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The role of lipids in host microbe interactions

Roland Lang[1], Jochen Mattner[1]

[1]Mikrobiologisches Institut, Klinische Mikrobiologie, Immunologie und Hygiene, Universitatsklinikum Erlangen and Friedrich-Alexander Universitat (FAU) Erlangen-Nurnberg, Wasserturmstr. 3/5, D-91054 Erlangen, Germany

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1. ABSTRACT

Lipids are one of the major subcellular constituents and serve as signal molecules, energy sources, metabolic precursors and structural membrane components in various organisms. The function of lipids can be modified by multiple biochemical processes such as (de-)phosphorylation or (de-)glycosylation, and the organization of fatty acids into distinct cellular pools and subcellular compartments plays a pivotal role for the morphology and function of various cell populations. Thus, lipids regulate, for example, phagosome formation and maturation within host cells and thus, are critical for the elimination of microbial pathogens. Vice versa, microbial pathogens can manipulate the lipid composition of phagosomal membranes in host cells, and thus avoid their delivery to phagolysosomes. Lipids of microbial origin belong also to the strongest and most versatile inducers of mammalian immune responses upon engagement of distinct receptors on myeloid and lymphoid cells. Furthermore, microbial lipid toxins can induce membrane injuries and cell death. Thus, we will review here selected examples for mutual host-microbe interactions within the broad and divergent universe of lipids in microbial defense, tissue injury and immune evasion.

2. INTRODUCTION

Lipids are membrane-bound compounds found in all living organisms. They might exist in complex forms, for example linked to polysaccharides or in simple free forms. Thus, lipid structures are very divergent and range, to name a few, from saturated or unsaturated fatty acids to glyco- (sphingo-) lipids, phosphoglycolipids, glycerophospholipids, lysophospholipids or cholesterol esthers. Glycerophospholipids represent the main structural constituent, particularly within the inner leaflet of biological membranes and thus, form the main barrier between the cell´s interior and its environment. Depending on the cell type, the lipid composition of each leaflet can significantly vary. Plasmalogens, a type of phosphoglyceride, for example, are key

components of muscle and nerve membranes. The cytosolic leaflet side in the plasma membrane of human erythrocytes consists instead mainly of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol in contrast to the *exoplasmic* side which predominantly cointains phosphatidylcholine and sphingomyelin. While plasma membranes of animal cells consist also of cholesterol, cholesterol is not part of bacterial or mitochondrial membranes (1), which originate from alphaproteobacteria (endosymbiont theory).

Many lipids and glycolipids are also found in the cell wall of bacteria and fungi. Bacteria, for example, have distinct cell walls. The envelope of Gram-negative bacteria such as *Escherichia coli* and *Salmonella enterica*, is composed of two distinct lipid membranes. While the inner membrane consists similar as in mammalian cells mainly of glycerophospholipids such as phosphatidylethanolamine, phosphatidylglycerol and cardiolipin, the highly distinctive outer membrane is an asymmetric bilayer; its outer leaflet contains in most species predominantly lipopolysaccharides (LPS) (2). However, there exists several Gram negative bacterial genera that do not contain any LPS. These include *Ehrlichia*, which utilze, for example, the host´s cholesterol for their membranes (3, 4) or *Sphingomonas* species, which replace LPS by Glycosphingolipids (5-7). Other bacteria such as *Streptococci*, *Borrelia* or *Mycobacteria* express also different and distinct lipids in their cell walls. Those include diacylglyceroles and cholesteryl galactosides in *Streptococci* (8) and *Borrelia* (9, 10). Major classes of mycobacterial glycolipids are glycopeptidolipids, trehalose containing lipooligosaccharides, lipoarabinomannan and phenolic glycolipids, which represent highly immunogenic antigens (11, 12). In contrast to Gram-negative bacteria, teichoic acids are major constituents of the cell wall of most Gram-positive bacteria. They can be linked to the peptidoglycan layer, but also to lipid achors and are referred to then as lipoteichoic acids (LTA). Similar as observed for LPS in Gram-negative bacteria, the structure of LTA varies also between the different species of Gram-positive bacteria.

