in this way the cytoskeletal organization is affected [130,131]. Furthermore, IpgD is required for the formation of PI(3)-kinase products during *S. flexneri* invasion, possibly by activating a class I PI(3)-kinase [132].

Other pathogens that manipulate the phosphorylation at the 3 position of phosphoinositides to disrupt phagosome maturation include (i) Enteropathogenic *E. coli* that secretes EspF to block host PI(3) kinase activity [133,134]; (ii) *Helicobacter pylori* that strongly activates PI(3) kinases to disrupt actin cytoskeleton regulation and to cause a delayed phagocytosis [135]; and (iii) *Mycobacterium tuberculosis* that targets phosphoinositide metabolism is several ways. Via an unknown mechanism, *M. tuberculosis* secretes SapM, a lipid phosphatase that hydrolyzes PI(3)P (Fig. 4) and inhibits phagosome-late endosome fusion *in vitro*, thereby arresting phagosomal maturation [136]. In addition, *M. tuberculosis* interferes with PI-3 kinase hVPS34 by using phosphatidylinositol homologues [137] (Fig. 4). This will be further discussed in Section 4.

3.3. Sphingolipid signaling

Sphingolipids are a major class of membrane lipids, virtually absent from mitochondria and the ER, but constituting 20–35 mol% of plasma membrane lipids [138]. As a bulk lipid, sphingomyelin is involved in the stability of the lipid scaffold of lipid-enriched microdomains or lipid rafts. These microdomains are important targets for pathogens and will be dealt with separately (see Section 6). During the past two decades it became clear that sphingosine and related lipids containing a sphingoid base have an important role in cellular signaling, in cell function including the cell cycle, and in apoptosis [104]. The sphingolipid metabolites ceramide (Cer) and sphingosine (Sph) are associated with growth arrest and apoptosis. Many stress stimuli increase the levels of Cer and Sph, whereas suppression of apoptosis is associated with increased intracellular levels of sphingosine-1-phosphate (S1P). The balance between the apoptotic signals of Cer and Sph and the pro-survival signal of S1P is referred to as the "sphingolipid rheostat" [139].

3.4. Pathogen targeting of sphingosine-1-phosphate

S1P directly stimulates Ca^{2+} release from the endoplasmic reticulum (ER) [140,141]. The sphingosine kinase 1-dependent increase in cytosolic [Ca^{2+}] is necessary for the fusion and fission events during phagosomal maturation [142]. S1P also activates phagosome actin assembly required for killing of pathogenic mycobacteria [108]. Hence, inhibition of S1P may benefit pathogen survival in host cells and indeed, lysates from macrophages that were treated with *M. tuberculosis* inhibited sphingosine kinase activity, indicating direct inhibition of the enzyme by mycobacterial components [142] (Fig. 4). In addition, *M. tuberculosis* interferes with sphingosine kinase 1 translocation to the Mycobacterial phagosome, thereby inhibiting S1P accumulation and phagosomal maturation [143].

In contrast, some viruses that do depend on the phagocytic process, such as respiratory syncytial virus (RSV), stimulate sphingosine kinase activity resulting in increased levels of S1P. The pro-survival signal of S1P delays host cell death and results in increased viral replication [144]. Cytomegalovirus also activates sphingosine kinase, but in this case an elevated level of dihydrosphingosine-1-phosphate (dhS1P, also known as sphinganine-1-phosphate) was observed [145]. Sphingosine kinase can use both sphingosine and dihydrosphingosine (dhSph) as a substrate, resulting in the synthesis of S1P or dhS1P, respectively. As dhSph is a temporary intermediate in early steps of the synthesis of sphingolipids in the ER, levels of dhSph and its metabolites such as dhS1P are usually very low. Hence little is known about their potential biological (signaling) activity. dhS1P has been shown to be an S1P receptor agonist [146]. Opposite effects of dhS1P and S1P have been observed in transforming growth factor-beta/Smad signaling [147]. Thus, potential overlap and divergence in their biological activities have yet to be defined. It is interesting to note that the lipid composition of HIV-1, an enveloped retrovirus, shows an enrichment of the unusual sphingolipid dihydrosphingomyelin [148].

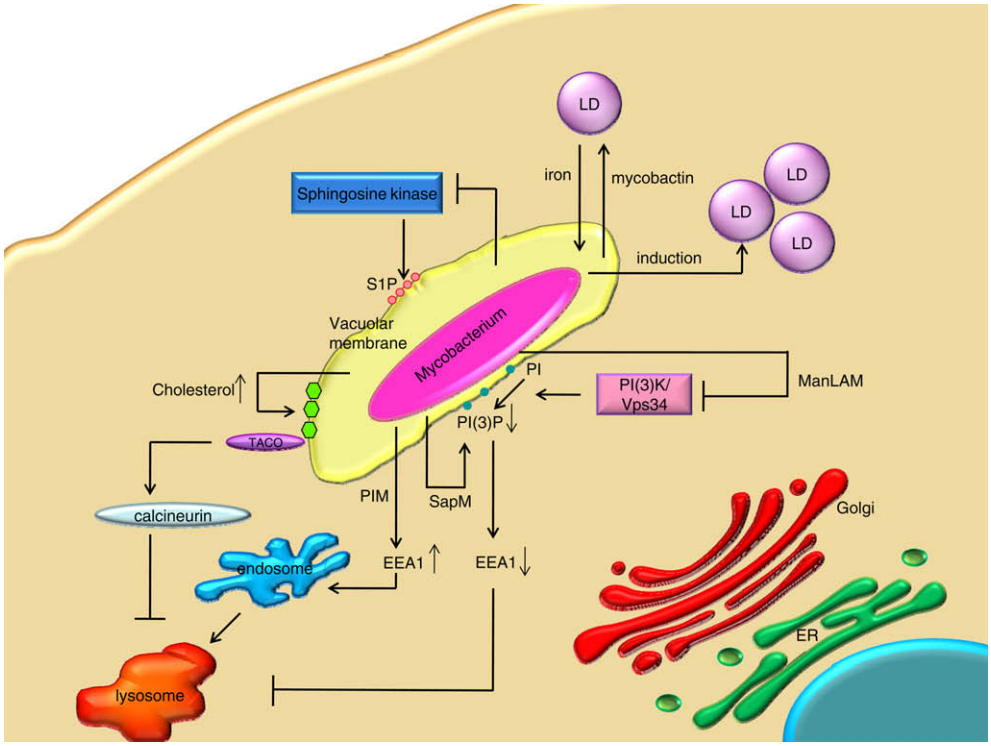

Fig. 4. Effects of mycobacteria on the host cell lipidome. LD: lipid droplet; S1P: sphingosine-1-phosphate; SapM: *M. tuberculosis* effector molecule; PI(3)P: phosphatidylinositol-3-phosphate; EEA1: Early endosomal antigen 1; PI(3)K/Vps34: phosphoinositide 3- kinase; PIM: phosphatidylinositol mannoside; ManLam: mannose-capped form of Lam (lipoarabinomannan); ER: endoplasmic reticulum; TACO: tryptophan aspartate containing coat protein.

3.5. Pathogens aid pharmaceutical targeting of sphingolipid metabolism

Many naturally occurring and synthetic sphingoid base-like compounds have been identified that interfere with various steps of the complex interconversion of sphingolipid metabolism [149]. These compounds bear promise for therapeutic interventions for cancer cells and pathogenic microorganisms. For example, specific inhibition of serine palmitoyltransferase (SPT), the first step in the synthesis of sphingolipids, suppresses virus replication [150–152]. In several of these cases, it is not clear whether the inhibition is the result of inhibition of cellular signaling, or is due to an altered interaction of pathogens with lipid rafts, in which sphingolipids play an important role (see Section 6).

