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**Antimicrobial lipids: Emerging effector molecules of innate host defense**

Porter E *et al*. Lipids to the defense

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**Abstract**

The antimicrobial properties of host derived lipids have become increasingly recognized and evidence is mounting that antimicrobial lipids, 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 antimicrobial lipid 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 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 antimicrobial lipids are provided. Before it is possible to utilize antimicrobial lipids 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; Cystic fibrosis; Infectious disease; Mucosa

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**Core tip:** The antimicrobial properties of host derived lipids have become increasingly recognized. This review develops the concept of antimicrobial lipids 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 antimicrobial lipid 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 ĸB (NFĸB) and interferon response factors (IRFs).

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 groups and varying additional groups such as choline, an alcohol, or amines. Phosphosphingolipids such as sphingomyelin use sphingosine instead of the diglyceride. Free fatty acids 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 CoA. For more detailed information on their classification refer to Fahy and colleagues[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 antimicrobial lipids and earmarks the enzymes for which evidence of regulation by innate immune pathways is available.

The initial and committed step in the fatty acid synthesis pathway is mediated by acetyl-CoA carboxylase 1 (ACC1) that catalyzes the addition of CO2 to themethyl 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 (FASN) 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 (SCD) also known as delta-9-desaturase catalyzes the synthesis of monounsaturated fatty acids (MUFAs). Biosynthesis of monounsaturated fatty acids occurs through the introduction of the first cis double bond in the 9 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 (ACSL) activate free long-chain fatty acids by converting them into fatty acyl-coenzyme A (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 ACAT1), 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 polyunsaturated fatty acids 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 free fatty acids 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* skin infections. In the stratum corneum of lamellar ichthyosis patients who are at higher risk of contracting chronic dermatophytosis[21], the amount of free fatty acids 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 antimicrobial lipids**

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 this 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 free fatty acids 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 free fatty acids, 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 free fatty acids.

***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 (TAG), 25 diacylglycerols (DAG), numerous wax esters and squalenes, a total of 9 cholesterol esters, and more than 48 free fatty acids (FFA) in sebum. Antimicrobial activity has been attributed to fatty alcohols, monoglycerides, sphingolipids including D-sphingosine, phospholipids, and in particular free fatty acids 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, sphingosin 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***

Sphingosin, 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 free fatty acids, cholesterol, cholesterol esters, triglycerides, wax esters and squalene. The neutral lipid concentration was determined to be in the low μg/mL range. Overall, free fatty acids, 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 free fatty acids, 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 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 antimicrobial lipids**

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 free fatty acids. 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 antimicrobial lipids 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. Free fatty acids have been substantially investigated in this respect, and a detailed review on this subject has been authored by Debois 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 free fatty acids 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 antimicrobial lipids 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 antimicrobial peptides and proteins**

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. Antimicrobial peptides and proteins share many of the mechanisms described for antimicrobial lipids, 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 free fatty acids 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 free fatty acids 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 antimicrobial lipid production**

Reports on lipid profile changes in sepsis[73,74] have suggested that antimicrobial lipid 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 antimicrobial lipids by conserved pathways of innate immunity was provided by Georgel and colleagues[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 NFB 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 and colleagues have recently shown that overexpression of fatty acid desaturases increases resistance to infection in zebrafish[76]. 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 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]. A direct evidence for the regulation of SOAT1 by inflammation was recently provided by Yin and colleagues[79], who showed that oxLDL activates TLR4 and induces the expression of SOAT1 (referred to as ACAT-1 ) *via* MyD88 and NFB. 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 antimicrobial lipids 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 antimicrobial lipids**

Bearing in mind the hydrophobic nature of antimicrobial lipids 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 (CETP) 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 (SPLUNC-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 antimicrobial lipids 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 antimicrobial lipids. 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 and colleagues 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*[89]*.* 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* andother 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.

**Antimicrobial lipids as effector molecules of epithelial innate defense**

Based on the evidence laid out above, we propose that antimicrobial lipids take part in the innate epithelial defense controlled by regulatory pathways like antimicrobial proteins and functioning in synergism (Figure 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 antimicrobial lipids 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 antimicrobial lipids delivered to pathogens? Do carrier proteins assume this task or do exosomes serve this purpose? Can antimicrobial lipids be incorporated in novel drug design? Is resistance to antimicrobial lipids a pathogenicity factor that could be targeted in the management of infectious diseases? Are certain chronic and recurrent infectious diseases linked to defective antimicrobial lipid production and/or delivery? Can the lipid mediated arm of host defense be integrated in novel vaccine strategies?

