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EDITORIAL

Antimicrobial lipids: Emerging effector molecules of innate host defense

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Abstract

The antimicrobial properties of host-derived derived lipids have become increasingly recognized and evidence is mounting that antimicrobial lipids (AMLs), like antimicrobial peptides, are effector molecules of the innate immune system and are regulated by its conserved pathways. This review, with primary focus on the human body, provides some background on the biochemistry of lipids, summarizes their biological functions, expands on their antimicrobial properties and site-specific composition, presents modes of synergism with antimicrobial peptides, and highlights the more recent reports on the regulation of AML production as well as bacterial resistance mechanisms. Based on extant data a concept of innate epithelial defense is proposed where epithelial cells, in response to microbial products and proinflammatory cytokines and through activation of conserved innate signaling pathways, increase their lipid uptake and up-regulate transcription of enzymes involved in antimicrobial lipid biosynthesis, and induce transcription of antimicrobial peptides as well as cytokines and chemokines. The subsequently secreted antimicrobial peptides and lipids then attack and eliminate the invader, assisted by or in synergism with other antimicrobial molecules delivered by other defense cells that have been recruited to the site of infection, in most of the cases. This review invites

reconsideration of the interpretation of cholesteryl ester accumulation in macrophage lipid droplets in response to infection as a solely proinflammatory event, and proposes a direct antimicrobial role of lipid droplet-associated cholesteryl esters. Finally, for the interested, but new- to- the-field investigator some starting points for the characterization of AMLs are provided. Before it is possible to utilize AMLs for anti-infectious therapeutic and prophylactic approaches, we need to better understand pathogen responses to these lipids and their role in the pathogenesis of chronic infectious disease.

Key words: Atopic dermatitis; Cholesterol; Infectious disease; Cystic fibrosis; Mucosa

Core tip: The antimicrobial properties of host-derived derived lipids have become increasingly recognized. This review develops the concept of antimicrobial lipids (AMLs) as effectors of the innate immune response that work together with antimicrobial peptides to prevent infection, and highlights more recent reports on the regulation of AML production as well as bacterial resistance mechanisms. Furthermore, this review invites reconsideration of the interpretation of cholesteryl ester accumulation in macrophage lipid droplets in response to infection as a solely proinflammatory event, and proposes a direct antimicrobial role of lipid droplet-associated cholesteryl esters.

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INNATE IMMUNITY

Innate immunity is the first line of host defense; it engages pattern recognition receptors as opposed to highly variable antigen specific receptors utilized by the adaptive immune system; its response is preformed or rapidly induced within minutes to hours after pathogen contact; it provides no memory, but is essential for priming the adaptive immune response; and in return it can be augmented by effectors of the adaptive immune response[1,2]. The innate immune response is activated by microbial products and proinflammatory cytokines when general physical and chemical defense mechanisms on body surfaces have failed to eliminate potential intruders. Ligand-binding to surface-expressed and intracellular pattern recognition and cytokine receptors leads to increased output of antimicrobial effector molecules, chemokines, and cytokines to attack the pathogen, recruit, and activate additional immune cells, respectively. The associated signaling pathways are conserved and utilize common central transcription factors including nuclear factor κB and interferon response factors.

Key effector cells of the innate immune response are epithelial cells, granulocytes, monocytes, macrophages, dendritic cells, and natural killer cells. In particular, macrophages and dendritic cells are important for the initiation of the adaptive immune response. In addition, the more recently recognized innate lymphocytes facilitate the cross talk between innate and adaptive immune responses[3]. Key effector molecules with direct antimicrobial action include the complement system, antimicrobial peptides and proteins (AMPs), and, increasingly recognized, antimicrobial lipids (AMLs). This review aims to introduce the concept of lipids as antimicrobial effector molecules in the innate epithelial cell defense. The reader is directed to Thormar and Hilmarsson 2007[4], Drake *et al*[5], 2008, and Thormar[6] 2012 for more extensive previous reviews on antimicrobial properties of lipids.