Several of the naturally occurring sphingoid base-like compounds that interfere with sphingolipid metabolism are produced by pathogenic fungi (for an excellent review, see [149]). *Fusarium moniliforme* (=*F. verticillioides*) for example produces fumonisins which mimic metabolites of sphingolipid metabolism. Fumonisins inhibit ceramide synthase and are major toxins involved in inducing apoptosis [153–156]. The effects of the compounds produced by fungi are very diverse, ranging from causing disease to immunosuppression [157]. Some of these compounds act as (irreversible) enzyme inhibitors, whereas others act as molecular mimics, closely resembling host lipids (see Section 4).

3.6. Other signaling lipids

Upon host cell infection with *Chlamydia*, diacylglycerol (DAG) accumulates at the inclusion vacuole (Fig. 5), as detected by the expression of a fluorescently tagged C1 domain that specifically interacts with DAG [158]. The accumulation of DAG at the vacuole results in the recruitment of protein kinase Cδ, resulting in an anti apoptotic effect of bacterial infection.

Leishmania major induces cholesterol depletion in host cells [159]. Recent evidence suggests that this affects the DC40 signaling complex (signalosome) composition and effector function of this parasite [160]. This is the first time that a pathogen is shown to be capable of modulating lipid levels to such an extent, that the biophysical properties of biological membranes are altered. These biophysical properties are likely to be mediated via lipid rafts which are discussed below (see Section 6).

4. Molecular mimicry of lipids

4.1. Sphingolipids

One of the most complex forms of molecular mimicry occurs in sphingolipid metabolism. Sphingolipids play an important role in the regulation of the delicate balance between the pathogen and the host. Pathogens that cannot produce sphingolipids themselves are often capable of utilizing host sphingolipids to promote their virulence [161,162]. This is illustrated by *Chlamydia trachomatis*. The bacterial membrane contains up to 4% of (modified) sphingolipids although the bacterium is not capable of sphingolipid synthesis *de novo* [163]. The observations implying that pathogens can take up and modify host cell sphingolipids will be further discussed below (see Section 8). Pathogens capable of producing sphingolipids often produce types of sphingolipid species that are not present in mammalian hosts and that are specific to plants and fungi, including phytoceramide, inositolphosphorylceramide (IPC), and products thereof [156,161,162]. The functions of these pathogen-specific sphingolipids are not fully understood. Some are essential for pathogen function itself, as is seen for example in *Leishmania*. In this case, inhibition of sphingolipid synthesis results in an accumulation of small intracellular vesicles in internal vacuoles and the flagellar pocket [164,165]. The small vesicles in internal vacuoles resemble those occurring within the multivesicular bodies or multivesicular tubules of the endocytic pathway. These observations are consistent with the enrichment of sphingolipids in the endocytic pathway and multivesicular bodies [138]. Another potential function for these pathogen-specific

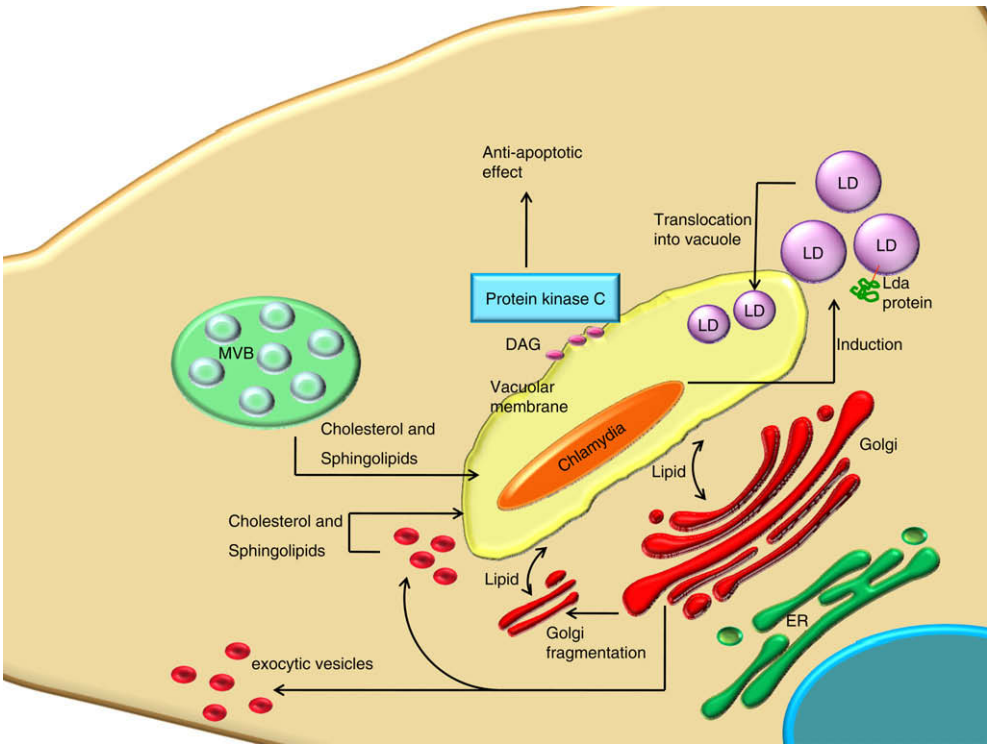

Fig. 5. Effects of *Chlamydia trachomatis* on the host cell lipidome. LD: lipid droplet; DAG: diacylglycerol; ER: endoplasmic reticulum; MVB: multivesicular body.

sphingolipids is that they contribute to virulence. In this respect it is interesting to note that pathogen-specific sphingolipids are not a substrate for host cell enzymes involved in sphingolipid metabolism and thus are not converted to other sphingolipid metabolites in host cells [162]. This does not exclude the possibility, however, that several of these pathogen-specific sphingolipids can bind to host cell enzymes involved in sphingolipid metabolism. In fact, by molecular mimicry several of these compounds are highly efficient inhibitors of host cell enzymes involved in sphingolipid metabolism [149]. Given the crucial role of sphingolipids in host cell function, including proliferation, differentiation and apoptosis, these compounds can effectively affect the host organisms, and cause disease or death. In fundamental and applied research, these inhibitors are used to study the involvement of sphingolipid metabolism in biological processes. For pharmaceutical research these compounds offer great potential for the development of anti-infectious disease drugs.

4.2. Phosphoinositides

Phosphatidylinositol with a mannose attached to the inositol headgroup at the 2 position (PIM) is one of the most common phospholipids in the mycobacterial envelope [166]. Additional mannose groups can be attached at the 6 position of the inositol ring, giving rise to PIM2-PIM6. PIM stimulates Rab5 and EEA1 to enhance fusion of phagosomes with early endosomes (Fig. 4) instead of fusion with lytic organelles [136]. PIM is the precursor of lipoarabinomannan (LAM), which is a heavily glycosylated form of PI, and of ManLAM (mannose-capped form of LAM). ManLAM interferes with a PI(3) kinase that synthesizes PI(3)P and thereby with subsequent recruitment of EEA1, affecting late endosomal and lysosomal trafficking events [137,167,168] (Fig. 4). The molecular mechanism(s) of action of PIMs and LAMs are not known. *M. tuberculosis* may disrupt phosphoinositide signaling by molecular mimicry of phosphoinositides. It has been suggested that especially the structure of PIM1 and PIM2 may resemble that of phosphoinositides, with the mannose residues replacing the phosphate groups [169]. The effect of PIM is independent of PI(3)K as it stimulates early endosomal fusion in the presence of wortmannin [170]. However, it is difficult to envision the binding of PIMs to phosphoinositide-specific protein domains in which electrostatic interactions of the phosphate groups play an important role [171]. Deretic and colleagues [170] suggest that PIMs may act either by insertion in membranes and subsequent recruitment of effector proteins [172], or by modifying the biophysical properties of the membrane bilayer.

