Thematic Review Series: Skin Lipids

Antimicrobial lipids at the skin surface

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Abstract  The skin surface represents our interface with the external environment, and as such, is our first line of defense against microbial colonization and infection. Lipids at the skin surface are thought to underlie at least part of an antimicrobial barrier. Some of these lipids are synthesized in the epidermis and are carried to the surface as cells differentiate, whereas others are secreted onto the surface from the sebaceous glands. One such group, free sphingoid bases, are known to have broad antimicrobial activity, and our previous studies demonstrate their presence at the skin surface. Free sphingoid bases may be generated by enzymatic hydrolysis of epidermal ceramides. In addition, our preliminary results demonstrate potent antibacterial activity associated with two specific fatty acids derived from sebaceous triglycerides. Most remarkably, one of these fatty acids (sapienic acid, $\text{C}_{16}:1\Delta6$), in combination with a low concentration of ethanol, is very effective against methicillin-resistant Staphylococcus aureus (MRSA). In fact, this combination was far more effective than mupirocin with or without ethanol. Mupirocin is a “gold standard” for activity against MRSA.—Drake, D. R., K. A. Brogden, D. V. Dawson, and P. W. Wertz. Antimicrobial lipids at the skin surface. J. Lipid Res. 2008. 49: 4–11.

Supplementary key words  long-chain base • sphingosine • fatty acid • sebum • epidermis

INNATE IMMUNE MECHANISMS

The innate immune system in mammals and other vertebrates plays a number of crucial roles (1, 2). Specifically, it: a) provides first-line recognition of microbial organisms (3, 4); b) contains the infection prior to the induction of adaptive immune responses, which can take 4 to 5 days, and c) controls the activation of adaptive immunity and determines the type of effector responses appropriate for the infecting pathogen (2, 5, 6). These functions critically depend on the ability of the innate immune system to detect the presence of infectious microorganisms and to induce a set of endogenous signals like the secretion of cytokines by macrophages and natural killer cells (7, 8).

Noncellular innate host defense elements range from simple inorganic molecules (e.g., hydrochloric acid, peroxidases, and nitric oxide) to complement (9), C-reactive protein, extracellular collectins (lung surfactant proteins A and D, conglutinin, and collectin-43) (3), and germline-encoded receptors recognizing pathogen-associated molecular patterns (e.g., lipopolysaccharide, lipoteichoic acid, lipoarabinomannan, mannans, etc.) (4, 10–12) and antimicrobial proteins and peptides. These include lysozyme (13, 14), lactoferrin (15, 16), acidic proline-rich proteins (17, 18), plunc (19, 20), saliva mucus glycoprotein (16, 21), histatin (13, 17, 22), defensin HBD1 (23, 24), defensin HBD2 (25–27), defensin HBD3 (26, 27), defensin HBD4 (26, 27), and hCAP18/LL-37 (28). Fatty acids and long-chain bases found at the skin surface have strong bactericidal activity (29–33). The purpose of this review is to summarize what is known about antimicrobial lipids found at the skin surface. Some points will be illustrated with our own preliminary results.

In recent years, much attention has focused on the epithelial production of antimicrobial cationic peptides as part of an innate immune system, an ancient first line of defense in all surface epithelia (29).

Burtenshaw showed in the 1940s that lipid extracts from the skin surface have the ability to kill Staphylococcus aureus in vitro, and it was thought that free fatty acids were the active agent (30); however, this proposition was not extensively tested with fatty acids actually found at the human skin surface until recently (31, 32). In addition to a potential role for antimicrobial fatty acids, studies have demonstrated significant levels of free sphingosines, dihydrosphingosines, and 6-hydroxydihydrosphingosines in the stratum corneum (33, 34). Several studies have demonstrated that these long-chain bases are potent antimicrobials (35–39).
The chemical structures of the antimicrobial lipids known to present at the human skin surface are given in Fig. 1.

**COMPOSITION OF LIPIDS AT THE SKIN SURFACE**

In epidermis, lipids accumulate with increasing cellular differentiation (40, 41). Much of the accumulating lipid is packaged in the form of small membranous organelles known as lamellar granules (42–46). The lamellar granules are round to ovoid in shape and consist of a unit-bounding membrane surrounding one or several stacks of lamellar disks. Isolated lamellar granules contain abundant phospholipids, cholesterol, and glycolipids (43–46). A number of hydrolytic enzymes are also associated with the granules (44, 45). Near the boundary between the stratum corneum and the granular layer, the contents of the lamellar granules are exocytosed into the intercellular spaces. The hydrolytic enzymes act on the phospholipids to produce ceramide from sphingomyelin and glucosylceramides and a mixture of saturated fatty acids and monounsaturated cholesterol esters from the phosphoglycerides. Ceramides, cholesterol, and free fatty acids are the principal lipids of the stratum corneum (40, 41, 47, 48).

When the contents of the lamellar granules are extruded into the intercellular spaces, the keratin filaments inside the cell condense under the influence of the histidine-rich protein filaggrin, and the cells become extremely flattened. All of the internal organelles are degraded. Simultaneously, a thick band of protein is deposited at the periphery of the corneocyte (48). This peripheral protein becomes a polymerized cross-linked protein layer through the formation of both disulfide linkages and isopeptide linkages and is known as the cornified envelope. In epidermis, ω-hydroxyacylceramide molecules derived from the bounding membrane of the lamellar granule become covalently attached to the outer surface of the cornified envelope (50–52). This covalently bound lipid consists mainly of 30- through 34-carbon ω-hydroxyacids amide-linked to sphingosine bases. Owing to the unusual length of the ω-hydroxyacylcereamide molecule, this lipid layer has the dimensions of a typical bilayer (50, 52). Thus, epidermal stratum corneum consists of an array of extremely flat, keratin-filled cells bounded by a cornified envelope and embedded in a lipid matrix. This structure provides a permeability barrier that prevents desiccation.