BIOCHEMICAL CHARACTERISTICS OF LIPIDS

Lipids are a widely heterogeneous group of molecules that share hydrophobic or mixed hydrophobic/hydrophilic properties. They are composed of hydrocarbon chains to which additional functional groups are linked which affects the degree of hydrophobicity. The major lipid classes are: fatty acids, tri-, di- and mono-acylglycerols consisting of the alcohol glycerol and fatty acid chains, cholesterol and cholesteryl esters, phospholipids and sphingolipids. Mostly, fatty acids, acyl chains with a carboxy group, are incorporated into more complex lipids. For example, sphingolipids like sphingosines consist of a fatty acid residue linked to an amino alcohol and cholesteryl esters are formed through esterification of a fatty acid to cholesterol. Phospholipids typically consist of a glycerol with two fatty acid residues attached, a phosphate group and varying additional groups such as choline, an alcohol, or amines. Phosphosphingolipids such as sphingomyelin use sphingosine instead of the diglyceride. Free fatty acids (FFA) are less abundant in the body, and among them palmitic, stearic, oleic, linoleic (the latter three differing in the number of double bonds) and docosahexaenoic acid are possibly the most important in the current context. Linoleic acid and its metabolite arachidonic acid are essential and cannot be synthesized by humans. Otherwise, our body generates all other fatty acids by two-carbon chain additions to acetyl-coenzyme A (CoA). For more detailed information on their classification refer to Fahy *et al*[7] and Christie and Xianlin[8].

Though lipid biosynthesis is quantitatively most active in hepatocytes and adipose tissue, every nucleated cell is capable of it. Figure 1 gives an overview of lipid biosynthesis as it relates to the production of AMLs and earmarks the enzymes for which evidence of regulation by innate immune pathways is available.

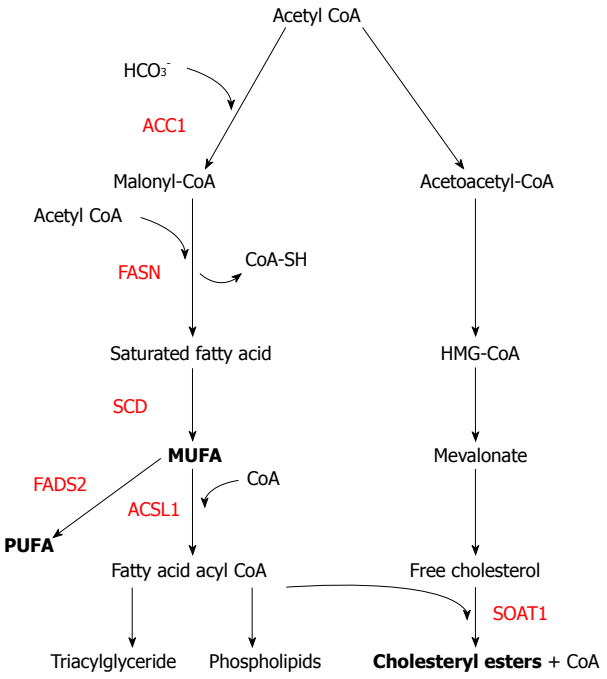

Figure 1 Simplified lipid biosynthesis pathway highlighting the lipids and the enzymes with a putative role in innate immunity. Lipid classes with documented antibacterial activity are in bold, key enzymes that may be induced in response to infection and inflammation (*homo sapiens* nomenclature) are in red. MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; ACC1: Acetyl-CoA carboxylase 1; FASN: Fatty acid synthase; SCD: Stearoyl-CoA desaturase-1; ACSL1: Acyl-CoA synthetase long-chain family member 1; FADS2: Fatty acid desaturase 2; SOAT1: Sterol O-acyltransferase 1 (SOAT1, also known as acyl-Coenzyme A: Cholesterol acyltransferase 1 or ACAT 1); HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA.

The initial and committed step in the fatty acid synthesis pathway is mediated by acetyl-CoA carboxylase 1 that catalyzes the addition of CO_2 to the methyl group of acetyl CoA generating malonyl-CoA. Malonyl-CoA serves as the donor of two carbon acetyl groups during each round of the fatty acid synthesis reaction cycle. Fatty acid synthase is a multifunctional enzyme that catalyzes seven different reactions where two carbon units from malonyl-CoA are linked together ultimately resulting in the formation of saturated fatty acids. Terminal desaturases then generate unsaturated fatty acids. Stearoyl-CoA desaturase also known as delta-9-desaturase catalyzes the synthesis of monounsaturated fatty acids (MUFAs). Biosynthesis of MUFAs occurs through the introduction of the first cis double bond in the Δ9 position between carbons 9 and 10. Fatty acid desaturase 2, encoded by FADS2 and also known as delta-6 desaturase, is required for the synthesis of polyunsaturated fatty acids (PUFAs). FADS2 is classified as a front-end desaturase because it introduces a double bond between the pre-existing double bond and the carboxyl end of the fatty acid. Long-chain-fatty-acid-CoA ligase 1 is encoded by ACSL1 and converts free long-chain fatty acids into fatty acyl-CoA esters. Acyl-CoA synthetases activate free long-chain fatty acids by converting them into fatty acyl-CoA esters. Fatty acyl-CoA esters are substrates for multiple fatty acid metabolic pathways, including mitochondrial β-oxidation and phospholipid and triacylglycerol synthesis. Sterol O-acyltransferase 1 (SOAT1, also known as acyl-Coenzyme A: cholesterol acyltransferase 1 or cholesterol acyltransferase 1), catalyzes the esterification of fatty acids to cholesterol. An ester bond is formed between the carboxylate group of a fatty acid and the hydroxyl group of cholesterol. *De novo* synthesis of free cholesterol *via* the mevalonate pathway also begins with acetyl CoA. Acetyl-CoA undergoes condensation with another acetyl-CoA subunit *via* acetyl-CoA transferase to form acetoacetyl-CoA. Acetyl-CoA condenses with acetoacetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA is reduced to mevalonate with consumption of NADPH, and after sequential reactions producing the intermediates mevalonate-P, isopentenyl-PP, farnesyl-PP, squalene, lanosterol, and 7-dehydrocholesterol, free cholesterol has been generated.