4.3. Cholesterol

As will be discussed below (see Section 6), cholesterol plays an important role in host–pathogen interactions. In the case of Mycobacteria, cholesterol is involved in mycobacterial entry and survival [173,174]. Mycolic acids are major components of the cell wall of Mycobacteria [166]. These α-alkyl β-hydroxy long-chain fatty acids may resemble the structure of cholesterol according to several criteria, including binding to cholesterol-specific antibodies, binding to Amphotericin B, and binding to each other [175]. Thus, free mycolic acids from microbacterial origin seem to be capable of mimicking cholesterol. It is not clear how a small subset of mycolic acids via their cholesterol-like hydrophobic properties contribute to mycobacterial pathogenesis.

4.4. Lipid membrane mimicry

Many highly pathogenic viruses including influenza virus, vaccinia virus, and HIV are enveloped, *i.e.* they have a membrane around the nucleocapsid containing the viral genome. Whereas the proteins on the viral envelope are almost exclusively virally encoded, all lipids originate from the host and are recruited from host membranes. Thus, the lipid composition of the virus resembles the composition of the host cell membrane [176,177]. This form of mimicry may allow viruses to avoid immune detection. An example illustrating this principle is the recent observation that two rare antibodies against HIV-1 with potential clinical application were in fact reactive with the phospholipid cardiolipin [178]. Because of autoantigen mimicry, current HIV-1 vaccines may not induce these types of antibodies.

Vaccinia uses membrane mimicry to enter host cells [179]. The uptake of the virus by the host cell is critically dependent on the presence of phosphatidylserine in the viral membrane. By having phosphatidylserine at the outside of the membrane, Vaccinia mimics apoptotic bodies. This mimicking of apoptotic bodies causes efficient uptake of Vaccinia due to macropinocytosis by phagocytosing cells.

Schistosoma mansoni binds and ingests LDL particles, and breaks them down to serve as a source for lipids [180,181]. Next to lipid acquisition *S. mansoni* also uses LDL for immune evasion, by covering its surface with LDL to mask its own antigens [180].

4.5. Glycosylphosphatidylinositol (GPI)

GPI is a complex structure comprising a phosphoethanolamine linker, a glycan core, and a phosphatidylinositol tail. It is well known as a C-terminal post-translational modification of proteins [182]. Much less is known about the function of free glycosylphosphatidylinositol molecules that abundantly cover the cell surface of several parasites, including *Leishmania*, *Trypanosoma*, *T. gondii*, and malaria parasites (*P. falciparum*). The free GPIs of these protozoan pathogens closely resemble the mammalian GPI anchor, but differ from each other with respect to the composition of the glycan head group and/or the fatty acid-moieties of the lipid anchor [183,184]. Differences between free GPI structures are responsible for sometimes opposite biological effects. Depending on the isolation of GPIs from *T. Brucei*, *P. falciparum,* or *Leishmania*, the GPI isolates activated or inhibited macrophage function [185–187]. Very little is known about the mechanism of action of free GPI on host cells. If free GPI from pathogens would act by molecular mimicry on host cells, this would suggest a signaling role of GPI in mammalian cells. So far, however, the only function ascribed to GPI in mammalian cells is to serve as anchoring device for peripheral proteins. Alternatively, the signal of pathogen-derived GPI might be mediated via Toll-like receptors (TLR) and be involved in induction of secretion of the cytokine tumor necrosis factor (TNF). Indeed, parasite GPIs have been shown to activate TLR1, TLR2, TLR4, and TLR6 [188–190] A glycosylphosphatidylinositol-based treatment alleviates *Trypanosomiasis*-associated immunopathology [191].

5. Cholesterol and pathogens

Cholesterol is a ubiquitous component of all mammalian membranes. It influences the biophysical properties of biological membranes and is central to the organization, dynamics, function, and sorting of lipid bilayers *in vivo* [192]. Cholesterol is enriched in the plasma membrane of eukaryotic cells, where it is non-uniformly distributed. Within the plasma membrane, it is enriched in microdomains that play an important role in cellular signaling [193,194]. Because of the crucial roles of cholesterol, its (sub)cellular levels are carefully maintained [192,195–197]. The crucial roles played by cholesterol also make this lipid an attractive target for many pathogens via which they can influence host cell dynamics. In addition, the absence of cholesterol from prokaryotes makes it

an almost ideal biomarker that pathogens can use to recognize and infect mammalian host cells. Hence, pathogens have developed various ingenious ways to make use of the presence of cholesterol for the recognition of and interaction with host cell membranes. The interaction of pathogens with lipid rafts will be discussed in the next section. Here we will discuss indications for raft-independent interactions of pathogens with cholesterol.

5.1. Effector binding to cholesterol

Several type 3 secretion systems (T3SSs) have now been identified in Gram-negative bacteria to deliver virulence effector proteins into eukaryotic target cells via a needle-like structure. They have been classified into three distinct phylogenetic groups in which *e.g. Salmonella* and *Shigella* entry-associated T3SSs are closely related and the T3SSs of *EPEC* and *Pseudomonas* are more divergent [198]. At the tip of the needle, bacterial proteins insert into the host cell membrane to form a translocon that perturbs the bilayer [199,200]. The translocon components SipB and IpaB of *Salmonella* and *Shigella*, respectively, are cholesterol-binding proteins. Cholesterol is required for the binding of SipB to the plasma membrane, and secretion of effectors by the T3SSs of *Salmonella*, *Shigella* and *EPEC* depends on cholesterol [201]. Of the *Pseudomonas* translocon components PopB and PopD, PopB directly binds cholesterol, while in addition both PopB and PopD can bind phosphatidylserine [202]. PopB together with PopD causes a cholesterol-dependent aggregation of liposomes [203]. This aggregation occurs in liposomes containing 15% cholesterol and is independent of the presence of sphingomyelin, suggesting that this effect is lipid raft-independent. The biological significance of the aggregation observed in this assay is not clear. Under different experimental conditions, PopB and PopD synergistically permeabilize vesicles without causing aggregation in a cholesterol-independent manner [202].

5.2. Cholesterol accumulation on pathogen-containing vacuoles

Host cell cholesterol accumulates at parasitophorous vacuoles of several intracellular pathogens (see Section 8). At later stages of infection the *Salmonella*-containing vacuole (SCV) contains up to 30% of the cellular cholesterol pool in both epithelial cells and macrophages [204]. The function of cholesterol recruitment to the pathogen-containing vacuoles is not clear.

First, it may be used as a nutrient, as was recently shown for mycobacteria [205]. See Section 8 for further details.

Second, cholesterol may influence the interaction of pathogen-containing vacuoles with other cellular organelles, which depends on membrane-trafficking pathways [206]. Various intracellular trafficking pathways of eukaryotic cells are sensitive to cholesterol, including ER to Golgi transport [207], intra-Golgi transport [208], endosomal transport [209–211], and phagosomal maturation [212,213]. Cholesterol levels may affect intracellular trafficking in various ways, including the recruitment of proteins and lipids to the pathogen-containing vacuole. For example, cholesterol was found to mediate the phagosomal association of TACO/coronin-1, affecting the degradation of mycobacteria in lysosomes [174] (Fig. 4). Coronin-1 is required for activation of the Ca^{2+}-dependent phosphatase calcineurin, thereby blocking lysosomal delivery of mycobacteria [214]. This mechanism acts in concert with the effect of mycobacterium on sphingosine kinase (discussed in Section 3.4 and elsewhere [215]).