Over the years, lipids have not only been perceived as structural component of membrane bilayers or efficient energy stores, but are also now recognized as signaling molecules and mediators of apoptosis or inflammation. Furthermore, lipids play an important role in microbial pathogenesis and even act as virulence factors. Thus, lipids influence the immune system and host-microbe interactions in multiple ways. Lipids regulate, for example, phagosome formation, antigen presentation or cytokine production. Many of the cell populations expressing lipid-reactive receptors share innate (-like) immune features. Here, we will describe the function of distinct immune cells with specific lipid-reactive receptors and the effects of undirected lipid actions on a few examples.

3. ROLE OF HOST LIPIDS

As indicated above, lipids contribute to many (patho-)physiological processes and are part of complex signaling and functional networks with multiple nodes of interaction and cross-regulation. Due to their specific molecular structure, tissue location, modifications and embedding into cellular components/networks, lipids exert distinct functions. Thus, for example, ceramides and sphingosines act pro-apoptotic and antiproliferative; however, when sphingosine is converted into sphingosine-1-phosphate (S1P), cell growth and proliferation are promoted (13-17). Similar as sphingosine, lysophosphatidic acid (LPA) binds to a family of G protein coupled receptors and thus, functions in an autocrine and paracrine manner (18, 19). In addition, LPA, together with specific eicosanoids, binds to lipid-sensing transcription activators and thus, can modulate the repression of genes encoding, for example, fibrinogen or IFN-gamma (20-23). Imbalances in these pathways contribute to the pathogenesis and progression of multiple diseases (13). Despite the before mentioned additional functions of lipids within host cells, we will confine our discussion to lipid-mediated mechanisms of microbe digestion and immune cell signaling.

3.1. Role of host lipids in phagosome maturation

Phagocytosis is a pivotal mechanism for the removal of microbial pathogens and cell debris from the body. Phagocytes such as macrophages or neutrophilic granulocytes engulf microbial invaders or apoptotic cells into plasma membrane-derived phagosomes. These phagosome sequentially fuse with lysosomes as well as early or late endosomes and mature into phagolysosomes. This maturation process represents a carefully choreographed sequence of membrane fusion and fission events, which is coupled to the acquisition and activation of a panoply of oxidative, acidifying and hydrolytic enzymes, leading to the elimination of the ingested microbes or apoptotic cells (24, 25).

Lipids are known to exert numerous roles in phagosome formation and maturation. Notably phosphoinositides are involved in signaling, actin assembly and the cytoskeletal rearrangements that underlie engulfment. In addition, phosphoinositides and other lipids such as lyso*bis*phosphatidic acid (LBPA) or cholesterol contribute to the regulation of multiple membrane budding, fission and fusion events required for maturation. Some bacterial

pathogens such as *Listeria*, *Mycobacterium*, *Shigella*, *Salmonella*, *Legionella* and *Yersinia* manipulate the phosphoinositide (PIP) composition of phagosomal membranes and are not delivered to phagolysosomes, pointing at an emerging role of PIPs in phagosome maturation (26-28).

The recognition of lipids from dying cells is also critical for the initation of phagocytosis by macrophages or neutrophils. An exposure of phospholipids such as phosphatidylserine (PS) or phosphatidylethanolamine (PE) outside the cell membrane is a major factor in the recognition of apoptotic cells by phagocytes, involving membrane-bound receptors, such as the PS-receptor (29) or scavenger receptors (30, 31), and soluble opsonins which can connect apoptotic cells with phagocytes (32).

3.2. Role of host lipids in autophagy

Autophagy plays a critical role in the physiology of cells: it protects them against starvation, enables them to recycle nutrients from digested organelles or macromolecules and removes damaged or aberrantly folded proteins. Thus, similar like phagocytosis autophagy contributes to the maintenance of tissue homeostasis. Accordingly, perturbation(s) of autophagy have been linked to various disorders (33, 34).

While there exist different forms of autophagy – macroautophagy, microautophagy and chaperone-mediated autophagy – we will focus on macroautophagy, which represents the most prevalent form of autophagy (35). The most distinguishing feature of macroautophagy is the formation of a double-membrane bound autophagosome. In particular, the characterization of autophagy (Atg) genes and the respective encoded proteins was not only used to mark the autophagsome, but led also to the identification of four major complexes required for the formation of the autophagosome: a) the Atg1/Unc-51-like kinase (ULK) complex, a key signaling intermediate for the target of rapamycin (TOR); (b) the autophagy-specific class III phosphatidylinositol 3-kinase (PI3K) complexes, which are citical for the formation and maturation of the autophagosome; (ic) the transmembrane protein Atg9/mAtg9 and its trafficking machinery; and (d) the ubiquitin-like proteins Atg12 and Atg8/LC3 and their conjugation machinery leading to the lipidation of Atg8/LC3 with phosphatidylethanolamine (PE) (36-38).