**How to work with lipids?**

Tools to study antimicrobial lipids are relatively underdeveloped compared to the extensive repertoire for proteomics and genomics. An essential technique for qualitative analysis and the ability to assess a 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 antimicrobial lipids, in particular nonpolar lipids like cholesteryl esters, is their low solubility in aqueous media used for antimicrobial activity testing. For free fatty acids 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**

Antimicrobial lipids 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 antimicrobial lipid 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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**REFERENCES**

1 **Parker D**, Prince A. Innate immunity in the respiratory epithelium. *Am J Respir Cell Mol Biol* 2011; **45**: 189-201 [PMID: 21330463 DOI: 10.1165/rcmb.2011-0011RT]

2 **Tossi A**. Host defense peptides: roles and applications. *Curr Protein Pept Sci* 2005; **6**: 1-3 [PMID: 15638764]

3 **Annunziato F**, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. *J Allergy Clin Immunol* 2015; **135**: 626-635 [PMID: 25528359 DOI: 10.1016/j.jaci.2014.11.001]

4 **Thormar H**, Hilmarsson H. The role of microbicidal lipids in host defense against pathogens and their potential as therapeutic agents. *Chem Phys Lipids* 2007; **150**: 1-11 [PMID: 17686469 DOI: 10.1016/j.chemphyslip.2007.06.220]

5 **Drake DR**, Brogden KA, Dawson DV, Wertz PW. Thematic review series: skin lipids. Antimicrobial lipids at the skin surface. *J Lipid Res* 2008; **49**: 4-11 [PMID: 17906220 DOI: 10.1194/jlr.R700016-JLR200]

6 Lipids and Essential Oils as Antimicrobial Agents (ed H. Thormar). Chichester, UK: John Wiley & Sons Ltd., 2011

7 **Fahy E**, Subramaniam S, Brown HA, Glass CK, Merrill AH, Murphy RC, Raetz CR, Russell DW, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze MS, White SH, Witztum JL, Dennis EA. A comprehensive classification system for lipids. *J Lipid Res* 2005; **46**: 839-861 [PMID: 15722563 DOI: 10.1194/jlr.E400004-JLR200]

8 **Christie WW**, Xianlin H. Lipid analysis. 4 ed. Oily Press, 2010

9 **He J**, Cheng Q, Xie W. Minireview: Nuclear receptor-controlled steroid hormone synthesis and metabolism. *Mol Endocrinol* 2010; **24**: 11-21 [PMID: 19762543 DOI: 10.1210/me.2009-0212]

10 **Simons K**, Sampaio JL. Membrane organization and lipid rafts. *Cold Spring Harb Perspect Biol* 2011; **3**: a004697 [PMID: 21628426 DOI: 10.1101/cshperspect.a004697]

11 **Sonnino S**, Prinetti A. Membrane domains and the "lipid raft" concept. *Curr Med Chem* 2013; **20**: 4-21 [PMID: 23150999]

12 **Reeves VL**, Thomas CM, Smart EJ. Lipid rafts, caveolae and GPI-linked proteins. *Adv Exp Med Biol* 2012; **729**: 3-13 [PMID: 22411310 DOI: 10.1007/978-1-4614-1222-9\_1]

13 **Shireen T**, Basu A, Sarkar M, Mukhopadhyay K. Lipid composition is an important determinant of antimicrobial activity of alpha-melanocyte stimulating hormone. *Biophys Chem* 2015; **196**: 33-39 [PMID: 25282663 DOI: 10.1016/j.bpc.2014.09.002]

14 **Glukhov E**, Stark M, Burrows LL, Deber CM. Basis for selectivity of cationic antimicrobial peptides for bacterial versus mammalian membranes. *J Biol Chem* 2005; **280**: 33960-33967 [PMID: 16043484 DOI: 10.1074/jbc.M507042200]

15 **Lohner K**. New strategies for novel antibiotics: peptides targeting bacterial cell membranes. *Gen Physiol Biophys* 2009; **28**: 105-116 [PMID: 19592707]

16 **Weylandt KH**, Kang JX, Wiedenmann B, Baumgart DC. Lipoxins and resolvins in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 797-799 [PMID: 17262807 DOI: 10.1002/ibd.20109]