BIOLOGICAL FUNCTIONS OF LIPIDS

Lipids are used as a form of energy storage, are precursors for steroid hormones[9], and have important structural functions. Cell membranes are composed of a phospholipid bilayer and transmembrane receptor signaling is dependent on the specific lipid composition of the cell membrane in the vicinity of these receptors. These specialized regions are referred to as lipid rafts and caveolae[10-12]. There are substantial differences in the phospholipid composition of bacterial and mammalian cell membranes, likely contributing to the preferential action of host defense molecules against bacterial targets[13-15]. Furthermore, lipids liberated from cellular membranes have been found to be strong modulators of inflammation. Initially, they were identified as strong proinflammatory second messengers such as prostaglandins and leukotrienes which are synthesized from arachidonic acid. However, in the last decade an important down regulatory role of membrane-derived lipids has been discovered. These inflammation resolving lipids are derivatives of the essential omega-6 and omega-3 PUFAs and include resolvins (coined after their inflammation resolving function), lipoxins, protectins and maresins[16-18]. Moreover, there is new evidence that lipids may also trigger increased antimicrobial peptide production as shown for the sphingolipid S1P which increased CAMP production[19], or for sebum FFA which induced beta-defensin production[18]. However, lipids can also exert direct antimicrobial activity, which is not only supported by *in vitro* testing but also by the association of some infectious diseases with defects in lipid metabolism.

CLINICAL CORRELATIONS BETWEEN LIPID ALTERATIONS AND INFECTIONS

Several chronic infectious diseases are associated with altered lipid composition in skin and in the airways.

For example, Arikawa *et al*[20] reported reduced sphingosine levels in keratinocytes in patients with atopic dermatitis and recurrent *Staphylococcus aureus* (*S. aureus*) skin infections. In the stratum corneum of lamellar ichthyosis patients who are at higher risk of contracting chronic dermatophytosis[21], the amount of FFA is reduced and the ceramide profile is altered[22,23]. In cystic fibrosis, patients suffer from chronic lung infections with *S. aureus*, *Burkholderia cepacia* complex, *Stenotrophomonas maltophilia*, and, most importantly, *Pseudomonas aeruginosa*[24-27]. In these patients, altered fatty acid levels including reduced levels of docosahexaenoic acid[28] have been described and docosahexaenoic acid supplementation improved the clinical status in some studies[29]. Other lipid anomalies in cystic fibrosis are altered cholesterol homeostasis[30], and elevated cholesteryl ester concentrations in tracheobronchial secretions[31]. We have found an increased cholesteryl ester representation in the lipid content of bronchoalveolar lavages obtained from pediatric cystic fibrosis patients[32]. Furthermore, elevated cholesteryl linoleate levels were found in sinus washes in chronic rhinosinusitis[33].

BODY SITES AND FLUIDS WITH AMLS

Lipids have been well characterized in all body surfaces and tissues whereby extraction and identification method influences the outcome and caution should be applied when comparing results from different studies. Recognition of the antimicrobial activity of certain lipids and improved analytical instrumentation have invited additional surveys many of which are compiled in Thormar[6] 2011. Analysis of the lipid composition of the intestinal tract is complicated by nutritional lipids and lipids synthesized by the normal endemic microbiota and thus, is not considered in the present review.