Although these major complexes consist predominantly of proteins, lipids and lipid metabolizing enzymes have been increasingly implicated in the control of biochemical processes and membrane remodeling underlying autophagic processes. First, phosphatidylinositol-3,4,5-trisphosphate, for example, the product of class I PI3Ks, regulates signaling cascades converging onto the mammalian TOR (mTOR) pathway, which, in turn, negatively regulates the initiation of autophagy (39). Second, lipids such as PI3P act as membrane-bound localized signals controlling membrane dynamics due to the recruitment of cytosolic protein effectors that mediate membrane deformation, expansion and vesicle transport and thus control the biogenesis and maturation of the autophagosome (40, 41). Third, covalent binding of amine-containing phospholipids, such as PE, to Atg8/LC3 family members confers a unique mode of regulation by stably anchoring these critical factors to the membrane of phagophores that mediate their elongation and closure (36). Finally, lipids directly affect the physicochemical properties of lipid bilayers independently of proteins. Examples of this regulation of membrane dynamics include phosphatidic acid (PA), diacylglycerol or cholesterol, which promotes or stabilizes membrane bilayers (38).

3.3. Role of host lipids in signaling processes

Lipids are part of complex signaling networks and thus, regulate profound physiological responses. Lipids with signaling function such as eicosanoids, phosphoinositides, sphingolipids or fatty acids control cell growth, proliferation, apoptosis, metabolism and motility (13, 42-44). Vice versa, extracellular signals from cytokines, growth factors and nutrients modify the activity of several pivotal lipid-modifying enzymes such as phospholipases, prostaglandin synthase, 5-lipoxygenase, phosphoinositide 3-kinase, sphingosine kinase and sphingomyelinase (13) and thus the availability of the respective signaling lipids.

A modulation of lipids or lipid-metabolizing enzymes which influence inflammatory processes has been a target for therapeutic intervention for centuries. The pharmacological inhibition of the enzyme cyclooxygenase-2 (Cox-2), which catabolizes the formation of inflammatory lipid mediators such as prostaglandins, represents the best-known example in this context. In contrast, other arachidonic acid derivates such as lipoxins exert anti-inflammatory and pro-resolution activities. Thus, the goal would be to amplify the availability of these promising molecules in the context of an anti-inflammatory therapy. However, metabolic pathways that induce adverse (side) effects as well as cross-regulatory or para-/autocrine effects as exemplified for sphingosine above, need to be carefully considered. Thus, in addition to the biosynthesis of lipoxins by host cells, microbes might contribute. For example, pathogens such as *Pseudomonas aeruginosa* or *Toxoplasma gondii* express the enzyme 15-lipoxygenase which can metabolize linoleic acid,

alpha-linolenic acid, gamma-linolenic acid, arachidonic acid, eicosapentaenoic acid, and/or docosahexaenoic acid as physiological substrates. Thus, microbes potentially interact with endogenous biosynthetic circuits of the host in multiple ways and thus can divert the host´s immune defence (45).

Glycosphingolipids represent another group of lipids which transmit multiple signals. Glycosphingolipids in combination with protein receptors are organized in glycolipoprotein microdomains termed lipid rafts (46, 47). These specialized membrane microdomains compartmentalize cellular processes by serving as organizing centers for the assembly of signaling molecules, influencing membrane fluidity and membrane protein trafficking, and regulating neurotransmission and receptor trafficking (47, 48). Lipid rafts are more ordered and tightly packed than the surrounding bilayer, but freely float within the membrane bilayer (49). Although more common in plasma membrane, lipid rafts have also been reported in other parts of the cell, such as the Golgi apparatus and the lysosomes.