17 **Serhan CN**. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* 2007; **25**: 101-137 [PMID: 17090225 DOI: 10.1146/annurev.immunol.25.022106.141647]

18 **Serhan CN**. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 2014; **510**: 92-101 [PMID: 24899309 DOI: 10.1038/nature13479]

19 **Park K**, Elias PM, Shin KO, Lee YM, Hupe M, Borkowski AW, Gallo RL, Saba J, Holleran WM, Uchida Y. A novel role of a lipid species, sphingosine-1-phosphate, in epithelial innate immunity. *Mol Cell Biol* 2013; **33**: 752-762 [PMID: 23230267 DOI: 10.1128/mcb.01103-12]

20 **Arikawa J**, Ishibashi M, Kawashima M, Takagi Y, Ichikawa Y, Imokawa G. Decreased levels of sphingosine, a natural antimicrobial agent, may be associated with vulnerability of the stratum corneum from patients with atopic dermatitis to colonization by Staphylococcus aureus. *J Invest Dermatol* 2002; **119**: 433-439 [PMID: 12190867 DOI: 10.1046/j.1523-1747.2002.01846.x]

21 **Scheers C**, Andre J, Thompson C, Rebuffat E, Harag S, Kolivras A. Refractory Trichophyton rubrum infection in lamellar ichthyosis. *Pediatr Dermatol* 2013; **30**: e200-e203 [PMID: 23679236 DOI: 10.1111/pde.12160]

22 **Bouwstra JA**, Ponec M. The skin barrier in healthy and diseased state. *Biochim Biophys Acta* 2006; **1758**: 2080-2095 [PMID: 16945325 DOI: 10.1016/j.bbamem.2006.06.021]

23 **Pilgram GS**, Vissers DC, van der Meulen H, Pavel S, Lavrijsen SP, Bouwstra JA, Koerten HK. Aberrant lipid organization in stratum corneum of patients with atopic dermatitis and lamellar ichthyosis. *J Invest Dermatol* 2001; **117**: 710-717 [PMID: 11564181 DOI: 10.1046/j.0022-202x.2001.01455.x]

24 **Conway SP**, Brownlee KG, Denton M, Peckham DG. Antibiotic treatment of multidrug-resistant organisms in cystic fibrosis. *Am J Respir Med* 2003; **2**: 321-332 [PMID: 14719998]

25 **Saiman L**, Siegel J. Infection control in cystic fibrosis. *Clin Microbiol Rev* 2004; **17**: 57-71 [PMID: 14726455]

26 **Gibson RL**, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003; **168**: 918-951 [PMID: 14555458 DOI: 10.1164/rccm.200304-505SO]

27 **Waters V**, Ratjen F. Multidrug-resistant organisms in cystic fibrosis: management and infection-control issues. *Expert Rev Anti Infect Ther* 2006; **4**: 807-819 [PMID: 17140357 DOI: 10.1586/14787210.4.5.807]

28 **Strandvik B**, Gronowitz E, Enlund F, Martinsson T, Wahlström J. Essential fatty acid deficiency in relation to genotype in patients with cystic fibrosis. *J Pediatr* 2001; **139**: 650-655 [PMID: 11713441 DOI: 10.1067/mpd.2001.118890]

29 **Coste TC**, Armand M, Lebacq J, Lebecque P, Wallemacq P, Leal T. An overview of monitoring and supplementation of omega 3 fatty acids in cystic fibrosis. *Clin Biochem* 2007; **40**: 511-520 [PMID: 17316592 DOI: 10.1016/j.clinbiochem.2007.01.002]

30 **White NM**, Jiang D, Burgess JD, Bederman IR, Previs SF, Kelley TJ. Altered cholesterol homeostasis in cultured and in vivo models of cystic fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2007; **292**: L476-L486 [PMID: 17085523 DOI: 10.1152/ajplung.00262.2006]

31 **Slomiany A**, Murty VL, Aono M, Snyder CE, Herp A, Slomiany BL. Lipid composition of tracheobronchial secretions from normal individuals and patients with cystic fibrosis. *Biochim Biophys Acta* 1982; **710**: 106-111 [PMID: 7055590]