Third, the *Salmonella* effector SseJ has been described to use cholesterol as an acceptor of acyl chains and might therefore use cholesterol at SCVs as a substrate [40,41]. In addition, this function may also imply cholesterol-specific recruitment of SseJ to the SCVs. Vice versa, SseJ may be involved in the recruitment of cholesterol to the SCV, as overexpression of SseJ results in formation of cholesterol-rich membrane structures [42].

5.3. Pathogen uptake and modification of cholesterol

Cholesterol in the membranes of *Helicobacter* spp. [216–218], *S. aureus* [217], *Anaplasma phagocytophilum* [219] and *Chlamydia* EB and RB membranes [163] is of host origin. A sterol-binding protein in *Toxoplasma* was recently identified that may optimize pathogen handling of host cell derived cholesterol [220]. *Helicobacter pylori* extracts cholesterol from epithelial cells and converts it into cholesteryl α-glucosides [221]. Cholesterol and cholesteryl glucosides represent 1.6% and 25%, respectively, of the total lipids of *H. pylori* [222]. The abundant presence of glucosylated cholesterol inhibits phagocytosis of and T-cell activation by *H. Pylori*, providing an immune escape mechanism for this pathogen.

6. Lipid rafts

The organization of biological membranes is based on interactions between membrane proteins and lipids. Recent evidence suggests that in these membranes specialized microdomains (so called lipid rafts) exist that are characterized by an enrichment of cholesterol, sphingolipids, and a specific subset of proteins. By selective inclusion and exclusion of proteins, rafts are involved in many cellular processes such as endocytosis, signaling, protein sorting and intracellular membrane trafficking. It is beyond the scope of this review to describe details and to discuss controversies surrounding the biological function and properties of lipid rafts. These topics are covered by many review articles [10,223–230].

The biophysical properties and the high concentration of signaling molecules make lipid rafts a natural target for pathogens through which they communicate with host cells and hijack membrane-trafficking pathways. This topic has also been the focus of several reviews to which the reader is referred [231–235]. An updated list now includes over 100 pathogens that have been suggested to interact with lipid rafts (Tables 1–4). Not in all cases the involvement of rafts, rather than *e.g.* cholesterol, in the host–pathogen interaction has been unambiguously established. The early methodology to show an involvement of lipid rafts in biological processes is rather diffuse, and uses detergents and cholesterol-extracting agents. Only a combination of several independent methods, which also include morphological and functional studies, can establish the involvement of lipids rafts in host–pathogen interactions. Hence, especially data from early publications, when these additional methods were not yet available, should be interpreted with some caution.

The basic property of lipid rafts that is exploited by pathogens is their intrinsic ability to transiently oligomerize. This property is abused by pathogens, resulting in stabilized, altered or disrupted oligomeric structures which (i) provide a mechanism for pathogen uptake, (ii) affect host cell signaling; (iii) alter intracellular trafficking including pathogen vacuole maturation, and (iv) offer strategies for exit from the host cell. Here we will focus on the role of lipids in the raft-mediated interactions between host cells and pathogens.

6.1. Raft lipids as receptors for pathogens

Several pathogens directly target sphingolipids in raft structures (Table 1). Recently, VacA was identified as a bacterial virulence factor, secreted by the gastric pathogen *Helicobacter pylori* that exploits a plasma membrane sphingolipid, sphingomyelin (SM), as a cellular receptor [298]. *Helicobacter pylori* may exploit the capacity of SM to partition into lipid rafts in

Table 1
Bacteria interacting with lipid rafts.

Bacterium	Involved in	Pathogen molecule involved	Raft molecule involved	Refs.
Anaplasma phagocytophilum	Entry		GPI-anchored proteins	[236]
Afipia felis	Entry via macropinocytosis			[237]
Brucella abortus	Entry Phagosomal maturation	1,2 cyclic glycan	Class A scavenger receptor	[238–240]
Brucella suis	Entry/ intracellular survival			[238]
Campylobacter jejuni	Entry			[241]
Chlamydia pneumonia	Entry			[242]
Chlamydia psittaci	Entry			[242]
Chlamydia trachomatis (E and F)	Entry			[242–244]
Ehrlichia chaffeensis	Entry		GPI-anchored protein	[236]
Escherichia coli FimH expressing	Entry/survival	FimH	CD48/CD55 uroplakin-1a	[245–249]
Escherichia coli Enteropathogenic	Induces lipid rafts during pedestal formation			[250,251]
Escherichia coli Uropathogenic	Transcellular translocation			[252]
Escherichia coli diffusely adhering (DAEC)	Entry	AfaE	CD55	[253], but see also [254]
Francisella tularensis	Entry /survival			[255]
Helicobacter pylori	Adhesion	HpaA	LacCer	[256]
Legionella pneumophila	Intracellular survival			[257]
Listeria monocytogenes	Signaling	Internalin	E-cadherin	[258]
Mycobacterium avium	Entry	Polar lipid fraction		[259]
Mycobacterium kansasii	Entry		CR3 in association with GPI-anchored protein(s)	[260]
Mycobaterium tuberculosis	Entry/survival	Receptor-C_k		[174,261]
Mycobaterium bovis	Entry			[174]
Mycoplasma fermentans				[262]
Porphyromonas gingivalis	Entry/intracellular survival			[263,264]
Pseudomonas aeruginosa	Entry/signaling			[265–267]
Salmonella typhimurium	Entry/intracellular survival Effector proteins	SipB (T3SS) PipB and PipB2	Cholesterol	[201,204,268,269]
Shigella flexneri	Entry	Components of the T3SS: IpaB	CD44	[270,271]
Yersinia enterolitica	Intracellular replication			[272]
Neisseria gonorrhoeae	Entry		CEACAM	[273,274]
Staphylococcus aureus	Entry		asialoGM1/TLR2	[275]
Gram-negative bacteria	Binding/Entry	Lipopolysaccheride (LPS)	CD14	[276]

Table 2
Bacterial toxins interacting with lipid rafts.

Bacterial toxin[a]				
Actinobacillus actinomycetemcomitus	Binding	Cytolethal distending toxin		[277,278]
Aeromonas hydrophila	Binding–oligomerization	Aerolysin	CD14 (GPI-anchored)	[279,280]
Bacillus anthracis	Oligomerization – Clathrin-coated pits	anthrax toxin (protective antigen) Anthrolysin O (a CDC)	Anthrax toxin receptor (ATR)	[281,282]
Bacillus thuringiensis	Binding/oligomerization	cry1A toxin	Aminopeptidase N	[283–285]
Clostridium botulinum	Binding	Neurotoxin		[286,287]
Clostridium difficilus	Binding	Toxin TcdA and TcdB		[288]
Clostridium perfringens	Binding/oligomerization	epsilon-Toxin		[289]
Clostridium perfringens	Binding/internalization	iota-Toxin		[290]
Clostridium perfringens	Binding	theta-Toxin (CDC)[b] (=perfringolysin)	Cholesterol	[291,292]
Clostridium septicum	Binding	α-Toxin	GPI-anchored proteins	[293]
Clostridium tetani	Binding/internalization	Tetanus toxin		[294]
Escherichia coli	Binding/oligomerization	Heat-labile enterotoxin LTII	GD1	[295]
Escherichia coli	Binding/oligomerization	Heat-labile enterotoxin LTI	GM1	[296]
Helicobacter pylori	Binding/oligomerization	VacA	SM[c]	[297,298]
Listeria monocytogenes	Binding/oligomerization Vacuole lysis (via T3SS-raft interaction)	Listeriolysin (a CDC)[b]	Cholesterol	[258,299,300]
Shigella dysenteriae	Binding/oligomerization	Shiga toxin	Gb3	[301,302]
Streptococcus pyogenes	Oligomerization	streptolysin (CDC)[b]		[303]
Vibrio cholera	Binding/Caveolae	Vibrio cholera cytolysin (CDC)[b] Cholera toxin	Cholesterol GM1	[303,304]