The importance and complexity of signaling through lipids rafts is exemplified here on mast cells, one of the key cell populations in allergic and anaphylactic immune reactions. Complexes consisting of antigen and immunoglobulin E (IgE) complexes, for example, trigger the crosslinking of high-affinity IgE receptors and their association with lipid rafts on mast cells, initiating a cascade of protein tyrosine kinases. These phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) on the cytosolic part of the IgE receptor and cause the translocation of the kinase Syk through its two Src-homology-2 (SH2) domains (50). Further phosphorylation of membrane-anchored proteins (such as linker for activation of T cells (LAT) and non-T-cell activation linker (NTAL)) and soluble adaptor proteins (such as GAB2) starts a first wave of lipid signalling by recruitment of so-called class IA PI3K heterodimers that comprise the regulatory subunit p85 and catalytic subunit of PI3K (PI3K$_c$).

The catalytic subunit of PI3Kδ (p110δ) has an important role downstream of the IgE receptor (51), but it also relays signals from the B-cell receptor and, to a lesser extent, the T-cell receptor (52). Elevation of levels of PtdIns(3,4,5)P_3 by PI3K is crucial for the progression of antigen receptor signalling because the lipid recruits and activates proteins that contain pleckstrin homology (PH) domains - among them, Bruton's tyrosine kinase (Btk) and PLCγ. Activation of PLCγ through the generation of Ins(1,4,5)P_3 causes Ca^{2+} release from internal stores. Usually, overstimulation of immune cells at low antigen concentrations is prevented by the SH2-domain-containing inositol 5-phosphatase-1 (SHIP1), which reduces the levels of PtdIns(3,4,5)P_3 (53).

4. RECOGNITION OF LIPIDS BY DISTINCT RECEPTORS

4.1. Lipid reactive receptors on myeloid cells

Lipid structures on the microbial surface are directly accessible to macrophages and other phagocytes during the initial stages of phagocytosis. Thus, it is therefore not surprising that several microbial glycolipids function as pathogen associated molecular patterns (PAMPs) and induce innate immune activation upon recognition by transmembrane pattern recognition receptors on the surface of myeloid cells. Several Toll-like receptors (TLR) sense lipid-containing PAMPs, e.g. TLR4 which binds lipopolysaccharide (LPS) in cooperation with MD2, and TLR2 which is activated by mycobacterial lipoarabinomannan (LAM) (54, 55) or lipoteichoic acid (LTA). Another important group of cell surface-localized PRR are Syk-coupled signaling C-type lectin receptors (CLR) of the Dectin-1 and Dectin-2 families (56). While Dectin-1, the founding member of this group, binds beta-glucans from fungal and bacterial cell walls (57), several other signaling CLR directly interact with lipid-containing ligands found in bacterial, mycobacterial and fungal cell walls.

Glycolipids represent another interesting group of biological active lipids. As amphiphilic components of cell membranes, glycolipids are composed of a hydrophilic polar sugar headgroup and a hydrophobic apolar lipid moiety which anchors the molecule in the cell membrane. According to their detailed chemical structure, glycolipids exert a variety of biological functions in the recognition, adhesion and signaling of cells and influence membrane parameters such as domain formation (lipid rafts) similar as glycosphingolipids. While mammalian glycolipids might serve as transmitters and storage elements of information, glycolipids of microbial origin belong to the strongest activators of immune responses (58). Although lipids interfere with numerous pathways and processes, we will confine here our discussion to three prominent lipid-receptor interactions.

4.1.1. Lipopolysaccharide (LPS) and TLR4

Lipopolysaccharides (LPS) are not only the key components of the Gram-negative cell wall, but are also required for the survival, the virulence or the immunogenicity of many Gram-negative bacteria. Once released from the bacterial cell wall, LPS elicits strong immunogenic responses and triggers, for example, the release of many inflammatory cytokines such as tumor necrosis factor alpha (TNFalpha) and interleukin-1beta (IL-1beta). Therefore LPS has been implicated as etiological agent for a variety of pathologies including septic shock, organ failure and death (59).

The common basic structure of LPS consists of three distinct components: a covalently bound, hydrophobic lipid component, termed lipid A and a hydrophilic heteropolysaccharide unit consisting of a core region and an O-specific oligosaccharide (O antigen) which is highly variable and antigenic. The main virulence determinant of LPS resides within lipid A, which is recognized as part of the innate immune response (60).

Lipid A ties the LPS molecule to the bacterial cell membrane, while the polysaccharide component interacts with the external environment. Lipid A is a unique and distinctive phosphoglycolipid; its structure is highly conserved among most bacterial species. However, there exist exceptions from the common basic structure in *Helicobacter pylori* (61-64), different *Francisella* species (65-67) or *Yersinia pestis* (68-70). The respective modifications within the lipid A structure influence the biological activity and toxicity of LPS and thus, promote the pathogenesis of all three microorganisms. These variations in the fine structure can arise from the degree of phosphorylation, the type of hexosamine present, the chain length, the presence of phosphate substituents as well as the number and the position of the acyl groups.

