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Minireview

Lipidomics of host–pathogen interactions

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Abstract The cell biology of intracellular pathogens (viruses, bacteria, eukaryotic parasites) has provided us with molecular information of host–pathogen interactions. As a result it is becoming increasingly evident that lipids play important roles at various stages of host–pathogen interactions. They act in first line recognition and host cell signaling during pathogen docking, invasion and intracellular trafficking. Lipid metabolism is a housekeeping function in energy homeostasis and biomembrane synthesis during pathogen replication and persistence. Lipids of enormous chemical diversity play roles as immunomodulatory factors. Thus, novel biochemical analytics in combination with cell and molecular biology are a promising recipe for dissecting the roles of lipids in host–pathogen interactions.

Keywords: Lipidomics; Host–pathogen; Membrane traffic; Mass spectrometry

1. Introduction

Infectious diseases are a continuous burden on global health. AIDS, Malaria and Tuberculosis are the top three infectious diseases by scale of mortality. While these diseases typically are limited to endemic areas, other forms of infections, such as avian flu or the SARS, have been posing immediate threats based on localized outbreaks followed by rapid global spreading. All of these infectious diseases are caused by intracellular pathogens, enveloped viruses, mycobacteria and eukaryotic parasites.

The biosynthesis of mammalian lipids is fairly well understood. This is not at all the case however for pathogen lipids where many of the genes which encode for lipid enzymes have not been identified and characterized in detail. In addition, despite many decades of biochemical research, we only have a vague picture of membrane and cell wall composition of many pathogens and the role of lipids in microbial pathogenesis [1,2]. This review should act to stimulate a thought process on the potential functions of lipids during interaction of important intracellular pathogens with their mammalian (human) host cells. In an attempt to conceptualize a format for future update and reference the host–pathogen interaction process is divided into various steps (Fig. 1) at which different classes of lipids [3] have been shown to play a role (Table 1). Eicosanoids and derivatives, well known immunomodulatory lipids [4], are not discussed in detail here. Instead the focus lies in membrane components and lipids involved in energy homeostasis.

2. A lipidomic view of pathogen replication cycles

Extracellular (free) forms of pathogens depend on lipids for structural and functional integrity (Fig. 1, stage 1). Uptake of pathogens is accompanied by general phenomena including re-organization of the cytoskeleton, membrane ruffling and invagination at the site of pathogen entry. Obviously, these processes are orchestrated, in part, by signaling cascades including lipid kinases, phosphatases and lipases. What is not evident is the precise interconnectivity between pathogen lipid metabolism and host lipid metabolism (Fig. 1, stages 2–5).

2.1. Enveloped viruses (human immunodeficiency virus)

Enveloped viruses are critically dependent on their lipid shell which is evidenced by the high sensitivity to detergent. Indeed, delipidation of viruses is used in novel antiviral therapeutics [5]. It is intriguing that, so far, not much interest has been devoted to the lipid inventory of viruses, much in contrast to viral genomes and proteins. This is largely due to the fact that only very few virus encoded lipid enzymes are known [6]. Instead, viruses derive their lipids from the host. Biochemical analysis of purified viruses shows that certain classes of lipids, even specific molecular species, are present and enriched in viral particles [7,8]. It will be interesting to see how lipids act at the molecular level during virus particle formation and propagation (Fig. 1, stage 1).

The precise signaling reactions at docking sites remain unclear yet recent observations suggest that binding of murine leukemia virus is followed by rapid, actin-mediated movement along filopodia [9]. Pathogen recognition at the cell periphery might be a trigger event that leads to clustering of lipids [10] and proteins at the site of docking, followed by signaling (e.g. via protein kinases and lipid kinases) and subsequent uptake. Phosphatidylserine and (complex) glycolipids have been implicated but the precise assembly of such ligand–receptor pairs remains poorly understood. It will be interesting to learn whether lipids act as co-factors to the well studied protein–protein interactions that mediate viral docking. It is conceivable that such interactions play a role given the high density of epitope-rich glycolipids on mammalian cell surfaces (Fig. 1, stage 2).