[a] For a complete overview of CDC, secreted by 26 different pathogens, the reader is referred to a recent review by Rosado et al. [305].
[b] CDC: Cholesterol-dependent cytolysin.
[c] For a list additional toxins that interact with sphingomyelin (SM) but are secreted by other types of pathogens, the reader is referred to a recent review by Shogomori and Kobayashi [306].

order to access the raft-associated cellular machinery. Other pathogens that exploit lipid-binding toxins to localise to lipid rafts include toxigenic *E. coli*, which secrete the heat-labile enterotoxin LTI targeting GM1, or which secrete the heat-labile enterotoxin LTII targeting GD1, *Shigella dysenteriae* that secrete Shiga toxin targeting Gb3, and *Vibrio cholera* that secrete cholera

Table 3
Viruses interacting with lipid rafts.

Virus				
Avian sarcoma and leukosis virus	Entry		GPI-anchored receptor TVA800	[307]
Bluetongue virus	Protein localisation	VP5		[308]
Coxsackievirus	Entry/Golgi targeting			[309]
Dengue virus	Entry		Entry: HSP70/90	[310,311]
	Association	Association: non-structural glycoprotein NS1		
Ebola virus	Entry	Entry: fusion glycoprotein GP2		[312–314]
	Budding/assembly	Budding/assembly: VP40	Budding/assembly: Recruitment of TSG101	
Echovirus 1	Entry/non-caveolar endocytosis/ caveosomes/trafficking		alpha2beta1 integrin	[315–317]
Echovirus 6	Entry		DAF GPI-anchored)	[318]
Echovirus 11	Entry raft dependent		DAF for raft-dependent entry	[319]
Ecotropic murine leukemia virus	Entry/budding			[320]
Epstein-barr virus	Signaling			[321,322]
Hepatitis C virus	Entry		Entry: CD81 and class B scavenger receptor	[323–326]
	Replication	Replication: NS proteins	Replication: hVAP-33	
Herpes simplex virus	Entry/binding	Entry: glycoprotein B		[327,328]
	Budding	Budding: UL11		
Herpesvirus saimiri	Down regulation TCR	Tip		[329]
HIV	Entry	Binding: gp120	Binding: CD4/CXCR4	[148,330–340]
	Transcytosis	Transcytosis: Gp41	Transcytosis: GalCer	
	Assembly/budding/release	Assembly: Gag	Assembly: Annexin2	
Human herpes virus-6	Entry	Glycoprotein Q1	CD46	[341]
Human herpesvirus 8 = Kaposi's sarcoma-associated herpes virus	Manipulation of signaling			[342]
Human T-cell leukemia virus	Entry/budding/			[343–345]
	Signaling in T-cell proliferation	Signaling: Tax1	Signaling: IκB	
Influenza virus	Entry/budding/ assembly	hemagglutinin and neuraminidase		[346–348]
Marburg virus	Entry/budding			[313]
Measles virus	Budding/assembly			[349–351]
Newcastle disease virus	Assembly/release			[352,353]
Pseudorabies virus	Entry/proteins localisation	Us9		[354–356]
Respiratory syncitial virus	Entry in caveolae	F protein		[357–359]
	Assembly			
Rhinovirus	Entry			[360]
rotaviruses	Entry/replication	VP4		[361–363]
SARS CoV	Entry	S-protein	ACE-2	[364,365]
Sendai Virus	Budding/assembly			[366,367]
Semliki forest virus	Entry and exit	Entry: Fusion peptide		[368,369]
Simian virus 40	Entry/Caveolae/trafficking/		MHCI	[370,371]
Vaccinia virus	Entry			[372,373]
Varicella zoster virus	Viral envelope integrity			[374]
West Nile virus	Entry			[375]

Table 4
Protozoa, fungi and other pathogens interacting with lipid rafts.

Protozoon			
Cryptosporidium parvum	Entry/attachment		[376]
Entamoeba histolytica	Attachment		[377]
Leishmania	Intracellular survival	Lipophosphoglycan	[378]
Plasmodium falciparum	Entry		[379–381,101]
Theileria parva	Signaling		[382]
Toxoplasma gondii	Invasion/Intracellular survival		[383]
Fungus			
Paracoccidiodis brasiliensis	Adhesion/signaling		[384]
Other			
Prion protein PrP^{C}	Localisation to host cell membrane	PrP^{sc}	[385,386]

toxin targeting GM1. The multimeric toxins have the potential to cluster rafts, thus allowing high affinity toxin-binding through relatively weak individual binding forces of carbohydrate binding sites [387,388]. In addition, the raft-dependent interaction also determines their mechanism of uptake. It is becoming increasingly clear that there are clathrin-dependent and clathrin-independent endocytic pathways which differ in their requirement of lipid rafts [389–392].

The family of cholesterol-dependent cytolysins (CDCs) binds cholesterol in membranes, resulting in oligomeric ring structures in membranes that create pores with diameters ranging from 1 to 50 nm [305]. CDCs localise to lipid rafts, but how this property relates to their mode of action is not clear, as the biophysical properties of lipid rafts hamper the interaction of cholesterol with CDCs [393].

6.2. Lipid-dependent reorganization of rafts

Several lipids are targeted by pathogens in order to change the oligomeric state of lipid rafts, resulting in (i) stabilization, (ii) alteration, or (iii) disruption of lipid rafts. Examples of the first possibility, that of stabilization of lipid rafts, are provided by *Neisseriae gonorrhoeae, P. aeruginosa, S. aureus*, rhinovirus or Sindbis virus, which activate the enzyme acid sphingomyelinase to release ceramide in the outer leaflet of the cell membrane [235]. Ceramide molecules have the tendency to induce the formation of ceramide-enriched microdomains, to function as a fusogen that triggers the spontaneous fusion of ceramide-enriched membrane microdomains, and to stabilize large ceramide-enriched membrane macrodomains [394–396]. The second possibility of lipid targeting by pathogens is that lipids cause a reorganization/alteration of lipid rafts. This possibility has been suggested for lipoarabinomannan (LAM), an integral component of the *M. tuberculosis* that markedly alters the morphology of lipid domains in membranes [397]. As a result phagosome maturation is inhibited [398]. The third possibility is that pathogens target lipids to cause a disruption of lipid raft structures. *Brucella abortus* secretes 1,2 cyclic glycan, a component that resembles cyclodextrins and extracts cholesterol from membranes, resulting in disruption of lipid rafts [399].

Together these results confirm that lipids are essential for the stability of microdomains in biological membranes. In model membranes that consist of only a specific subset of lipids, lipid rafts can easily be observed (see *e.g.* [400]). In biological membranes, the lipid composition is much more complex and heterogeneous, resulting in the suggestion that protein interactions are necessary to stabilize lipid rafts (see *e.g.* [229] for a discussion on this topic). The fact that pathogens can alter the oligomeric state of rafts in biological membranes by targeting lipids suggests that lipids provide a major driving force determining the biophysical state and hence physiological role of rafts.