32 **Ma DC**, Yoon AJ, Faull KF, Desharnais R, Zemanick ET, Porter E. Cholesteryl esters are elevated in the lipid fraction of bronchoalveolar lavage fluid collected from pediatric cystic fibrosis patients. *PLoS One* 2015; **10**: e0125326 [PMID: 25919295 DOI: 10.1371/journal.pone.0125326]

33 **Lee JT**, Jansen M, Yilma AN, Nguyen A, Desharnais R, Porter E. Antimicrobial lipids: novel innate defense molecules are elevated in sinus secretions of patients with chronic rhinosinusitis. *Am J Rhinol Allergy* 2010; **24**: 99-104 [PMID: 20338107 DOI: 10.2500/ajra.2010.24.3444]

34 **Thormar H**, Isaacs CE, Brown HR, Barshatzky MR, Pessolano T. Inactivation of enveloped viruses and killing of cells by fatty acids and monoglycerides. *Antimicrob Agents Chemother* 1987; **31**: 27-31 [PMID: 3032090]

35 **Isaacs CE**. Human milk inactivates pathogens individually, additively, and synergistically. *J Nutr* 2005; **135**: 1286-1288 [PMID: 15867325]

36 **Rissmann R**, Gooris G, Ponec M, Bouwstra J. Long periodicity phase in extracted lipids of vernix caseosa obtained with equilibration at physiological temperature. *Chem Phys Lipids* 2009; **158**: 32-38 [PMID: 18996362 DOI: 10.1016/j.chemphyslip.2008.10.001]

37 **Rissmann R**, Groenink HW, Weerheim AM, Hoath SB, Ponec M, Bouwstra JA. New insights into ultrastructure, lipid composition and organization of vernix caseosa. *J Invest Dermatol* 2006; **126**: 1823-1833 [PMID: 16628195 DOI: 10.1038/sj.jid.5700305]

38 **Tollin M**, Bergsson G, Kai-Larsen Y, Lengqvist J, Sjövall J, Griffiths W, Skúladóttir GV, Haraldsson A, Jörnvall H, Gudmundsson GH, Agerberth B. Vernix caseosa as a multi-component defence system based on polypeptides, lipids and their interactions. *Cell Mol Life Sci* 2005; **62**: 2390-2399 [PMID: 16179970 DOI: 10.1007/s00018-005-5260-7]

39 **Hoeger PH**, Schreiner V, Klaassen IA, Enzmann CC, Friedrichs K, Bleck O. Epidermal barrier lipids in human vernix caseosa: corresponding ceramide pattern in vernix and fetal skin. *Br J Dermatol* 2002; **146**: 194-201 [PMID: 11903227]

40 **Lee SH**, Jeong SK, Ahn SK. An update of the defensive barrier function of skin. *Yonsei Med J* 2006; **47**: 293-306 [PMID: 16807977]

41 **Camera E**, Ludovici M, Galante M, Sinagra JL, Picardo M. Comprehensive analysis of the major lipid classes in sebum by rapid resolution high-performance liquid chromatography and electrospray mass spectrometry. *J Lipid Res* 2010; **51**: 3377-3388 [PMID: 20719760 DOI: 10.1194/jlr.D008391]

42 **Feingold KR**. The outer frontier: the importance of lipid metabolism in the skin. *J Lipid Res* 2009; **50** Suppl: S417-S422 [PMID: 18980941 DOI: 10.1194/jlr.R800039-JLR200]

43 **Fischer CL**, Drake DR, Dawson DV, Blanchette DR, Brogden KA, Wertz PW. Antibacterial activity of sphingoid bases and fatty acids against Gram-positive and Gram-negative bacteria. *Antimicrob Agents Chemother* 2012; **56**: 1157-1161 [PMID: 22155833 DOI: 10.1128/aac.05151-11]

44 **Butovich IA**. Cholesteryl esters as a depot for very long chain fatty acids in human meibum. *J Lipid Res* 2009; **50**: 501-513 [PMID: 18836212 DOI: 10.1194/jlr.M800426-JLR200]

45 **Rantamäki AH**, Seppänen-Laakso T, Oresic M, Jauhiainen M, Holopainen JM. Human tear fluid lipidome: from composition to function. *PLoS One* 2011; **6**: e19553 [PMID: 21573170 DOI: 10.1371/journal.pone.0019553]

46 **Mudgil P**. Antimicrobial role of human meibomian lipids at the ocular surface. *Invest Ophthalmol Vis Sci* 2014; **55**: 7272-7277 [PMID: 25316725 DOI: 10.1167/iovs.14-15512]