In each bacterial species, the heterosaccharide unit consists of two parts - an inner core region and an outer '*O*-specific' chain. This '*O*-specific' chain is a complex oligosaccharide polymer, which determines the serological and/or antigenic specificity of the respective lipopolysaccharide. For example, bacterial colonies expressing the '*O*-specific' chain appear 'smooth'. In contrast, the 'rough' LPS type lacks the *O*-specific chain, while a 'semi-rough' or short-chain type contains only one *O*-chain repeating unit. The *O*-chains or O antigens of LPS constitute the basis for the serotype classification of bacterial genera, as they interact with the immune system of the host protecting the bacterium against its defense mechanisms or against antibiotic actions. However, when separated from lipid A, the *O* antigens do not display any endotoxic activity.

During bacterial replication, death and/or destruction, heat-stable lipopolysaccharides are released. Those have been named endotoxins and can be differentiated from heat-labile exotoxins released by live bacteria. In particular, lipid A perpetuates the toxic effects observed during infections with Gram-negative bacteria. The conserved LPS structure of diverse Gram-negative bacteria is recognized by toll-like receptor 4 (TLR4). An engagement of TLR4 on myeloid cells such as macrophages, neutrophils and dendritic cells promotes the release of pro-inflammatory cytokines which contribute to bacterial clearance. The ligation of many TLR4 receptors, triggered due to a massive release of LPS during infection with Gram-negative pathogens, instead can lead to septic shock and multi-organ failure. However, lipid A modulates also immune responses and induces resistance to both bacterial and viral pathogens at low concentrations. Thus, for example, the application of LPS or LTA (see below) by oral gavage protected broad-spectrum antibiotics-treated mice from colitis-induced mortality, morbidity, and severe colonic bleeding (71). In addition, a previous exposure to low amounts of LPS causes myeloid cells to become refractory to a subsequent endotoxin challenge, a phenomenon termed, endotoxin or LPS tolerance (72). Thus, as indicated by these few examples, the response to LPS is highly complex, and it has been shown that hundreds of genes are affected in immune cells exposed to LPS.

4.1.2. Lipoteichoic acid (LTA) and TLR2

Lipoteichoic acids (LTAs) are major constitutents of the cell wall of Gram-positive bacteria. Most bacteria containing LTA also synthesize wall teichoic acid (WTA). In contrast to WTA, which is covalently linked to the peptidoglycan layer (73), LTA is tied via lipid anchors to the membranes of Gram-positive bacteria. Furthermore, the synthesis of LTA is intimately involved in the biosynthesis and turnover of lipids in bacteria (74). LTAs have been also reported to regulate muramidases, autolytic cell wall enzymes which are critical for the maintenance of bacterial cell wall integrity. Although the exact biological role of LTA is unknown, bacterial mutant strains display significant defects in growth and physiology, similar as observed for bacterial LPS mutant strains.

Similar as LPS LTA exhibits also antigenic properties when released from bacteria after bacteriolysis. LTA may bind to target cells non-specifically through membrane phospholipids, or specifically to CD14 and toll-like receptors such as TLR-2. Specific immune responses engaged upon TLR2-ligation include the expression of the central transcription factor NF-κB, the release of cytokines such as interleukin-10 (IL-10) and the induction of both pro- and anti-apoptotic genes. Additionally, modifications of the LTA backbone structure can protect bacteria against cationic antimicrobial peptides of the host (75). The release of LTA can be inhibited by non-bacteriolytic antibiotics and by polysulfates such as heparin *in vitro*. Furthermore, LTA can interact with circulating antibodies and activate the complement cascade. It also triggers the release of reactive oxygen and nitrogen species or bactericidal proteins and enzymes from neutrophils and macrophages. In animal studies, LTA has induced arthritis, nephritis, uveitis, encephalomyelitis, meningitis, and periodontal lesions, and also triggered cascades resulting in septic shock and multi-organ failure. Therefore, LTA shares many pathogenic similarities with endotoxins

(lipopolysaccharide). LTA or LTA synthesis pathways represent also promising targets for antibiotics (76, 77).