6.3. Lipid rafts in budding of viruses

It appears that several enveloped viruses select raft-like domains to exit from cells. HIV viral membranes contain raft markers GM1, Thy-1 and CD59 [334]. HIV viral membranes are Triton X-100 resistant and contain typical raft lipids [148]. Other viruses known to assemble on or bud from lipid rafts in host cell membranes include influenza virus [176], measles virus [401] and Newcastle disease virus [352] (see Table 1 for a comprehensive list). As HIV-1 particles excluded a bona fide raft marker (flotillin-1), this indicates that the virus buds from a subset of cellular microdomains. The function of lipid rafts in virus assembly can be manifold, including recruitment of a subset of host lipids and proteins into viral membranes and determination of the oligomeric state of viral proteins. Another surprising possibility was suggested by the finding that assembly of the non-enveloped Rotavirus is dependent on cholesterol, suggesting that rafts may control the proper incorporation of viral proteins into virions [402].

7. Lipid droplets

Lipid droplets (LDs), also termed lipid bodies or adiposomes, are lipid storage organelles found in animals, plants and microorganisms [403]. Lipid droplets are thought to be formed by accumulation of neutral lipids between the membrane leaflets of the ER and bud from there [403–405], although some variations of this model have been presented [406,407]. Lipid droplets consist of a neutral core composed of triacylglycerols, cholesterol esters, retinol esters and diacylglycerols surrounded by a monolayer of phospholipids. In addition, lipid droplets contain a specific and dynamic subset of structural and functional proteins that are involved in the biogenesis, turnover, and biological functions of these organelles. Recent proteomic approaches have identified a large variety of proteins associated with lipid droplets, which indicates that they are much more than lipid storage depots. A wide range of cellular functions have been suggested, including an involvement of lipid droplets in lipid transport and metabolism, membrane trafficking, intracellular signaling, production of inflammatory mediators, and in protein folding and aggregation [408,409].

These properties make them attractive targets for pathogens and indeed, recent work shows that several pathogens interact with lipid droplets.

7.1. Pathogen induction of lipid droplet formation

A variety of pathogens induce the formation of lipid droplets in host cells. For example, bacterial lipopolysaccharide (LPS), present in all Gram-negative bacteria, induces lipid droplet accumulation in host cells in a Toll-like receptor 4 (TLR4)-dependent way, which, in case of macrophages, results in formation of foam cells [410,411]. Other examples include the induction of lipid droplet formation by *Plasmodium berghei* in mouse kidney and liver cells [412,413] and the induction of lipid droplet formation by *Candida albicans* in macrophages and hepatocytes by use of an extracellular lipase [414]. The induction of lipid droplet formation by hepatitis C virus (HCV), *Trypanosoma cruzi, M. tuberculosis*, *Chlamydia trachomatis*, and *Mycobacterium bovis* (*M. bovis*) will be discussed in some detail below.

7.2. Lipid droplets and pathogen assembly

Hepatitis C virus induces the formation of more and larger lipid droplets in hepatic cells. In addition, structural proteins of HCV have been found to localise to lipid droplets [415]. The HCV capsid protein (core protein) localises to the LDs and recruits non-structural proteins and replication complexes, which appears critical for the production of virus particles [416,417]. The core protein most likely replaces adipose differentiation-related protein (ADRP), a major protein associated with the surface of lipid droplets, by progressively coating the entire surface. The absence of ADRP then results in lipid droplet aggregation around the nucleus [418]. The presence of the replication machinery and the observation of virions in close proximity to the LDs might indicate virus assembly at or in close proximity to lipid droplets. Recent work from Roingeard et al. [419] indicates that the overexpression of HCV core protein induces virion budding from ER membranes in close proximity to the LD rather than at membranes directly apposed to the lipid droplets.

7.3. Pathogen-vacuole interactions with lipid droplets

Trypanosoma cruzi induces both lipid droplet formation and eicosanoid production in macrophages [420]. This suggests that lipid droplets may have a role in eicosanoid production. Indeed, subsequent work showed that lipid droplets in cells infected with *T. cruzi* are found in close proximity to, attached to, and even internalized into the parasite-containing vacuole. Thus, a close interaction of lipid droplets with the pathogen vacuole allows a possible discharge of lipid droplet content [421]. A direct interaction

between lipid droplets and pathogen vacuoles is, however, not always necessary for the induction eicosanoid production: the membrane compound LAM of *Mycobacterium* induces a TLR2-dependent, but phagocytosis-independent induction of lipid droplets and eicosanoids in macrophages [422].

Cells infected with *M. tuberculosis* also show a close association of the mycobacterial phagosome with lipid droplets. In this case the close association of lipid droplets with the pathogen-containing vacuole is hijacked for the acquisition of iron (Fig. 4). *M. tuberculosis* secretes the metal-free siderophore mycobactin and after binding to host cell cytosolic iron, mycobactin is targeted to lipid droplets. The close interaction with lipid droplets subsequently allows the pathogen to acquire host cell iron [423].

Lipid droplets are also crucial in *Chlamydia trachomatis* intracellular survival and replication. In epithelial cells three *Chlamydia* proteins (Lda) were found to localise to host cell lipid droplets (Fig. 5). Similar to what had been observed for HCV, Lda association with lipid droplets occurred with a concomitant decrease of ADRP, suggesting a replacement of lipid droplet coat protein with a pathogen protein, allowing functional control of this organelle. For example, lipid droplet-associated Lda may be involved in the observed accumulation of lipid droplets around the *Chlamydia*-containing vacuole (inclusion) [424]. Alternatively, the chlamydial Lda proteins may function in the recently described translocation of lipid droplets into the *Chlamydia* inclusion, probably for the acquisition of nutrients [425]. See Section 8 for further details.

7.4. Pathogen-localised lipid droplets in detoxification

As a consequence of a hemoglobin diet for blood-feeding organisms such as *P. falciparum,* they produce large amounts of heme, a toxic molecule that can disrupt membranes, inhibit enzymatic processes and initiate oxidative damage. *P. falciparum* uses lipid droplets in close proximity to its digestive vacuole to detoxify heme by formation of haemozoin crystals within the hydrophobic environment of lipid droplets [426,427]. Lipid droplet-mediated detoxification of host derived heme has also been suggested for *Schistosoma mansoni* and *Rhodnius prolixus*, allowing the formation of multicrystalline assemblies in the guts of these organisms [428].

In summary LDs are involved in a wide variety of host–pathogen interactions, such as inflammatory responses, pathogen assembly, pathogen nutrient acquisition and detoxification processes. As we are only beginning to understand the biological functions of lipid droplets, studies on the involvement of lipid droplets in host–pathogen interactions will also contribute to this area of research.

8. Lipid acquisition

The host provides an appealing habitat for numerous microorganisms and excluding them from critical nutritive resources has its role in host defense [429]. This nutrient restriction also provides an interesting pharmaceutical target, as many pathogen enzymes that utilize host cell lipids bear resemblance to host cell enzymes, but distinct differences allow the design of specific drugs to selectively inhibit pathogen enzymes. A recent publication by Brinster et al. [430], however, casts some doubts on this strategy, as inhibition of bacterial fatty acid synthesis is fully bypassed by the uptake of exogenous fatty acids. There is an enormous amount of literature available that indicates that pathogens require host cell lipids and it is beyond the scope of this review to give an extensive overview. For certain pathogens, it may seem obvious why they acquire host cell lipids as they are auxotroph for fatty acids (*e.g.* [431]), or cholesterol [205]. In many other cases, however, the reason for lipid acquisition from the host cell is not clear (*e.g.* [432]). Here, we will focus on the general principles, illustrated with prominent and recent examples.