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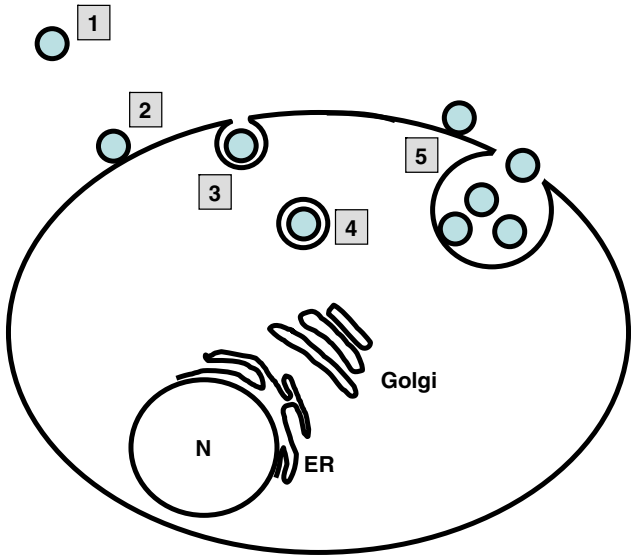

Fig. 1. Stages of host–pathogen interactions. The cartoon illustrates in general terms the various stages of host–pathogen interactions which are discussed in the text. Numerous roles of lipids, both of pathogen as well as of host origin, are summarized in Table 1. 1, Extracellular/free state; 2, docking; 3, invasion/uptake; 4, intracellular state/persistence/fusion/replication; 5, budding/escape from host cell.

Specialized membrane microdomains are entry portals for many intracellular pathogens. This is also the case for enveloped viruses, the majority of which enter via clathrin mediated endocytosis (human immunodeficiency virus, HIV; vesicular stomatitis virus, VSV) or caveolae/raft mediated uptake (e.g. Simian Virus 40, SV40). The roles of lipids in these uptake processes has been studied intensively and phosphorylated forms of phosphatidylinositol (GPIns), the phosphoinositides (PIs), cholesterol and sphingomyelin have been critically implicated in clathrin- and caveolae-mediated endocytosis (Fig. 1, stage 3).

There is growing evidence that lipids play an important role in the regulation of the infection cycle of enveloped viruses, in particular during entry, packaging, and release from the host cell [11]. Genomic analysis of the early host response to hepatitis C virus infection revealed that a large number of genes involved in lipid metabolism are differentially regulated underscoring the hitherto unappreciated important role of lipids in viral life cycles [12]. Implicated functions range from fusion of endosomal membranes [13] to transcriptional control of viral mRNA [14], thus spanning a wide range of biological activities. Hepatitis C virus RNA replication requires the synthesis of fatty acids. Addition of exogenous saturated fatty acyls to cells leads to increased transcription, whereas polyunsaturated fatty acyls have the opposite effect [15]. The mecha-

Table 1
Implications of different lipid classes at various steps of host–pathogen interactions

Stage (Fig. 1)	Enveloped viruses (e.g. HIV)	Bacteria (e.g. mycobacteria)	Apicomplexa (e.g. *Toxoplasma*, *Plasmodium*)	
1		Mycolic acids and waxes form an impermeable layer around the plasma membrane of mycobacteria [80,81]		FA
		Growth state dependent metabolism of triglycerides in mycobacteria in vitro [82–84]		GL
			Toxoplasma is independent from host in aminophospholipid synthesis. Phosphatidylcholine accounts to ~75% of total phospholipid [85]	GP
	Viral envelopes are enriched in sphingomyelin [8,86]			SP
	Envelope cholesterol is critical for structure and infectivity [87–89]			ST
2	Phosphatidylserine stimulates entry of enveloped viruses [90,91]	Mannose receptor binds to the ManLAM mannose caps of mycobacteria and mediates phagocytosis [92]	GPI anchored proteins (host [35,36] and parasite [37]) act as receptors for *P. falciparum* attachment and entry	GP
	(Complex) glycolipids act as receptors for viral envelope proteins [93]			SP
	Elevated levels of ceramide and GM3 confer resistance to HIV type 1 fusion [94,95]			
		Lipid (A) [22] recognition by TLR receptors [56,96]		SL
3	Signaling via lipid kinases is activated during viral entry [97,98]			GP
	Glycolipids promote CD4-dependent fusion of HIV [99,100]			SP
	Cholesterol in target membrane is required for fusion and infection [101,102]	Cholesterol is required for entry of mycobacteria into macrophages [103]	Cholesterol (host) controls *T. gondii* entry [38]. Lipid rafts of red blood cell membranes are involved in formation of the parasitophorous vacuolar membrane around *P. falciparum* [36]	ST