4.1.3. Trehalose-6,6'-dimycolate (TDM/Cord factor) and mincle

The most abundant glycolipid of the mycobacterial cell wall is Trehalose-6,6'-dimycolate (TDM), also known as cord factor, as the formation of large clumps of mycobacterial cells is attributed to its hydrophobic nature. The cord factor has been known for a long time to trigger important aspects of the host response in tuberculosis, as (a) injection of TDM into mice is sufficient to induce granuloma-like structures (78), (b) TDM has the highest capacity of mycobacterial cell (glyco)lipids to induce pro-inflammatory cytokine production from macrophages (79), and (c) cord factor is – similar to Complete Freund's adjuvant – a potent adjuvant for Th1/Th17 responses (80). Thus, the cord factor acts as a PAMP activating innate immunity and directing the differentiation of developing T cell responses towards beneficial Th1/Th17 phenotypes (81, 82). On the other hand, there exists also evidence that TDM may contribute to immune evasion by delaying phagosomal maturation (83) and by attenuating the production of some cytokines and/or the expression of costimulatory molecules (84, 85).

Mincle is the receptor for TDM, as it directly binds to it and is required for macrophage activation *in vitro* (86, 87), as well as granuloma formation to cord factor *in vivo*. Mincle also binds to and is activated by the synthetic cord factor analogue Trehalose-6,6'-dibehenate, an adjuvant where the complex mycolic acid esters are replaced by a simple C22-fatty acid (87, 88). The crystal structure of Mincle in complex with trehalose is available and shows Ca^{2+}-dependent binding in a sugar binding pocket with an adjacent hydrophobic groove thought to accommodate the lipid portion of TDM or TDB (89, 90). Other synthetic glycolipids based on trehalose- and glucose-esters of mycolates, corynomycolates (as found in the cell wall of corynebacteria, belonging to the Order Actinobacteria as mycobacteria), and fatty acids, have been developed and tested for Mincle binding and activation (91-93). Of note, Mincle binds also Glucose-monomycolate which is also a CD1b-restricted ligand for T cells with germ line-encoded T cell receptors (TCRs) (see below) (94), an interesting case of a dual function of a glycolipid as PAMP for innate immune activation and antigen for T cell responses. Among the fungi binding to Mincle and activating macrophages and other immune cells in a Mincle-dependent manner, the exact chemical nature of the ligands has been defined only for *Malassezia furfur* as glyceroglycolipid and complex fatty acids linked to mannitol (95).

Beyond its role as pattern recognition for microbial danger, Mincle detects also endogenous ligands. It was first shown to bind the ribonucleoprotein SAP130, an alarmin released from necrotic cells, triggering an inflammatory response of myeloid cells to cell death. The SAP130-Mincle interaction appears to drive the development of immune suppressive monocyte populations in a mouse model of pancreatic cancer (96). Cholesterol crystals are another recent addition to the growing list of Mincle ligands, in this case only for the human form of the receptor (97). It remains to be investigated whether this interaction plays a role in the development or course of atherosclerosis.

The CLR Mcl (Clec4d) is a close relative of Mincle and is located next to it in the Dectin-2 gene cluster. While Mcl binds TDM only weakly, its signaling appears to be sufficient to enhance Mincle expression for higher affinity binding of the cord factor. Mcl and Mincle proteins interact with each other, likely forming heterodimers on the macrophage surface and strongly mutually increasing their surface levels (98-100). Mcl also can form heterodimers with Dectin-2, a third member of this CLR cluster.

Dectin-2 is like Mincle an FcRγ-coupled receptor; it binds a wide range of fungi (including *Aspergillus fumigatus*, *Trichophoyton rubrum*, *Cryptococcus spp.*, *Histoplasma capsulatum*, *Candida albicans*, and *Malassezia furfur*) and several bacteria (mycobacteria, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*) (Ostrop and Lang, in press). Dectin-2 ligands on fungi include mannans of the Candida albicans cell wall and a mannosylated protein from *Malassezia furfur*. In contrast, the mycobacterial cell contains mannosylated lipoarabinomannan (manLAM) which is bound by Dectin-2 and triggers cytokine production from macrophages (101).