8.1. Metabolic fate of acquired lipids

One function of acquired lipids is to modulate host–pathogen interactions and virulence, as has been discussed in other sections of this review (see also *e.g.* [433]). A second function of acquired lipids is to serve as an energy source. For example, *Corynebacterium jeikeium* cannot synthesize fatty acids, but upon their acquisition they are utilized for subsequent β-oxidation, as the bacterium contains all the enzymes required for this process [431]. Even cholesterol can be used as a substrate for energy production, for example by *Rhodococcus* species, soil bacteria related to *M. tuberculosis* [434]. To ensure expedited energy supply, Gram-negative bacteria can induce the release of fatty acids from host cells by stimulating adipose lipolysis via the lipid A moiety of LPS [435]. A third function of acquired lipids is to provide building blocks for pathogen assembly. In general, pathogens can synthesize the typical prokaryotic lipids phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol and must acquire lipids generally associated with eukaryotes such as sphingomyelin, cholesterol, phosphatidylcholine and phosphatidylinositol [163]. Hence, several pathogens are auxotrophic for these lipids (*e.g.* sphingolipids [162] or cholesterol [99,219]). Host cell lipids can also be used as a carbon source, as has been described for *e.g. M. tuberculosis* [205,429], *Salmonella* [436], *Candida albicans* [437], and *Cryptococcus neoformans* [438]. The acquisition of a viral envelope from host cell membranes could also be considered as an effective mechanism to acquire building blocks for pathogen assembly. This has been discussed elsewhere in this review (Section 4).

Thus, there are various ways by which pathogens utilize host cell lipids. In the next section we will discuss which lipids are known to be acquired by pathogens via interactions with a host cell and describe the mechanisms involved.

8.2. Lipid acquisition by extracellular pathogens

Extracellular pathogens often reside in the host's nutrient-rich body fluids. *Trypanosoma brucei* acquires lysophosphatidylcholine from plasma pools. Lysophospholipids are more soluble in aqueous solution when compared to di-acylated phospholipids and are more readily imported by the parasite. After their uptake, two lysophosphatidylcholine molecules are then used for the generation of one molecule of phosphatidylcholine by 3 consecutive enzymatic steps involving a lyso-phospholipase A1, an acyl-CoA ligase and a lysophosphatidylcholine:acyl-CoA acyltransferase [439,440].

T. brucei also incorporates host cholesterol from plasma into its membranes. Cholesterol is not water soluble, but in this case *T. brucei* selective absorbs LDL particles from the plasma by receptor-mediated endocytosis [441,442] and acquires cholesterol from these particles. *Trichomonas vaginalis* and *Schistosoma mansoni* also internalize LDL particles for the acquisition of essential host lipids [180,181,443]. The endocytic uptake by *T. vaginalis* is mediated by a receptor for apolipoprotein CIII.

Extracellular pathogens acquire lipids from other body fluids as well. For example, *P. aeruginosa* acquires lipids from lung surfactant [444] and *Salmonella* ssp. might utilize phospholipids from intestinal mucus [445], although this has not been confirmed *in vivo*. Finally, extracellular pathogens can obtain lipids from the extracellular leaflet of the plasma membrane, as has been shown for *Helicobacter pylori,* which acquires cholesterol from lipid rafts in the plasma membrane [221].

8.3. Lipid acquisition inside a host cell via the pathogen vacuole

Many pathogens reside within membrane-enclosed vacuoles and determine the properties of these structures to avoid host cell-mediated degradation. The vacuolar membrane is the barrier between host and pathogen, and can at the same time function as source of lipids to the pathogen. Indeed, several host cell-derived lipids accumulate in the membrane of pathogen vacuoles. Cholesterol accumulates in the vacuoles of *e.g. Coxiella* [446], *Salmonella* [204], *Chlamydia* [447], *T. gondii* [448–450], and *M. tuberculosis* [212]. Sphingomyelin accumulates in *Chlamydia* inclusions [451] and *T. gondii* vacuoles [452]. Diacylglycerol and the phospholipids PI and PC accumulate in *Chlamydia* inclusions [158,163]. *T. gondii* also preferentially recruits PC over other phospholipids to its parasitophorous vacuole [453]. How are these lipids delivered to the vacuole? In the case of *Chlamydia*, the observation that host transmembrane proteins are not delivered to the vacuole has intriguing implications for the mechanisms of transport [454]. Only for a few pathogens, including *Chlamydia* and *T. gondii*, the molecular mechanisms of lipid transport to the pathogen vacuole have been described in some detail. These pathogens will be discussed in some more detail, with evidence from other pathogens added as additional examples.

8.4. Lipid acquisition via vesicular trafficking to the pathogen vacuole

Chlamydia intercepts exocytic vesicles from the secretory pathway to acquire host cell lipid nutrients. In an elegant series of experiments, the group of Hackstadt provided evidence for the diversion of Golgi-derived vesicles, en route to the plasma membrane, to the pathogen vacuole (Fig. 5) (reviewed in [455]). The transport of both sphingomyelin [451] and cholesterol [447] was shown to be Brefeldin A-sensitive, suggesting a Golgi origin of these vesicles. In contrast, Golgi-derived glycoproteins en route to the plasma membrane were not intercepted by the pathogen [456]. These results indicate that only a subfraction of vesicles is targeted for delivery to the pathogen vacuole. Indeed, recent evidence suggests that *Chlamydia* preferentially intercepts basolaterally-directed sphingomyelin-containing exocytic vesicles [457]. Whether the intercepted vesicles represent bona fide exocytic vesicles derived from the Golgi complex remain to be established, as it cannot be excluded that *Chlamydia* induces an alternative or modified exocytic pathway from the Golgi to the plasma membrane. Recently, it was shown that *Chlamydia trachomatis* causes fragmentation of the Golgi complex and the generation of Golgi ministacks surrounding the bacterial inclusion [458]. This process is mediated by bacteria-induced proteolytic cleavage of the Golgi matrix protein Golgin-84. This Golgi fragmentation is involved in lipid acquisition, as inhibition of the cleavage of Golgin-84 also inhibited the transport of lipids to the pathogen vacuole. These observations open the possibility that lipid transport is not only mediated by exocytic vesicles, but also by Golgi fragments (Fig. 5). *T. gondii* also induces Golgi fragmentation in the host cell, but in this case, the Golgi fragments seem dispensable for parasite invasion and replication [459]. It will interesting to determine whether the lipids from these Golgi fragments are transported by fusion of the Golgi fragments with the vacuole or by the generation of contact sites between the Golgi fragments and the bacterial vacuole (see also next Section 8.5). The Golgi fragments generated by knockdown of Golgin-84 are different from the Golgi fragments caused by Brefeldin A. First, in the presence of Brefeldin A, *de novo* synthesized sphingomyelin and cholesterol from the exocytic pathway are no longer delivered to the *Chlamydia* vacuole [447,451]. Second, whereas Golgin-84-induced Golgi fragmentation enhances bacterial replication [458], Brefeldin A does not affect bacterial multiplication [447,460]. The ineffectiveness of Brefeldin A on bacterial multiplication indicates that alternative pathways must be available to provide *Chlamydia* with lipids. Indeed, recent evidence suggests that *Chlamydia* can also recruit lipids from multivesicular bodies that are rich in cholesterol and sphingomyelin [461,462] (Fig. 5).