Table 1 (*continued*)

Stage (Fig. 1)	Enveloped viruses (e.g. HIV)	Bacteria (e.g. mycobacteria)	Apicomplexa (e.g. *Toxoplasma*, *Plasmodium*)	
4	Fatty acyl synthesis is required for Hepatitis C virus RNA replication [12,15]	Intracellular growth and virulence of listeria require host-derived lipoic acid [105]	Anti type II FAS inhibitors (herbicides and antibiotics) inhibit apicomplexa [107]	FA
	Geranylgeranylation is required for Hepatitis C virus RNA replication [104]	Trehalose mycolates induce a proinflammatory cascade that influences granuloma formation [106]		
		Isocitrate lyases (ICL1 and ICL2) are required for intracellular replication [31]		
			Triacylglycerol formation is essential for intraerythrocytic replication of *P. falciparum* [108–110]	GL
	Lysobisphosphatidic acid and phosphoinositides regulate release of VSV nucleocapsid into cytoplasm [13]	SigD/SopB is a type III bacterial derived phosphoinositide phosphatase [29,112]	Molecular species of phosphatidylcholine and phosphatidylethanolamine are altered in host plasmamembrane after *Plasmodium* infection [116]	GP
	Ethanolamine phospholipids required for Sindbis virus production [111]	Intracellular mycobacteria release a heterogeneous mixture of lipids [27,113]	Growth arrest of *Plasmodium* [117] and *Toxoplasma* [85] by disruption of phosphatidylcholine synthesis	
	Semliki Forest virus mRNA capping enzyme requires association with anionic membrane phospholipids for activity [14]	Phosphatidylinositol mannosides stimulate fusion of early endosomes with mycobacterial phagosomes [30]		
		Glycosylated phosphatidylinositol causes phagosome maturation arrest [114]		
		SapM, a mycobacterial derived phosphatase hydrolyses PI3P contributing to inhibition of phagolysosome maturation [115]		
		Sphingosine 1-kinase is recruited to nascent phagosomes [118]	Inhibition of sphingolipid biosynthesis in *T. gondii* blocks replication [119]	SP
			Sphingomyelin metabolism important for *P. falciparum* development [120]	
	Inhibition of cholesterol biosynthesis inhibits Hepatitis C virus RNA replication [15]		Inhibition of cholesterol acquisition by the host lowers *T. gondii* replication [40]	ST
			Cholesterol esterification essential for optimal *T. gondii* proliferation [121,122]	
			Isopreonoid synthesis inhibitors with anti-malarial [123] and anti-*T. gondii* activity [124]	PR
			Block of protein farnesylation as antiapicomplexan therapies [125]	
5	Unsaturated fatty acids block HIV budding [126]	Mycobactin mediates iron supply within macrophages [32]		FA
	Affinity of HIV Gag protein is regulated by myristoyl switch [127]	Presentation of glycerophospholipids, sulfolipids and mycolates via CD1 receptors [58,132,133]		GP
	Golgi-derived viruses have different phospholipid (incl semilysobisphosphatidic acid) composition depending on the precise site of budding [7,128]			
	HIV-like particle assembly is regulated by inositol phosphates [129]			
	HIV-1 Gag targeting is regulated by PIP2 [130]			
	Vaccinia virus envelope protein p37 has lipase activity [131]			
	Nef transports cholesterol to site of HIV budding [134]			ST
	Budding occurs from glycolipid-enriched membrane lipid rafts [8,135–137]			

Table summarizing the reported involvement of lipids, both from the host as well as the pathogen, during various stages of host–pathogen interaction (Fig. 1). Lipids are classified according to Fahy et al. [3]. FA, fatty acyls (e.g. oleic acids, prostaglandins); GL, glycerolipids (e.g. triacylglycerol); GP, glycerophospholipids (e.g. phosphatidylinositol); SP, sphingolipids (e.g. sphingomyelin, ceramide); ST, sterol lipids (e.g. cholesterol); PR, prenol lipids (e.g. menaquinone); SL, saccharolipids (e.g. lipid A).

nisms of these effects are not known but appear to be independent of signaling via nuclear LXR receptors.