Very recently, a fourth member of the Dectin-2 family, DCAR (Clec4b1), was identified by Yamasaki and colleagues to bind to mycobacteria. In this case, acetylated phosphatidylmannositol (acPIM2) could be identifed as the ligand in the cell wall by fractionation of lipids. DCAR an activating receptor that signals via FcRγ similar to Mincle and Dectin-2, and contributes to induction of a Th1 response during mycobacterial infection (Toyonaga K. et al., in press). Together, the recognition of mycobacterial cell wall glycolipids appears to be mediated to a significant extent by the members of the Dectin-2 family of signaling CLR.

Mincle (Clec4e) requires association with the ITAM-containing adapter FcRγ chain for membrane localization and Syk activation. Mincle binds in addition to mycobacteria including *Mycobacterium tuberculosis* complex as described above to a diverse array of bacteria: *Streptococcus pneumoniae*, *Helicobacter pylori*, *Klebsiella pneumoniae*, and fungi, such as *Candida albicans*, *Fonsecaea spp*. and *Malassezia furfur*. However, to date only for mycobacteria and

Candida albicans the identity of Mincle ligands has been revealed in detail.

4.2. Lipid reactive receptors on lymphoid cells

Complexes consisting of peptides and MHC molecules are not the sole targets of T cell receptors. A substantial proportion of the T cell repertoire recognizes antigens other than peptides presented by non-polymorphic antigen-presenting molecules. There exist T cell populations which react to (glyco-) lipid rather than protein antigens. For example, the antigen-presenting molecules CD1a, CD1b, CD1c and CD1d are encoded outside the MHC locus (102-106) and display a broad range of lipid antigens to T cells (107). CD1 molecules in conjunction with CD1 presented lipid antigens are recognized by T cells expressing the alpha/beta or the gamma/delta T cell receptor (108-110).

Human CD1 genes are located on chromosome 1 (111) and encode five CD1 isoforms, which were assigned to group 1 (CD1a, CD1b, CD1c and CD1e) or group 2 (CD1d) on the basis of sequence homology (112). Mice, in contrast, express only CD1d. CD1e is a lipid transfer protein (113), whereas CD1a-CD1d are membrane bound molecules. While the expression of the group 1 CD1 molecules is inducible by microbial stimuli (114, 115), CD1d is constitutively expressed (116, 117). Each CD1 molecule traffics differently, scavenges distinct cellular compartments and exhibits a distinct expression pattern (116, 118). B cells, for example, express CD1c and CD1d; CD1a, CD1b, CD1c and CD1d are detected on myeloid dendritic cells (DCs) while CD1d is aslo expressed on epithelial cells. CD1a is a characteristic marker for Langerhans cells.

The CD1 family of MHC-class-I-like glycoproteins present both foreign and self lipids as cognate antigens to T cells. Group 1 CD1 molecules activate clonally diverse T cells that mediate adaptive immune responses to a vast range of microbial lipid antigens. In contrast, as part of the innate immune response invariant NKT (*i*NKT) cells release copiuos amonts of cytokines upon engagement of their (semi-) invariant T-cell receptor by lipid antigens presented by CD1d (group 2) molecules (119).

4.2.1. Lipid-reactive T cells

T cells reactive to CD1a, b and c molecules represented major discoveries (114, 120). Similar to conventional T lymphocytes group-1-CD1-restricted T cells exhibit a diverse T cell receptor repertoire and clonally expand upon antigen encounter. Unlike conventional T cells, however, these T cells react to a variety of lipid instead of protein antigens. Many of those antigens are detected in the unique cell wall of mycobacteria including *Mycobacterium tuberculosis*, the causative agent of tuberculosis.

Many of the unique lipids found in *Mycobacterium tuberculosis* are presented by CD1a, CD1b and CD1c. Those include different mycolates, such as free fatty acid mycolic acid or glucose monomycolate. Mycolic acid, a long-chain α-branched, beta-hydroxy fatty acid was identified as first antigen to be presented by CD1b (121). Lipoglycans such as lipoarabinomannan or phosphatidylinositol mannosides from *Mycobacterium tuberculosis* are also recognized by CD1b-restricted T cells (122). In contrast, CD1c presents lipids closely related to mannosyl-1-phosphomycoketides to T cells (123, 124). Other unique lipids of mycobacteria such as lipopeptides and sulpholipids can be presented by CD1a and CD1b, respectively (119). Interestingly, patients infected with *Mycobacterium tuberculosis* exhibit an enhanced reactivity against seveal of these lipids compared to healthy control individuals (123, 125, 126), implying a role for CD1-restricted T cells in the host defense against tuberculosis infection.