Breast milk and vernix caseosa

Breast milk was one of the first human body fluids investigated for its lipid content. Thormar *et al*[34] reported in 1987 that FFA and monoglycerides in milk exhibit antiviral activity. It appears that milk lipases release the bioactive lipids from more complex lipids. This work was subsequently extended to include activity against various bacteria and protozoa[35]. Unique to the newborn is vernix caseosa, the waxy coat formed during the last trimester of pregnancy that covers the new born infant. This lipid-rich film is primarily derived from the stratum corneum and sebaceous glands of the fetal skin. Ten percent of its content is represented by lipids, with a relative abundance of nonpolar species such as wax esters/sterol esters/squalene, and triacylglycerol. Other vernix caseosa lipids include FFA, fatty alcohols, cholesterol, diacylglycerol, monoacylglycerol, and phospholipids[36-39]. Antibacterial activity of total lipid extract was observed against the test strain *Bacillus megaterium* and was attributed to FFA.

Skin

Skin lipids (sebum) are from secretions by sebaceous glands and the stratum corneum, their composition is in part further shaped by the metabolic activities of the normal microbiota[4,40] and the exogenous application of lotions and cosmetics. Employing a combined LC/MS approach Camera *et al*[41] identified 95 triacylglycerols, 25 diacylglycerols, numerous wax esters and squalenes, a total of 9 cholesterol esters, and more than 48 FFA in sebum. Antimicrobial activity has been attributed to fatty alcohols, monoglycerides, sphingolipids including D-sphingosine, phospholipids, and in particular FFA such as sapienic acid and lauric acid[42,43].

Meibum

Very long chain wax esters and fatty acids have been of identified in meibum, the lipid rich component of tears[44]. Lipids in tear fluid reach micromolar concentrations and the most abundant species are phosphatidylcholine and phosphatidylethanolamine. Additional lipid classes are triglycerides, sphingosine and ceramides, as well as cholesteryl esters[45]. While a lubricant function has been primarily attributed to tear lipids, a recent study suggested growth inhibitory activity of whole tear lipid extracts against several Gram-positive and Gram-negative bacteria[46].

Oral mucosa

Sphingosine, sapienic and lauric acid have also been identified as key antimicrobial fatty acids in the oral mucosa[47]. Brasser *et al*[48] analyzed salvia from healthy adults and identified FFA, cholesterol, cholesterol esters, triglycerides, wax esters, and squalene. The neutral lipid concentration was determined to be in the low μg/mL range. Overall, FFA, triglycerides, and cholesteryl esters were the most abundant lipids in saliva.

Airways

In the airway lumen, surfactant is the main lipid source ascending from the alveolar space, its primary site of production, to the upper airways, where some local production also occurs[49]. Phospholipids comprise the majority of the lipids in surfactant, a lipoprotein complex[50], and are thought to mainly contribute to reducing lung surface tension and participate in a downregulation of immune responses. The antimicrobial properties of surfactant have been mainly attributed to surfactant proteins SP-A and SP-D[51,52]. Nasal fluid is rich in lipids with all major classes represented, namely FFA, phospholipids, triglycerides, cholesterol, and cholesteryl esters, and their origin can be at least in part attributed to epithelial cell secretions[53]. Selective removal of the non-polar portion of lipids resulted in a decreased inherent antibacterial activity against *P. aeruginosa* that was restored after supplementation with the extracted lipids. This suggests that lipids in nasal fluid contribute to the innate antimicrobial defense in the airways[53].

Urogenital tract

Information on the lipid composition of fluids of the urogenital tract is scarce. Urine contains predominantly phospholipids including glycerophospholipids, phosphatidylcholine, phosphatidyl serine and sphingomyelin, as well as triglycerides, but cholesterol and cholesteryl esters are also present[54,55]. Semen lipids include sphingomyelin, glycerophospholipids, and cholesterol[56,57]. A very recent metabolomics study on bacterial vaginosis suggested elevated eicosanoid levels in affected women but this study was designed to identify differentially represented metabolites in diseases patients and did not aim to provide a baseline lipid profile of healthy women[58]. To the best of our knowledge, information on the antimicrobial activity of lipids of the urogenital tract is not available.

SPECTRUM OF ACTIVITY OF AMLS

Among human lipids, fatty acids are the best characterized as antimicrobial agents, and their spectrum of activity as a whole is broad and spans from bacteria and viruses to fungi and protozoa[6]. Other human lipids with antimicrobial properties include sphingoid bases[43], that are active against Gram positive and Gram negative bacteria. Cholesteryl esters have long been thought to serve only as a storage and transport form for either cholesterol or FFA. However, cholesteryl linoleate and cholesteryl arachidonate, when formulated in liposomes, demonstrated growth inhibitory activity against several Gram positive and Gram negative bacteria[53].