The coordinated assembly of viral components and the release of functional particles from the host are final steps of viral replication (Fig. 1, step 5). Viruses are thus excellent tools to probe the lipid composition of the membrane site where budding occurs. Viruses which form on intracellular membranes (e.g. vaccinia at the Golgi apparatus) carry lipids which are indicative for these organelles (e.g. lysobisphosphatidic acids) [7]. Cell surface packaged viruses (such as HIV in most cases) are rich in cholesterol and sphingolipids [8]. This has implications for the properties of the envelope membrane. The envelope of HIV, for example, was found to be more ordered than that of the producer cell on average [16].

2.2. Pathogenic bacteria (*Mycobacteria, Salmonella*)

Mycobacterium tuberculosis is the causative agent of TB. With respect to lipids, it is unique among bacteria since it has approximately 250 lipid enzymes in its genome (compared to only 50 in *Escherichia coli*) [17], and an unusually high number of distinct (glyco)lipids in its cell membrane and wall [18]. The natures of major components are known. However, elucidation of the precise chemical composition of many lipid species and more importantly their biosynthetic pathways, transport [19] and dynamics leaves enormous room for further investigations. Such more detailed knowledge will enhance the drug development process which classically has been targeting cell wall biosynthesis.

Approaches which target specifically the delicate molecular machinery employed by bacteria during interactions with their host will add new ways of interfering with pathogenic bacteria [20,21]. Among those are many lipid related processes (Fig. 1, step 2). One of the classical ligands for Toll like receptors (TLR) is the lipid A portion of lipopolysaccharide (LPS), a bacterial glycolipid [22]. Many of the TLR bind lipid structures. Thus it can be expected that the advances made in the systems biology of TLRs [23], and chemical analysis of naturally occurring forms of LPS [24] will bring together knowledge from different fields in order to understand better the molecular mechanisms of pathogen recognition.

As for viruses, uptake of bacteria (Mycobacteria, Salmonella) occur at specialized membrane microdomains. This phagocytic process shares some similarity with endocytosis of viruses. A common strategy for bacteria (as well as eukaryotic pathogens, see below) is to avoid the degradative environment of lysosomes. In order to achieve this goal, these pathogens have developed sophisticated molecular machinery which interferes with host cell signaling, cytoskeletal rearrangements and membrane trafficking. The mechanisms employed include lipid and calcium signaling, small GTPase function, and are mediated by effector molecules which are introduced (e.g. via type III [25] or type IV [26] secretion) or released [27] by the pathogens during invasion of the host [20,21]. It should be noted that while many pathogens remain contained in a large (parasitophorous) vacuole (*Toxoplasma*, mycobacteria) others escape into the cytosol where they migrate using tails of cytoskeletal matrix (e.g. *Trypanosoma cruzi*) [28].

This concept of molecular mimicry in order to disguise and trick the host is also reflected at the level of lipid metabolism. *Salmonella typhimurium* uses its SigD/SopB phosphatase to interfere with phosphoinositide metabolism of the host. This leads to the generation of a spacious replicative vacuole and blocking of maturation into phagolysosomes [29]. Intracellular bacteria release chemically distinct mixtures of lipids which interfere with membrane trafficking. It is intriguing that these biologically highly active compounds share some structural similarity to endogenous lipids which act as prominent signaling molecules in our body (Fig. 2). Mannosylated forms of GPIns (Fig. 2D), reminiscent to the doubly phosphorylated