47 **Dawson DV**, Drake DR, Hill JR, Brogden KA, Fischer CL, Wertz PW. Organization, barrier function and antimicrobial lipids of the oral mucosa. *Int J Cosmet Sci* 2013; **35**: 220-223 [PMID: 23320785 DOI: 10.1111/ics.12038]

48 **Brasser AJ**, Barwacz CA, Dawson DV, Brogden KA, Drake DR, Wertz PW. Presence of wax esters and squalene in human saliva. *Arch Oral Biol* 2011; **56**: 588-591 [PMID: 21247555 DOI: 10.1016/j.archoralbio.2010.12.002]

49 **Woodworth BA**, Smythe N, Spicer SS, Schulte BA, Schlosser RJ. Presence of surfactant lamellar bodies in normal and diseased sinus mucosa. *ORL J Otorhinolaryngol Relat Spec* 2005; **67**: 199-202 [PMID: 16024936 DOI: 10.1159/000087093]

50 **Agassandian M**, Mallampalli RK. Surfactant phospholipid metabolism. *Biochim Biophys Acta* 2013; **1831**: 612-625 [PMID: 23026158 DOI: 10.1016/j.bbalip.2012.09.010]

51 **Wright JR**. Pulmonary surfactant: a front line of lung host defense. *J Clin Invest* 2003; **111**: 1453-1455 [PMID: 12750392 DOI: 10.1172/jci18650]

52 **Glasser JR**, Mallampalli RK. Surfactant and its role in the pathobiology of pulmonary infection. *Microbes Infect* 2012; **14**: 17-25 [PMID: 21945366 DOI: 10.1016/j.micinf.2011.08.019]

53 **Do TQ**, Moshkani S, Castillo P, Anunta S, Pogosyan A, Cheung A, Marbois B, Faull KF, Ernst W, Chiang SM, Fujii G, Clarke CF, Foster K, Porter E. Lipids including cholesteryl linoleate and cholesteryl arachidonate contribute to the inherent antibacterial activity of human nasal fluid. *J Immunol* 2008; **181**: 4177-4187 [PMID: 18768875]

54 **Jüngst D**, Weiser H, Siess E, Karl HJ. Urinary cholesterol: its association with a macromolecular protein-lipid complex. *J Lipid Res* 1984; **25**: 655-664 [PMID: 6434678]

55 **Khan SR**, Glenton PA, Backov R, Talham DR. Presence of lipids in urine, crystals and stones: implications for the formation of kidney stones. *Kidney Int* 2002; **62**: 2062-2072 [PMID: 12427130 DOI: 10.1046/j.1523-1755.2002.00676.x]

56 **Feki NC**, Thérond P, Couturier M, Liméa G, Legrand A, Jouannet P, Auger J. Human sperm lipid content is modified after migration into human cervical mucus. *Mol Hum Reprod* 2004; **10**: 137-142 [PMID: 14742699]

57 **Vignon F**, Clavert A, Cranz C, Koll-Back MH, Reville P. Alterations in the lipid composition of seminal plasma in patients with a chronic infection of the urogenital tract. *Urol Int* 1993; **50**: 36-38 [PMID: 8434424]

58 **Srinivasan S**, Morgan MT, Fiedler TL, Djukovic D, Hoffman NG, Raftery D, Marrazzo JM, Fredricks DN. Metabolic signatures of bacterial vaginosis. *MBio* 2015; **6**: [PMID: 25873373 DOI: 10.1128/mBio.00204-15]

59 **Desbois AP**, Smith VJ. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Appl Microbiol Biotechnol* 2010; **85**: 1629-1642 [PMID: 19956944 DOI: 10.1007/s00253-009-2355-3]

60 **Parsons JB**, Yao J, Frank MW, Jackson P, Rock CO. Membrane disruption by antimicrobial fatty acids releases low-molecular-weight proteins from Staphylococcus aureus. *J Bacteriol* 2012; **194**: 5294-5304 [PMID: 22843840 DOI: 10.1128/JB.00743-12]

61 **Choi JS**, Park NH, Hwang SY, Sohn JH, Kwak I, Cho KK, Choi IS. The antibacterial activity of various saturated and unsaturated fatty acids against several oral pathogens. *J Environ Biol* 2013; **34**: 673-676 [PMID: 24640241]