The first reports about group-1-CD1-restricted human T cells were examples of self-reactive responses (114). CD1a-presented autoantigens, for example, were identified as extremely hydrophobic skin oils such as wax esters, squalene and triacylglycerides (127). Sulphatide, a major component of the myelin sheath, is a promiscuous self-lipid antigen that could be presented by CD1a, CD1b and CD1c and activates clonally restricted human T cells (128). Furthermore, monosialotetrahexosylganglioside (GM1) and related gangliosides are presented by CD1b, suggesting that self-lipid antigens might be important targets for autoimmune T cells (129, 130).

Many more types of lipid antigens have been identified, including glycolipid, phospholipid, glycophospholipid, sulfolipid and lipopeptide antigens (131, 132). However, many of the (auto-)antigens these human T cell clones are reacting to are still unknown. Furthermore, due to the unavailability of suitable animal models, the biological role of the respective CD1-reactive T cell clones *in vivo* has remained unclear.

4.2.2. CD1d-restricted NKT cells

As mice lack group 1 CD1 proteins, most studies have focused on the recognition of CD1d by a population of CD1d-dependent αβ T cells. These cells are frequently summarized as natural killer T (NKT) cells, although they respresent a heterogenous population (133). Thus, NKT cells can be divided into two distinct subpopulations, namely type I and type II NKT cells (134), which recognize – in an innate (-like) pattern recognition like manner – a broad range of self-lipid and microbial-lipid antigens (135). Type I

NKT cells are defined by their CD1d restriction and alpha-GalCer-reactivity (136), but also recognize other glycolipids, diacylglycerols, glycoshingolipids, glycerophospholypids, lysophospholidids, and cholesterol esthers presented by the atypical MHC-I (-like) molecule CD1d on antigen presenting cells (APCs) (summarized in (117, 118, 135)). In contrast to the semi-invariant, canonical T cell receptor of type I NKT cells (Vα14Jα18/Vβ2,7,8 in mice and Vα24Jα18/Vβ11 in humans), type II NKT cells exhibit a more diverse T cell receptor repertoire and react to mammalian and microbial phospholipids and sulfatides (summarized in (135, 137-140). In addition to a TCR engagement NKT cells can be also activated by different cytokines such as IL-12, IL-18 and type I interferon (141-145). Those different pathways of activation have been described to shape the cytokine release of NKT cells in addition to their tissue localization, which can promote Th1, Th2 and/or Th17 responses (summarized in (146)). In addition to the agonist actions of the NKT cell antigens described so far, *Bacteroides fragilis* supplements the host's endogenous lipid antigen milieu with unique inhibitory sphingolipids, impedes iNKT cells proliferation and thus, confers protection against colitis (147).

Although type I NKT cells predominate in mice, about ten times less of these cells representing up to 3% of the total T lymphocyte population at a maximum (134) are recovered from the human tissues (148). In contrast, there are significantly more type II NKT cells detected in humans (149, 150), while those cells constitute for less than 5% of the total NKT cell population in mice (151, 152).

5. SUMMARY AND PERSPECTIVE

Lipids are involved in multiple processes regulating host microbe interactions. Those include the recognition of microbial lipids in various structural variations by a panel of immune receptors on myeloid and lymphoid cells and the regulation of anti-microbial defense mechanisms including phagocytosis and autophagy by lipids of the host. In addition, lipids are important constituents of cellular membranes and complex signaling networks in microbes and mammals. As lipids of microbial origin belong to the most potent activators of immune responses by the host, they and their receptors on the host side represent interesting targets for therapeutic intervention. Consequently, the analysis of the circuits of these host-microbe interactions and the underlying cellular and molecular mechanisms need to be carefully evaluated, to achieve an improved clinical outcome, but also to prevent side effects.

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Send correspondence to: Jochen Mattner, Mikrobiologisches Institut, Klinische Mikrobiologie, Immunologie und Hygiene, niversitatsklinikum Erlangen and Friedrich-Alexander Universitat (FAU) Erlangen-Nurnberg, Wasserturmstr. 3/5, D-91054 Erlangen, Germany, Tel: 49-9131-85-23640, Fax: 49-9131-85-1001, E-mail: jochen.mattner@uk-erlangen.de