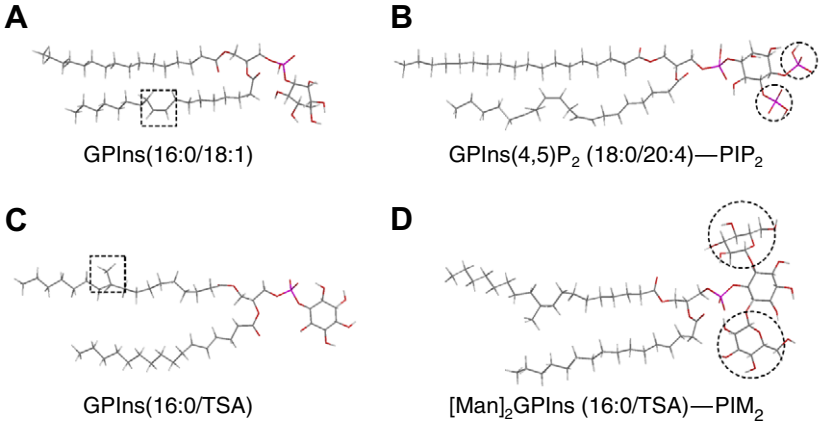

Fig. 2. Molecular species of mammalian and mycobacterial inositol lipids. Panels A and B show representative examples of phosphatidylinositols (GPIns) found in mammalian cells, while panels C and D display structurally reminiscent molecules found in mycobacteria. (A) Structure of GPIns with palmitoyl (C16:0) and oleoyl (C18:1) fatty acyls [GPIns(16:0/18:1)]. (B) Structure of phosphatidylinositol-bis-phosphate, GPIns(4,5)P_2, with stearic acid (C18:0) and arachidonic acid (C20:4), GPIns(4,5)P_2 (18:0/20:4). In mammalian cells fatty acyls with unsaturation are preferentially found in the sn-2 position (box in A) and reversible phosphorylation of inositol headgroup of GPIns leads to the generation of seven naturally known phosphoinositides (circles in B). (C) GPIns with palmitic acid and tuberculostearic acid (TSA, 10-methyl stearic acid, C19:0, box). Tuberculostearic acid is a marker for mycobacteria and in addition to GPIns is found in other phospholipids as well as in mannosylated forms of GPIns (panel D). (D) GPIns (16:0/TSA) with two mannose residues (circles) on the inositol headgroup [Man]$_2$GPIns (16:0/19:0) (PIM$_2$). PIMs are important structures in the envelope of mycobacteria and are bioactive during phagocytosis.

GPIns (e.g. PI(4,5)P2, Fig. 2B), interfere with endosomal fusion and phagosome maturation [30] (Fig. 1, stage 4). Often the lipid mimics employed by mycobacteria do not occur in the host cells and thus represent attractive sites for intervention.

The interaction between bacteria and their host at intracellular stages indeed deserves special attention. The parasites need to acquire resources from their hosts in order to survive, replicate or persist. Much of our knowledge in this area stems from research conducted on eukaryotic parasites (see below). There is increasing awareness however, also in the field of bacteriology, that supply with energy and resources is an aspect which might have been misjudged in its importance in the past. Energy production via beta oxidation of fatty acyls, using the glyoxylate cycle enzymes isocitrate lyases, is required for intracellular replication of mycobacteria [31]. Iron is required for growth of bacteria. Mycobacteria have thus developed siderophore based systems which supply them with iron. Mycobactin is one of these iron chelators and in infected cells localizes at cytosolic lipophilic structures in close proximity to the intracellular vacuoles which harbor bacteria [32,33]. The lipid tail of the mycobactin lipopeptide is likely to play an important anchor role during trafficking and localization of the iron carrier.

It should be stressed that commonly accepted wisdoms of bacterial lipidomes will have to be revisited. Phosphatidylethanolamine, phosphatidylglycerol and cardiolipin are major and important components. However, different classes of bacteria have developed very complex sets of chemically diverse lipid components to complement this basic inventory. The lipids of mycobacteria are a leading example but other clinically important pathogenic bacteria such as *Helicobacter pylori* are likely be investigated with enhanced scrutiny in this respect [34].

2.3. Eukaryotic parasites – apicomplexa

Apicomplexan parasites (e.g. *Toxoplasma*, *Plasmodium*) cause major diseases in animals and humans. *Toxoplasma gondii* is a particularly well studied member of apicomplexans due to the relative ease of genetic manipulation and cultivation in the laboratory. It is thus not surprising that much of what we know about apicomplexan lipids originates form work on *T. gondii*.