MECHANISMS OF ANTIMICROBIAL ACTION

Influenced by the three dimensional shape and saturation status of the acyl chains AMLs exert their action in different ways. These include disruption caused by interference with the cell membrane with ensuing permeability changes or interference with the activity of membrane bound enzyme complexes and events following lipid peroxidation with radical formation. FFA have been substantially investigated in this respect, and a detailed review on this subject has been authored by Desbois and Smith[59]. More recent studies describe rapid membrane depolarization in *S. aureus* treated with palmitoleate as well as when treated with glycerol ethers, sphingosine, and acyl-amines[60]. As demonstrated by scanning electron microscopy, meibomian lipids from tears cause major structural damage including distortion, loss or regular cell shape, and cell lysis in *S. aureus, P. aeruginosa,* and *Serratia marcescens*[46].

The more pronounced antimicrobial activity of unsaturated FFA compared to their saturated counterparts[61] may be at least in part attributed to lipid peroxidation. Spontaneous generation of a lipid radical at the unsaturated bond leads, under consumption of molecular oxygen, to the production of a lipid peroxyl radical that can react with nearby fatty acids leading to a lipid peroxidation chain reaction. Eventually, these radicals covalently modify adjacent macromolecules[62].

In addition, anti-adhesive effects of lipids have been reported. Milk fat globules from bovine and goat milk reduced attachment of *Salmonella* Enteritidis to HT-29 human adenocarcinoma cells and subsequent internalization[63]. Another more recently described effect of AMLs is inhibition of biofilm production. For example the milk monoglyceride monolaurin (also called lauricidin[64]) inhibits biofilm mass produced by Gram positive bacteria including *Streptococcus mutans* and *S. aureus*[65,66].

SYNERGISM WITH AMPS

Antimicrobial peptides are characterized by an amphipathic structure with cationic and hydrophobic domains and are typically less than 10 kDa in size. Antimicrobial proteins have similar amphipathic domains but are larger and typically consist of additional regions with unique functions, such as lysozyme that hydrolyzes peptidoglycan and lactoferrin that binds iron. AMPs share many of the mechanisms described for AMLs, in particular membrane disruption, and there are several studies documenting synergist activities between these two classes of antimicrobials. Tollin *et al*[38] reported synergistic activity between vernix caseosa lipids and the antimicrobial peptide LL37 whereby this effect was attributed to FFA in vernix. We found synergistic effects between nasal fluid lipid extracts and the antimicrobial peptide human neutrophil peptide HNP1[53], and between the free fatty acid docosahexaenoic acid and lysozyme[67]. The latter study demonstrated that in the presence of lysozyme, docosahexaenoic acid accumulates in the bacterial cell membrane. Nakatsuji *et al*[68] demonstrated synergistic effects between the free fatty acid lauric acid and the antimicrobial peptide HBD2 against *Propionibacterium acnes.* This study also showed that several sebum FFA up-regulate antimicrobial peptide production in sebocytes.

A different type of protein-lipid synergism has been described for human α-lactalbumin made lethal to tumor cells (HAMLET) from human milk, primarily known for its anti-tumor effects[69]. When complexed with oleic acid HAMLET exerts bactericidal effects against *S. pneumoniae via* calcium dependent membrane depolarization[70,71]. Furthermore, acetylation of cationic peptides has been shown to impart antimicrobial activity or increase their antimicrobial activity[72].

REGULATION OF AML PRODUCTION

Reports on lipid profile changes in sepsis[73,74] have suggested that AML production may be regulated in the context of infection that would involve TLR and other pattern recognition receptor signaling and signaling induced by proinflammatory cytokines like IL1β. Important evidence

for the regulation of AMLs by conserved pathways of innate immunity was provided by Georgel *et al*[75] investigating the regulation of stearoyl-CoA desaturase gene expression (*scd1* in mice and *scd* in humans), a rate limiting enzyme for the synthesis of monosaturated fatty acids. They found that the *scd1* gene has numerous NFκB elements in its promoter region and is strongly and specifically induced by TLR2 signaling and that *scd* expression is also induced by TLR2 signaling in a human sebocyte cell line. Furthermore, *scd1*$^{-/-}$ mice developed chronic skin infections.

Using a contrary approach, Wang *et al*[76] have recently shown that overexpression of fatty acid desaturases increases resistance to infection in zebrafish. Other findings that suggest that lipids are regulated by infection and inflammation include the activation of genes important for lipid synthesis in caseation of human tuberculosis granuloma[77].