62 **Catalá A**. Five decades with polyunsaturated Fatty acids: chemical synthesis, enzymatic formation, lipid peroxidation and its biological effects. *J Lipids* 2013; **2013**: 710290 [PMID: 24490074 DOI: 10.1155/2013/710290]

63 **Guri A**, Griffiths M, Khursigara CM, Corredig M. The effect of milk fat globules on adherence and internalization of Salmonella Enteritidis to HT-29 cells. *J Dairy Sci* 2012; **95**: 6937-6945 [PMID: 23021758 DOI: 10.3168/jds.2012-5734]

64 **Clarke NM**, May JT. Effect of antimicrobial factors in human milk on rhinoviruses and milk-borne cytomegalovirus in vitro. *J Med Microbiol* 2000; **49**: 719-723 [PMID: 10933257]

65 **Lester K**, Simmonds RS. Zoocin A and lauricidin in combination reduce Streptococcus mutans growth in a multispecies biofilm. *Caries Res* 2012; **46**: 185-193 [PMID: 22508519 DOI: 10.1159/000337307]

66 **Schlievert PM**, Peterson ML. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. *PLoS One* 2012; **7**: e40350 [PMID: 22808139 DOI: 10.1371/journal.pone.0040350]

67 **Martinez JG**, Waldon M, Huang Q, Alvarez S, Oren A, Sandoval N, Du M, Zhou F, Zenz A, Lohner K, Desharnais R, Porter E. Membrane-targeted synergistic activity of docosahexaenoic acid and lysozyme against Pseudomonas aeruginosa. *Biochem J* 2009; **419**: 193-200 [PMID: 19105793 DOI: 10.1042/BJ20081505]

68 **Nakatsuji T**, Kao MC, Zhang L, Zouboulis CC, Gallo RL, Huang CM. Sebum free fatty acids enhance the innate immune defense of human sebocytes by upregulating beta-defensin-2 expression. *J Invest Dermatol* 2010; **130**: 985-994 [PMID: 20032992 DOI: 10.1038/jid.2009.384]

69 **Mossberg AK**, Hun Mok K, Morozova-Roche LA, Svanborg C. Structure and function of human α-lactalbumin made lethal to tumor cells (HAMLET)-type complexes. *FEBS J* 2010; **277**: 4614-4625 [PMID: 20977665 DOI: 10.1111/j.1742-4658.2010.07890.x]

70 **Permyakov SE**, Knyazeva EL, Leonteva MV, Fadeev RS, Chekanov AV, Zhadan AP, Håkansson AP, Akatov VS, Permyakov EA. A novel method for preparation of HAMLET-like protein complexes. *Biochimie* 2011; **93**: 1495-1501 [PMID: 21596091 DOI: 10.1016/j.biochi.2011.05.002]

71 **Clementi EA**, Marks LR, Duffey ME, Hakansson AP. A novel initiation mechanism of death in Streptococcus pneumoniae induced by the human milk protein-lipid complex HAMLET and activated during physiological death. *J Biol Chem* 2012; **287**: 27168-27182 [PMID: 22700972 DOI: 10.1074/jbc.M112.371070]

72 **Malina A**, Shai Y. Conjugation of fatty acids with different lengths modulates the antibacterial and antifungal activity of a cationic biologically inactive peptide. *Biochem J* 2005; **390**: 695-702 [PMID: 15907192 DOI: 10.1042/bj20050520]

73 **Wendel M**, Paul R, Heller AR. Lipoproteins in inflammation and sepsis. II. Clinical aspects. *Intensive Care Med* 2007; **33**: 25-35 [PMID: 17093984 DOI: 10.1007/s00134-006-0433-x]

74 **Kruger PS**. Forget glucose: what about lipids in critical illness? *Crit Care Resusc* 2009; **11**: 305-309 [PMID: 20001883]

75 **Georgel P**, Crozat K, Lauth X, Makrantonaki E, Seltmann H, Sovath S, Hoebe K, Du X, Rutschmann S, Jiang Z, Bigby T, Nizet V, Zouboulis CC, Beutler B. A toll-like receptor 2-responsive lipid effector pathway protects mammals against skin infections with gram-positive bacteria. *Infect Immun* 2005; **73**: 4512-4521 [PMID: 16040962 DOI: 10.1128/IAI.73.8.4512-4521.2005]