Entry of eukaryotic parasites remains less well understood compared to endocytosis and phagocytosis of viruses and bacteria. In the case of apicomplexa this is due in part to the rapid kinetics of the invasion process. GPI anchored proteins in the host [35,36] or the parasite [37] act as receptors for *Plasmodium falciparum* attachment and entry. Targeting of the GPI anchor structure is indeed an avenue which is explored for anti-malarials with novel modes of action. The rhoptry, a specialized secretory organelle of *Toxoplasma*, discharges its content during entry into host cells. Rhoptries contain high levels of cholesterol (which *Toxoplasma* does not synthesize itself) but it is not precisely clear how this relates to function. It is intriguing that the same lipid which is required for the much slower endocytic and phagocytic uptake of viruses and bacteria is also implicated in the explosive generation of a membrane envelope around invading *Toxoplasma*. Host cholesterol, rather than *T. gondii* derived cholesterol, however, regulates entry [38]. With the rapid accumulation of knowledge in the field of multivesicular body (MVB) formation and viral budding, it can be expected that more light will be shed also on the pathway of rhoptry biogenesis. It has been hypothesized that the latter is reminiscent of MVB formation and includes secretory and endocytic pathways and adaptor based vesicular components (e.g. AP-1 and vps4) [39].

As obligate intracellular pathogens *Plasmodium* and *Toxoplasma* are dependent on nutrient access [40]. Phospholipid and neutral lipids are currently at a center stage of attention at intracellular stages of *Toxoplasma* but also of other parasites which feed small molecular metabolites from their hosts (Table 1). Neutral lipids (triglycerides, sterols and sterol esters) are stored in 'lipid bodies' which are observed at the ultrastructural level as droplets both in the parasites as well as in the cytosol of the host. The biogenesis of lipid droplets has attracted much interest in recent years because of their potential role in a variety of organisms and conditions on top of the more established function in adipose tissues [41]. In fact, they are now considered bonafide organelles with distinct metabolism. Their biogenesis is not fully understood and follows different pathways in bacteria and eukaryotic cells [42]. Triglycerides have been described in *Toxoplasma* [43] and in mycobacteria from sputum of patients with TB [44]. Functionally, the fatty acyls stored in triglycerides of fat bodies might be used (i) as a source of energy (e.g. upon resuscitation from dormant conditions) or (ii) as building blocks for membrane lipid synthesis during phases of active replication (see also below). Delineation between these options will be an important goal in future research. In the case of *Plasmodium* for example the usage of fatty acyls as a source of energy might not be very likely since its genome seems to lack homologies of enzymes involved in beta-oxidation [45]. In addition, the role of tricarboxylic acid cycle enzymes in the *Plasmodium* mitochondrion is unclear [46].

3. Novel approaches and tools to study the role of lipids during host–pathogen interactions

Over the past decade, research using genetic, cell biological and biochemical approaches has led to an enormous increase in molecular and mechanistic insight into interaction of pathogens with their hosts. Cell biological investigations, driven often by microscopy as a major technique, of fundamental microbiology proved particularly insightful since they added temporal and spatial elements to host–pathogen interactions. Initially, like in many other fields of biomedical research, the main focus was on identification of genes and proteins required for activity. Based on these findings there is now increased awareness that lipids, both of host as well as pathogen origin, play critical roles in regulating pathogen stability, entry into host cells as well as replication and persistence. Advancements in research and development are now fueled by increasing interests aimed at discovery of novel therapeutic interventions against major infectious diseases [47,48].

3.1. Cell and chemical biology

Classical experiments using pathogens as tools to investigate principles of membrane trafficking laid the foundation for an overlap between the fields of cell biology and microbiology [49]. These studies were complemented with biophysical characterization of membrane fusion and structural work on the

properties of fusion proteins. More recently, studies using (radio- and fluorescently)-labeled lipids and optical probes for localization of signaling lipids in living cells (e.g. PH domain fusions to green fluorescent protein) have provided us with information on early signaling events during pathogen entry and uptake of lipid (precursors) during replication. Rapid accumulation and degradation of phosphoinositides was followed using protein probes specific for PI(3)P, PI(4,5)P2, PI(3,4,5)P3, and diacylglycerol, alone or in combination, in living cells [29,50]. Careful comparison with morphological observations of endosomal membranes linked these 'waves' of lipid metabolism to functional activity which, in these cases were attenuation or arrest of phagosomal maturation, with a benefit for the pathogen [51].