SOAT1 is essential for cholesteryl ester synthesis and we have shown that non-polar lipids overall and specifically cholesteryl linoleate are elevated in sinus washes obtained from patients with chronic rhinosinusitis[33]. This data suggested an up-regulation of SOAT1 in the context of inflammation which was corroborated by a subsequent study showing increased SOAT1 mRNA expression in sinus mucosa of patients with chronic rhinosinusitis[78]. In addition, cholesteryl esters were increased within the lipid fraction and their concentrations correlated with human neutrophil peptides HNP1-3, markers of inflammation, in bronchoalveolar lavage collected from pediatric cystic fibrosis patients[32]. Direct evidence for the regulation of SOAT1 by inflammation was recently provided by Yin *et al*[79], who showed that oxLDL activates TLR4 and induces the expression of SOAT1 (referred to as ACAT-1) *via* MyD88 and NFκB. Thus, there is clinical and experimental evidence that *in vivo* cholesteryl ester biosynthesis is regulated by inflammation and infection. Additional data supporting the regulation of AMLs by TLR ligands and immunomodulatory cytokines can be found in the NCBI Gene Expression Omnibus (GEO Profiles) data base. Table 1 lists genes involved in lipid metabolism and transport which are regulated by TLR ligands and modulators of the immune system.

Other investigations propose that cholesterol and cholesteryl ester accumulation in response to inflammatory cytokines and infection serve perpetuation of inflammation. For example, Pessolano *et al*[80] described that IL1β increased cholesteryl ester accumulation in smooth muscle cells as part of cholesterol trafficking in atherosclerosis. Similarly, Tall and Yvan-Charvet[81] highlight the proinflammatory effects of increased cholesterol uptake through TLR signaling and inflammasome activation in macrophages. However, considering the direct antimicrobial activity of cholesteryl esters these studies could be revisited to investigate changes in the antimicrobial responses.

TRANSPORTERS OF AMLS

Bearing in mind the hydrophobic nature of AMLs and the aqueous milieu in body fluids, proteins with both hydrophilic domains and hydrophobic pockets likely serve as carriers. Albumin and fatty acid binding proteins are well established carriers for fatty acids. Sterol carrier protein 2 and cholesteryl ester transfer protein assume this role for cholesterol and cholesteryl esters, respectively[82]. In addition, in the airways, the highly hydrophobic protein short palate lung epithelial clone protein 1 binds certain phospholipids and sphingolipids[83,84] and may possibly also function as a cholesteryl ester carrier. However, much research is still needed to dissect the focused delivery of AMLs to the microbial target.

BACTERIAL MECHANISMS THAT MANIPULATE HOST-DERIVED LIPIDS

Host defense mechanisms are continuously challenged by microbial resistance factors and it would be surprising if successful pathogens do not have counter strategies that inactivate AMLs. Both, *S. aureus* and *S. saprophyticus* express a cell wall associated surface protein, SsaF and SssF, respectively, that mediates resistance to the free fatty acid linoleic acid[85,86]. Furthermore, cell wall teichoic acids of *S. aureus* confer resistance to fatty acids from skin sebaceous glands[87].

At this time it is still speculative whether a cholesterol esterase produced by *P. aeruginosa*[88] may represent an additional virulence factor aiding in the inactivation of host-derived antimicrobial cholesteryl esters. Of interest is the recent finding of Cadieux *et al*[89] who identified a lipase in a hypervirulent community-associated methicillin-resistant *S. aureus* strain USA300 that hydrolyzes triglycerides and liberates the free fatty acid linoleic acid with growth inhibitory activity against *S. aureus.* It is possible that the liberation of antibacterial linoleic acid is primarily targeted against other bacteria thereby conferring growth advantage to *S. aureus*. Such a mechanism has been proposed for *Salmonella* where the bacteria induce the production of antimicrobial proteins in the intestine that in turn altered the normal microbiota facilitating infection with the pathogen[90].

Successful pathogens subvert host defense mechanisms that normally control infection. Thus, the ability of *Mycobacterium tuberculosis*, *M. leprae* and other intracellular pathogens to import lipids from the cholesteryl ester-rich lipid droplets that they induce in their host cell[91,92] may be an example for subversion of antimicrobial cholesteryl ester accumulation as part of the innate defense.

AMLS AS EFFECTOR MOLECULES OF EPITHELIAL INNATE DEFENSE

Based on the evidence laid out above, we propose that AMLs take part in the innate epithelial defense controlled by regulatory pathways like antimicrobial proteins and functioning in synergism with AMPs (Figure

Table 1 Genes involved in lipid metabolism and transport regulated by innate immune pathways