76 **Wang YD**, Peng KC, Wu JL, Chen JY. Transgenic expression of salmon delta-5 and delta-6 desaturase in zebrafish muscle inhibits the growth of Vibrio alginolyticus and affects fish immunomodulatory activity. *Fish Shellfish Immunol* 2014; **39**: 223-230 [PMID: 24811009 DOI: 10.1016/j.fsi.2014.04.021]

77 **Kim MJ**, Wainwright HC, Locketz M, Bekker LG, Walther GB, Dittrich C, Visser A, Wang W, Hsu FF, Wiehart U, Tsenova L, Kaplan G, Russell DG. Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. *EMBO Mol Med* 2010; **2**: 258-274 [PMID: 20597103 DOI: 10.1002/emmm.201000079]

78 **Lee JT**, Escobar OH, Anouseyan R, Janisiewicz A, Eivers E, Blackwell KE, Keschner DB, Garg R, Porter E. Assessment of epithelial innate antimicrobial factors in sinus tissue from patients with and without chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2014; **4**: 893-900 [PMID: 25196914 DOI: 10.1002/alr.21404]

79 **Yin YW**, Liao SQ, Zhang MJ, Liu Y, Li BH, Zhou Y, Chen L, Gao CY, Li JC, Zhang LL. TLR4-mediated inflammation promotes foam cell formation of vascular smooth muscle cell by upregulating ACAT1 expression. *Cell Death Dis* 2014; **5**: e1574 [PMID: 25522268 DOI: 10.1038/cddis.2014.535]

80 **Pessolano LG**, Sullivan CP, Seidl SE, Rich CB, Liscum L, Stone PJ, Sipe JD, Schreiber BM. Trafficking of endogenous smooth muscle cell cholesterol: a role for serum amyloid A and interleukin-1β. *Arterioscler Thromb Vasc Biol* 2012; **32**: 2741-2750 [PMID: 22995521 DOI: 10.1161/ATVBAHA.112.300243]

81 **Tall AR**, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nat Rev Immunol* 2015; **15**: 104-116 [PMID: 25614320 DOI: 10.1038/nri3793]

82 **Stolowich NJ**, Petrescu AD, Huang H, Martin GG, Scott AI, Schroeder F. Sterol carrier protein-2: structure reveals function. *Cell Mol Life Sci* 2002; **59**: 193-212 [PMID: 11915938]

83 **Bartlett JA**, Gakhar L, Penterman J, Singh PK, Mallampalli RK, Porter E, McCray PB. PLUNC: a multifunctional surfactant of the airways. *Biochem Soc Trans* 2011; **39**: 1012-1016 [PMID: 21787339 DOI: 10.1042/BST0391012]

84 **Ning F**, Wang C, Berry KZ, Kandasamy P, Liu H, Murphy RC, Voelker DR, Nho CW, Pan CH, Dai S, Niu L, Chu HW, Zhang G. Structural characterization of the pulmonary innate immune protein SPLUNC1 and identification of lipid ligands. *FASEB J* 2014; **28**: 5349-5360 [PMID: 25223608 DOI: 10.1096/fj.14-259291]

85 **King NP**, Sakinç T, Ben Zakour NL, Totsika M, Heras B, Simerska P, Shepherd M, Gatermann SG, Beatson SA, Schembri MA. Characterisation of a cell wall-anchored protein of Staphylococcus saprophyticus associated with linoleic acid resistance. *BMC Microbiol* 2012; **12**: 8 [PMID: 22243671 DOI: 10.1186/1471-2180-12-8]

86 **Kenny JG**, Ward D, Josefsson E, Jonsson IM, Hinds J, Rees HH, Lindsay JA, Tarkowski A, Horsburgh MJ. The Staphylococcus aureus response to unsaturated long chain free fatty acids: survival mechanisms and virulence implications. *PLoS One* 2009; **4**: e4344 [PMID: 19183815 DOI: 10.1371/journal.pone.0004344]

87 **Kohler T**, Weidenmaier C, Peschel A. Wall teichoic acid protects Staphylococcus aureus against antimicrobial fatty acids from human skin. *J Bacteriol* 2009; **191**: 4482-4484 [PMID: 19429623 DOI: 10.1128/jb.00221-09]