In case of virus fusion certain lipids are now known to be important co-factors for docking and release at the cell surface as well as in intracellular compartments (Table 1). The precise molecular arrangements of lipid–protein complexes are not known however. It is conceivable that future developments of 'fusion/entry-inhibitors' will include a substantial amount of structure–function based considerations on lipid–peptide interactions. Some of these insights will come from structural analysis of fusion complexes but also high resolution mapping of small molecule inventories in viruses [8]. Virus-like particles (virosomes) with traffic and fusion characteristics similar to real viruses can be used for targeted delivery, enhanced transfection, and vaccine development [52,53].

Antibodies or protein modules specific for other proteins or ligands are traditional tools in cell biological research. While many lipid binding modules are known, their specificity is restricted mainly to phosphoinositides [54]. CD1, TLR and ML domain containing proteins have been shown recently to bind to various forms of complex lipids with a functional link to immunology [55–57]. The basic principle which governs specificity of recognition is not clear and in some cases is regulated by 'chaperone-like' proteins (e.g. saposins during CD1 loading [58], or ML proteins as co-factors of TLR in lipid A recognition [57]).

The antibody response which occurs in patients with AIDS is generally ineffective against the virus. Typically observed anti-lipid reactivities in patients infected with HIV include anti-cardiolipin, as well as anti-phosphatidylinositol and anti-phosphatidylserine antibodies [59,60]. The significance of this response is not clear and could involve reactions to cell debris (e.g. apoptotic cells and mitochondria which expose/liberate cardiolipin and phosphatidylserine). Interestingly, rare human antibodies which neutralize HIV are also reactive against cardiolipin [61].

Surprisingly, in fact, very few specific antibodies against lipids are currently known. In addition to the above there are a few diseases, mainly inflammatory disorders such as multiple sclerosis, systemic lupus erythematosus and anti-phospholipid syndrome, which are accompanied by lipid directed antibodies. Lipid microarrays (immobilized lipid chips) were recently used to detect antibodies against sulfatides, sphingomyelin and oxidized lipids from cerebrospinal fluids of patients with multiple sclerosis [62]. Caution is needed however in the use and expectations of these anti-lipid antibodies due to the fact that they are directed against serum proteins which bind phospholipids [63]. It can be anticipated that more anti-lipid antibodies, hopefully with better specificities, will be identified in the future due to (i) the investigations of the immunology of lipid related diseases and (ii) increased availability of milligram to gram quantities of pure and synthetic (glyco)lipids with high bioactivity [64,65].

3.2. Lipid profiling

Analysis of pathogen lipids in not something new. Major advances in analytical biochemistry have however added new momentum to such analysis. Liquid chromatography and mass spectrometry are fueling the field of lipidomics as major technological platforms. These approaches allow detection, characterization and quantification of many different classes of lipids, even though it is currently not possible to capture the full 'lipidome' of a cell or tissue in one single experiment. The large body of biochemical information of lipid inventories (e.g. of mycobacteria) is now being used to investigate the details of lipid biosynthetic enzymes and lipid transporters. This work will help to identify pathogen specific metabolic pathways.

Sphingolipids exist in different forms in eukaryotic cells (most bacteria do not synthesize sphingolipids though there are exceptions). In mammalian cells, sphingomyelin and glycosphingolipids are predominant forms of sphingolipids, whereas in plants, yeast and kinetoplastids these lipids typically carry inositol-glycosides on the ceramide backbone. De novo biosynthesis of sphingolipid has only very recently been described in *T. gondii* [66]. The precise chemical composition of inositol containing sphingolipids as well as methods for their detection are just being determined [67,68]. Mass spectrometry based analysis will thus help to understand better the inventory of pathogens with respect to bioactive lipids and their metabolism during interaction with the host. The apicoplast is a specialized organelle which has received lot of attention as a potential site of intervention in anti malaria control [69]. This organelle is essential for parasite survival and is not present in mammalian host cells. Since the apicoplast is a relict form of chloroplasts, some, but not all metabolic pathways are known. Several of the metabolic pathways, including lipid metabolism, seem to be fundamentally different from those found in human hosts [69].