Role	Gene name	Encoded protein	Function of the encoded protein	Cellular source	Regulators
Biosynthesis	*acc1*	Acetyl-CoA carboxylase 1	Catalyzes the rate limiting irreversible carboxylation of acetyl-CoA to produce malonyl-CoA	Hepatic tissue[1]	LPS *via* sterol regulatory element-binding protein-1c
	acsl1	Long-chain fatty-acid-coenzyme A ligase	Converts free long-chain fatty acids into fatty acyl-CoA esters	Mp, DC, EN, Mo	LPS, IFN-γ, TNF-α, IL22, Mtb-derived lipopeptide
	elov	Elongation of long chain fatty acids	Possibly implicated in tissue-specific synthesis of very long chain fatty acids and sphingolipids[3]	Mp, DC, CD34+, TE,B, F, EN	LPS, Zy, Schi, IL1, IFN-β, IFN-γ, IL10, TGF-β
	fad	Fatty acid desaturase	Catalyzes biosynthesis of highly unsaturated fatty acids. FADS2 catalyzes production of the mono-unsaturated fatty acid sapienate, the most abundant fatty acid in sebum	Mp,DC, CD34+, TE, B, EN	LPS, Zy, Schi, IL1, IFN-γ, IL10, TGF-β
	fasn	Fatty acid synthase	Catalyzes the formation of long-chain fatty acids from acetyl-CoA, malonyl-CoA and NADPH	Mp, DC, CD34+, TE, F, EN	LPS, Zy, Schi, IL1, IFN-γ, TGF-β
	lcat	Lecithin cholesterol acyltransferase[2]	Esterifies free cholesterol transported in plasma lipoproteins. Activated by apolipoprotein A-I	Mp, DC, CD8+ DC, B, F	LPS, Schi, IFN-β, IFN-γ, *Yersinia* + IFN-γ, Vit D3 + IFN-γ, IL10
	lipA	Lipase A[3]	Intracellular hydrolysis of internalized cholesteryl esters and triglycerides. Activation of endogenous cellular cholesteryl ester formation	Mo, Mp, DC, TE, EN, K, BrE, L, Mg	TLR agonists, IL1, Type Ⅰ and Ⅱ IFNs, γ, Diff/Polar
	scd[4]	Stearoyl-CoA desaturase	Catalyzes the desaturation of very long chain acyl-CoAs	Mo, Mp, L, CD8+ DC, TE, F, EN, K, BrE, Mg	LPS, Zy, TLR agonist, IL1, Type Ⅰ and Ⅱ IFNs, *Yersinia* + IFN-γ, Vit D3 + IFN-γ, Diff/Polar
	soat1[5]	Sterol o-acyltransferase[3]	Catalyzes the formation of fatty acid-cholesterol esters	Mo, Mp, DC, TE, EN, L, Mg	TLR agonists, Type Ⅰ and Ⅱ IFNs, IL1, Diff/Polar
Transport	*cetp*	Cholesteryl ester transfer protein[3]	Involved in the transfer of insoluble cholesteryl esters in the reverse transport of cholesterol	Mo, Mp, DC, TE, EN, K, L, Mg	TLR agonists, IL1,Type Ⅰ and Ⅱ IFNs, Diff/Polar
	fabp	Fatty acid binding proteins	Intracellular lipid transport	Mp, DC, CD34+, TE, B, F, EN	LPS, Zy, Schi, IL1, Type Ⅰ and Ⅱ IFNs, IL10, TGF-β
	ffar	Free fatty acid receptor	Receptor for short chain fatty acids (FFAR2) and medium to long fatty acids (FFAR1). FFAR2 is expressed at relatively high levels in peripheral blood leukocytes	Mp, DC, CD34+,TE, EN	LPS, Zy, Schi, IL1, IFN-γ, TGF-β
	slc27A	Solute carrier family 27	Translocation of long-chain fatty acids across the plasma membrane. Some involved in bile acid synthesis	Mp, DC, CD34+, TE, B, F, EN	LPS, Zy, Schi, IL1, IFN-γ, IFN-β, IL10, TGF-β

[1]Chen *et al*, J Pineal Res 2011 Nov; 51: 416-25 DOI: 10.1111/j.1600-079X.2011.00905.x; [2]Profiles for mouse only; [3]Profiles for human only; [4]*scd1* in mice; [5]Also known as *acat1* (acyl-Coenzyme A: Cholesterol acyltransferase 1). Data were extracted from NCBI Gene Expression Omnibus (GEO Profiles) and Swiss-Prot (http://www.uniprot.org/). Unless specified otherwise entries were for both mouse and human species. Mo: Monocytes; Mp: Macrophages; DC: Dendritic cells; TE: Thyroid epithelial cells; EN: Endothelial cells; L: Lung epithelial cells; Mg: Microglia; F: Fibroblasts; K: Keratinocytes; BrE: Bronchial epithelial cells; B: B-cells; Dex: Dexamethasone; Diff/Polar: Differentiation and polarization; IFN: Interferon; IL: Interleukin; LPS: Lipopolysaccharide; TGF-β: Transforming growth factor β; TNF-α: Tumor necrosis factor α; VitD3: Vitamin D3; Zy: Zymogen; Schi: *Schistosoma* antigen; Mtb: *Mycobacterium tuberculosis*.