88 **Sugihara A**, Shimada Y, Nomura A, Terai T, Imayasu M, Nagai Y, Nagao T, Watanabe Y, Tominaga Y. Purification and characterization of a novel cholesterol esterase from Pseudomonas aeruginosa, with its application to cleaning lipid-stained contact lenses. *Biosci Biotechnol Biochem* 2002; **66**: 2347-2355 [PMID: 12506971 DOI: 10.1271/bbb.66.2347]

89 **Cadieux B**, Vijayakumaran V, Bernards MA, McGavin MJ, Heinrichs DE. Role of lipase from community-associated methicillin-resistant Staphylococcus aureus strain USA300 in hydrolyzing triglycerides into growth-inhibitory free fatty acids. *J Bacteriol* 2014; **196**: 4044-4056 [PMID: 25225262 DOI: 10.1128/JB.02044-14]

90 **Behnsen J**, Jellbauer S, Wong CP, Edwards RA, George MD, Ouyang W, Raffatellu M. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. *Immunity* 2014; **40**: 262-273 [PMID: 24508234 DOI: 10.1016/j.immuni.2014.01.003]

91 **Elamin AA**, Stehr M, Singh M. Lipid Droplets and Mycobacterium leprae Infection. *J Pathog* 2012; **2012**: 361374 [PMID: 23209912 DOI: 10.1155/2012/361374]

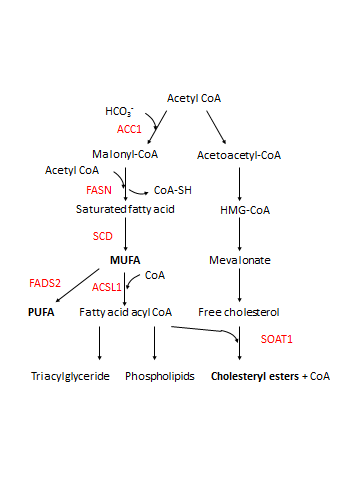
92 **Stehr M**, Elamin AA, Singh M. Cytosolic lipid inclusions formed during infection by viral and bacterial pathogens. *Microbes Infect* 2012; **14**: 1227-1237 [PMID: 22982567 DOI: 10.1016/j.micinf.2012.08.001]

93 **Bligh EG**, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959; **37**: 911-917 [PMID: 13671378]

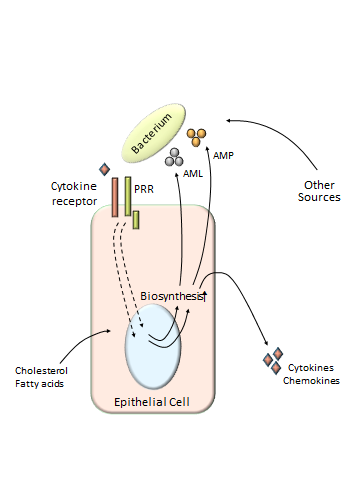
94 **Brecher P**, Chobanian J, Small DM, Chobanian AV. The use of phospholipid vesicles for in vitro studies on cholesteryl ester hydrolysis. *J Lipid Res* 1976; **17**: 239-247 [PMID: 6603]

95 **Hamilton JA**, Small DM. Solubilization and localization of cholesteryl oleate in egg phosphatidylcholine vesicles. A carbon 13 NMR study. *J Biol Chem* 1982; **257**: 7318-7321 [PMID: 7200981]

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**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).

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**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.

**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 tissue1 | LPS1 *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-, IL22, Mtb-derived lipopeptide |
|  | *elovl* | Elongation of long chain fatty acids | Possibly implicated in tissue-specific synthesis of very long chain fatty acids and sphingolipids3 | Mp, DC, CD34+, TE,B, F, EN | LPS, Zy, Schi, IL1, IFN-β IFN-γ, IL10, TGF- |
|  | *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 acyltransferase2 | 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 A3 | 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 |
|  | *scd4* | Acyl-coenzyme A oxidase | 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-acyltransferase3 | 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 protein3 | 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 |
|  | *slc27* | Solute carrier family 27 | **T**ranslocation 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 |

1Chen *et al*, *J Pineal Res* 2011 Nov; 51: 416-25 DOI: 10.1111/j.1600-079X.2011.00905.x; 2Profiles for mouse only; 3Profiles for human only; 4*scd1* in mice; 5Also 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-: Transforming growth factor ; TNF-β: Tumor necrosis factor β; VitD3: Vitamin D3; Zy: Zymogen; Schi: *Schistosoma* antigen; Mtb: *Mycobacterium tuberculosis*.