High resolution analysis of purified parasites will also help to learn more about how pathogens use their chemical composition to protect themselves during transmission within a host or from host to host. This could point to certain *classes of lipids* as major contributors of pathogenicity. Blood stream forms of *Trypanosoma brucei* for example are enriched in ether GPEtn and GPSer [70]. In these lipids the fatty acyls are bound to the glycerol backbone (typically in the sn-1 position) via an ether rather than ester bond. In some tissue and cell types ether lipids exist in considerable amount with their more common ester analogs. Platelet activating factor is a prominent representative of this class of lipids. Ether lipid analogs are used in antiviral therapy but their mechanism of action remains unknown [71]. It is possible that the elevated presence of ether lipids in free forms of pathogens might be related to a role in antioxidative protection. The control of the redox balance must be a challenge for pathogens as they travel from host to host. Indeed, redox cyclers have been proposed as tools to target viral cholesterol [72].

Identification of *unique lipid entities* with biological activity is an enormously promising area in infection biology and immunology. Biochemical fractionation of bacterial extracts followed by functional assays and analytical chemistry helped

to identify glycolipids which act as ligands for CD1 receptors, elicit proinflammatory cytokines [73] and which represent hypervirulence factors [74–76]. Given the complexity of lipids in mycobacteria but also in eukaryotic pathogens this is a promising approach which is likely to lead to the discovery of many more lipid structures and their biological activities.

A unique feature of many intracellular parasites is revolving life cycles in different organs of the host. Thus, it is possible that the regulation of various steps of parasite replication might depend, at least in part, on differences in lipid levels between organs. The appearance of an enveloped virus particle depends on the precise nature of the host cell and the site of budding. The lipid shell of HIV may be different between virus particles that originate from intracellular membranes as compared to those that bud from the cell periphery. Characteristics of the glycosylation apparatus between different host cells will affect decoration of proteins and lipids with sugar moieties. Thus a virus particle is likely to 'look' quite different at various stages of replication in the vector and the host. Metabolite profiling approaches will make important contributions in deciphering such differences.

'Pathogen lipid profiles' are the starting point for tests of functional relevance (pathway discovery [47]). They are also valuable diagnostic tools for monitoring of replication in laboratory animals (during the development of new medications) and in clinical isolates [77]. In a world of unprecedented mobility of man and pathogens, the speed of detection and development of effective vaccines, becomes a critical element in the control of disease outbreaks. Finally, novel diagnostics also need to be 'bush-proof' since many of the diseases discussed above are primarily diseases which occur in rural (tropical) areas which are medically and technologically poorly developed. Multi-metabolite readouts or kits for facile detection of pathogen related lipids (e.g. based on antibodies, see above) might represent important future options.

A major problem in many infectious diseases is latency. In case of a virus, this relates to inactivity in replication and maintenance of the viral genome. In case of pathogens which have energy metabolisms of their own, latency is a state of low metabolic inactivity. This is of course intimately linked to carbon/lipid metabolism. Comparative lipidomic profiling of pathogens in different physiological conditions (e.g. growing versus non-replicating mycobacteria) will help to identify pathways which may represent potential sites for therapeutic interventions. There is an urgent need to understand better the details of metabolically inactive conditions in particular under conditions which reflect the environment in vivo [78,79].

4. Conclusions

Cell biology of microbial pathogenesis has opened many doors for future research into the role of lipids in host–pathogen interactions. These interactions are very complex, dynamic and involve a multitude of mechanisms at different stages of the replication cycle. Lipids in host–pathogen interactions, play a role (i) as structural components (e.g. mycolic acids), in (ii) recognition (e.g. LPS), (iii) intracellular trafficking (e.g. PIM), and (iv) energy and resource homeostasis during reproduction (e.g. host lipids are building blocks, mycobactins, etc.). Many different chemical forms of lipids are involved ranging from fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterols and prenyl lipids making this a true heaven for lipidomic discovery. Novel approaches for lipid analysis in combination with the cell and molecular biology will help to dissect the complicated lipid signaling during host–pathogen interactions for applications in drug and biomarker development.

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