S. typhimurium is also known to redirect exocytic vesicles from the Golgi complex toward the *Salmonella*-containing vacuole [463]. This process is dependent on effectors encoded by the *Salmonella* pathogenicity island 2 (SPI-2). Similar to *Chlamydia*, the *Salmonella* vacuole is also in close proximity to the Golgi complex. In contrast to *Chlamydia*, however, *Salmonella* replication is affected by Brefeldin A [464]. *Legionella pneumophilla* also intercepts vesicular traffic, in this case from the endoplasmic reticulum exit sites, and the pathogen-containing vacuole becomes surrounded by these vesicles [465]. These vesicles attach and fuse with the *Legionella*-containing vacuole, allowing the acquisition of ER-derived proteins. Similar to *Legionella*, *Brucella abortus* interacts with the ER exit sites and its replication is inhibited by blockage of Sar1 activity, which disrupts the ER exit sites [466]. In the cases of *Salmonella*, *Legionella* and *Brucella,* it remains to be established whether, as a result of these specific interceptions of exocytic vesicles, the pathogens acquire lipids.

8.5. Lipid acquisition via novel translocation mechanisms across vacuolar membranes

Pathogen-containing vacuoles are often found in close proximity to other organelles (see also Fig. 3 and 5) such as the Golgi complex (*Chlamydia* [458], *Salmonella* [464]), the ER (*Brucella* [466], *Legionella* [465], *T. gondii* [467], and *Plasmodium* [468]), mitochondria (*T. gondii* [467]), lipid droplets (*Chlamydia* [424], *P. falciparum* [427], *Trypanosoma cruzi* [421]), multivesicular bodies (*Chlamydia* [462]) and possibly recycling endosomes (*Mycobacterium*, [205]). When the vacuole comes in close contact with another organelle, the two membranes may form local contact sites as an efficient means to transfer lipids from one membrane to the other [469,470].

Alternative models for lipid delivery to the pathogen have been suggested for cholesterol acquisition in the vacuole of *T. gondii*, as studied in an elegant series of experiments by Coppens and coworkers. The intracellular parasite *T. gondii* acquires cholesterol from the host. *T. gondii* scavenges cholesterol from LDL that is taken up by the host cell (Fig. 3) and does not utilize cholesterol synthesized *de novo* by the host cell. During *Toxoplasma* infection, cholesterol from LDL travels from the endosome to the parasitophorous vacuole and is taken up by the parasite in a microtubule dependent way [448,449]. The characteristics of cholesterol acquisition, such as temperature, energy, and microtubule dependency, supported a mechanism involving vesicular transport [449]. Recent evidence, however, allowed Coppens et al. [471] to propose an unconventional model that involves the inward budding of endosomal/lysosomal vesicles into the parasitophorous vacuole and subsequent fission of the vacuolar membrane, generating double membrane-layered vesicles in the vacuole.

A similar mechanism may apply to the appearance of lipid droplets in the *Chlamydia* vacuole [425]. Lipid droplets were found docked at the surface of the vacuole and can translocate from the host cell cytoplasm into the vacuolar lumen [425]. How entire organelles such as lipid droplets or vesicles derived from multivesicular bodies are translocated across the vacuolar membrane remains to be determined. Under physiological conditions, inward budding (i.e. into the lumen of an organelle) is so far only observed for multivesicular bodies. The ESCRT machinery provides the driving force for this process [472]. Pathogens such as HIV hijack this machinery to bud from the plasma membrane. It remains to be established whether the inward budding into the parasitophorous

vacuole also requires elements of the ESCRT machinery, or whether specific virulence effectors, secreted by the pathogens, are sufficient for this process. Clearly, the uptake of lipid droplets and vesicles from multivesicular bodies is dependent on pathogen-secreted factors [425,471]).

9. Conclusions and future perspectives

Lipids from host cells as well as from pathogens play important roles in the ability of pathogens to escape from the immune system. Pathogens make extensive use of the complexity of the host cell lipidome and have evolved very sophisticated mechanisms to use the diversity and complexity of the lipidome of their host to their advance. This is best illustrated by *Mycobacterium tuberculosis*, that uses a large portion of its coding capacity to the production of enzymes involved in lipogenesis and lipolysis [473]. Pathogens not only use lipids as building blocks or as a nutrient source, but also to influence the host cell physiology, enabling their survival and replication.

This review was written at the onset of the emergence of high-throughput lipidomic techniques. Lipidomic analysis of host–pathogen interactions by mass spectrometry will provide a great opportunity for future research and can be expected to reveal the specific roles of individual lipid species. This will greatly aid in the generation of more specific drugs against pathogens. An example of drug development that interferes with lipid metabolism is the statins. Statins have been reported to inhibit the replication of various pathogens in host cells, including viruses [29,474–483], bacteria [484–489] and parasites [490–493]. Clinical studies have been carried out concerning the use of statins for the management of septic patients [494]. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase and reduce the biosynthesis of cholesterol, but also that of farnesylpyrophosphate and geranylgeranylpyrophosphate. The latter two compounds serve as lipid attachments for a post-translational modification, termed prenylation, of a great number of regulatory proteins in eukaryotes, among which small GTP-binding proteins. These GTP-binding proteins have crucial roles in intracellular inflammatory signaling [495]. The inhibition by statins of the prenylation of regulatory proteins in the eukaryotic cells is in most cases thought to cause the suppressing effect of statins on the replication of the microorganisms. In the case of the suppression of Salmonella replication in host cells by statins [485], the inhibition of host cell prenylation of the bacterial effector protein SifA [496] may also play a role.

Another opportunity of lipidomic analysis is lipid profiling of host–pathogen interactions. Lipid profiling may become an important indicator for the metabolic condition of a cell or organism. The specific depletion, presence or modulation of certain lipid species may generate lipid fingerprints unique to infection by specific pathogens, allowing the identification of biomarkers.

The study on host–pathogen interactions is a two-edged sword: On the one hand, these studies advance our molecular understanding of normal cellular processes, as they reveal novel intracellular signaling and trafficking pathways/mechanisms. One such example is the surprising capacity of lipid droplet organelles to pass biological membranes. Many novel and unanticipated findings are expected to emerge from these studies, in combination with novel methods of lipidomic analysis. On the other hand, these studies generate knowledge about pathogen-mediated changes in host cell metabolism. In this respect it is revealing that most pathogens studied so far do not play a single trick with the host cell but simultaneously induce several changes in the host cell signaling and trafficking mechanisms, both at the level of the proteome and of the lipidome. In this review, we have described several pathogens, such as *T. gondii* (Fig. 3), *Mycobacteria* (Fig. 4), *Salmonella* and *Chlamydia* (Fig. 5), which appeared in multiple sections, illustrating that they take full advantage of the complexity of the host cell lipidome.

This may have important implications for drug development. The goal to develop one drug for one pathogen in order to control host cell infection may be too simplistic. For most pathogens, a multidrug approach that aims at several pathogen-derived effector molecules or their host cell targets may be more effective. Moreover, the pathogens described in this review change the host cell lipidome in a unique way, characteristic for that particular pathogen. Thus lipidomic techniques will soon provide us with a detailed view on changes of the host cell lipidome in response to pathogen infection. Although lipidomic methods are technically more challenging than proteomic techniques, they may soon catch up and bypass proteomic analyses. Most lipids exist across species, allowing the application of lipidomic analyses to all species, independently of the knowledge of the genomic organization. In the field of host–pathogen interactions, with many pathogen genomes still unknown, this is a great advantage. Thus, lipidomic analysis of host–pathogens can be expected to contribute significantly to the fight against infectious diseases.

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