2). Following activation of pattern recognition receptors and cytokine receptors, epithelial cells upsurge the uptake of cholesterol and fatty acids, increase the expression of antimicrobial peptides and enzymes for lipid biosynthesis, scale up the production and secretion of AMLs and antimicrobial peptides, and, combined with antimicrobial effectors from other sources such as macrophages, lead to membrane damage and other disrupting effects on the invading pathogen.

FUTURE DIRECTIONS

The recognition that host-derived lipids form part of the innate antimicrobial defense leads to new questions including the following: What are other microbial targets beyond bacteria and viruses? How are AMLs delivered to pathogens? Do carrier proteins assume this task or do exosomes serve this purpose? Can AMLs be incorporated in novel drug design? Is resistance to AMLs a pathogenicity factor that could be targeted in the management of infectious diseases? Are certain chronic and recurrent infectious diseases linked to defective AML production and/or delivery? Can the lipid mediated arm of host defense be integrated in novel vaccine strategies?

HOW TO WORK WITH LIPIDS?

Commercial tools to study AMLs are relatively under-developed compared to the extensive repertoire for proteomics and genomics. An essential technique for qualitative analysis and the ability to assess a

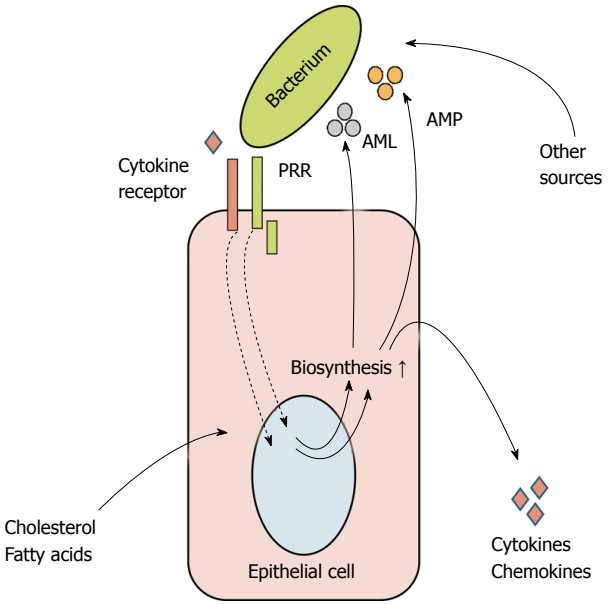

Figure 2 Working model of epithelial cell mediated innate defense. In response to microbial products and cytokines epithelial cells increase the production and secretion of antimicrobial lipids and antimicrobial proteins as well as cytokines and chemokine to eradicate infection in concert with other defense components of the body. PRR: Pattern recognition receptor for microbial products; AML: Antimicrobial lipids; AMP: Antimicrobial proteins. Other sources: Other defense cells recruited to the site of infections such as macrophages and neutrophils.

wide range of lipid classes is separation by thin layer chromatography with colorimetric visualization with a variety of reagents. Reversed phase high performance liquid chromatography with evaporative light scattering detection allows for more quantitative studies. Definitive and highly quantitative analysis is achieved with mass spectral analysis usually combined with gas chromatography or liquid chromatography. There are several web sites (accurate at the time of printing) that offer extensive hands-on information regarding lipid handling and analysis. These include the Cyberlipid Center (http://www.cyberlipid.org/), The American Oil Chemists' Society Lipid Library (http://lipidlibrary.aocs.org/), and the Lipidomics Gateway (http://www.lipidmaps.org/). Furthermore, some lipid manufacturers offer a wealth of technical support. Those who would like to take on the challenge of lipidomics will fare well by identifying a collaborator with a background in biochemistry and expertise in mass spectrometry and metabolomics.

While lipid extraction protocols are well established with one of the most frequently used one dating back to Bligh and Dyer[93], a major hurdle in investigating functional properties of AMLs, in particular nonpolar lipids like cholesteryl esters, is their low solubility in aqueous media used for antimicrobial activity testing. For FFA addition of low concentration of ethanol such as 0.05% allows for solubilization. However, for less polar and non-polar lipids embedding of the lipid of interest in liposomes prepared from various phospholipids has been proven successful for *in vitro* studies[6,94,95].

CONCLUSION

AMLs as effectors of the innate immune response and microbial counter strategies are an emerging field of study. New investigators are invited to enter the field to uncover the regulation of AML production, their delivery to pathogens and mechanism of action. We hope that this review has piqued the interest and will usher new investigators to this challenging and growing field.

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