The fatty acids in the stratum corneum are saturated, straight-chained, and mostly 20–28 carbons in length. The ceramides of the stratum corneum are structurally diverse and include all combinations of normal and α-hydroxyacids amide-linked to sphingosines and dihydrosphingosines, phytosphingosines and 6-hydroxysphingosines (53). In addition, there are acylceramides with 30- through 34-carbon-long ω-hydroxyacids amide-linked to long-chain bases and bearing linoleic acid ester-linked to the ω-hydroxyl group. These acylceramides are derived from analogous acylglucosylceramides associated with the lamellar granules and are thought to be of major importance for the physical organization of lipids in the stratum corneum. In addition, the aforementioned ω-hydroxyacylceramides on the cornified envelope are derived from acylglucosylceramide precursors. A shorthand nomenclature system has been introduced (54) in which N, A, or O indicates amide-linked normal fatty acid, amide-linked α-hydroxyacid, and amide-linked ω-hydroxyacid, respectively. S, P, and H indicate the presence of sphingosine, phytosphingosine, and 6-hydroxysphingosine, respectively. Sphingosine and dihydrosphingosine usually occur together. The presence of ester-linked fatty acid is indicated with the prefix E. Thus, for example, the acylceramide containing sphingosine bases would be represented CER EOS.

In addition to the major structural lipids, the stratum corneum contains free sphingosine bases. Concentrations of about 5 mg total long-chain base per gram of dry stratum corneum have been reported (55). The free long-chain bases found here mainly range from 16 through 20 carbons in length and include sphingosines, dihydrosphingosines, and 6-hydroxysphingosines (33, 34). There are also some unusually long chain dihydrosphingosines (34). Free sphingoid bases in the stratum corneum are produced through the action of ceramidases on ceramides. Ceramidases are found in both the viable portion of the epidermis and in the stratum corneum (56). In addition, a sphingosine gradient exists, with higher sphingosine concentrations in the stratum corneum (55). Sphingoid bases have been shown to have broad antibacterial and antifungal activities (35–39). In atopic dermatitis, ceramide concentrations in the stratum corneum are diminished owing to an error in sphingomyelin metabolism (57).

![Fig. 1. Chemical structures of antimicrobial lipids from the human skin surface.](image-url)
Individuals with atopic dermatitis generally have a greater carriage of bacteria, especially *S. aureus*, on the skin surface (58). Recently, it has been shown that this increased carriage of *S. aureus* in atopic dermatitis is correlated with the reduction in free sphingosine (59). In addition to an apparent contribution to the antimicrobial defenses of the skin surface, sphingosine is a potent inhibitor of protein kinase C (60). As such, the sphingosine gradient could play a significant role in regulation of the rate at which cell division and differentiation occur in the viable epidermis. The sphingosine gradient may provide a means for the stratum corneum to communicate with the viable portion of the epidermis.

In addition to the lipids of the stratum corneum, the skin surface of postpubertal humans is coated by a liquid-phase lipid mixture secreted by the sebaceous glands (61). The main components of sebum as it accumulates in the lumen of the gland are squalene, wax monoesters, and triglycerides. There are also minor amounts of cholesterol and cholesterol esters. As this mixture flows outward through the follicle and over the skin surface, variable proportions of the triglycerides undergo hydrolysis to yield free fatty acids. Some of the normal bacterial flora of the skin produce lipases capable of hydrolyzing sebaceous triglycerides; however, there is also an acid lipase delivered to the stratum corneum via the lamellar granules that could contribute significantly to triglyceride hydrolysis. The relative significance of bacterial triglyceride hydrolysis compared with hydrolysis by epidermal acid lipase remains unknown. In any case, the major fatty acid released from sebaceous triglycerides is sapienic acid, C16:1Δ6. Among the other fatty acids released from sebaceous triglycerides is lauric acid, C12:0. Both of these fatty acids have been shown to have antibacterial properties. The epidermal acid lipase presents a potential mechanism to regulate, at least in part, the extent of liberation of sapienic and lauric acids at the human skin surface.

**ANTIMICROBIAL EPIDERMAL LIPIDS**

Lipids at the skin surface have antibacterial activity against *S. aureus* (30). Free fatty acids, and possibly some of the other polar lipids from epidermal stratum corneum, have antibacterial activity against a range of gram-positive bacteria, but not against *Candida albicans* or a number of gram-negative bacteria (37). The organisms that are sensitive to fatty acids include *S. aureus*, *S. pyogenes*, *S. epidermidis* and *Micrococcus* sp. Our own preliminary studies demonstrate that certain fatty acids from the skin surface are more active than others. Specifically, lauric acid and sapienic acid are the most active. **Figure 2** illustrates the determination of the minimum inhibitory concentration (MIC) for sapienic acid versus a laboratory strain of *S. aureus*. The MIC is the concentration of an agent that is just sufficient to prevent growth. In this case, the MIC is 0.03 mg/ml, or 30 μg/ml.

**Figure 3** illustrates the relatively rapid killing of a methicillin-resistant strain of *Staphylococcus aureus* (MRSA) by sapienic acid in combination with ethanol. This and subsequent experiments were done using MRSA isolated from skin wounds. These isolates proved less sensitive to sapienic acid than laboratory strains; however, these isolates were also not completely resistant. It was found that ethanol and sapienic acid act synergistically, such that complete killing could be obtained with combinations of sapienic acid and ethanol, an effect that neither agent alone had. The combination of ethanol and sapienic acid was generally far more effective than mupirocin, the active ingredient in the commercial skin disinfectant Bactroban. Similar results were obtained with several other antibiotic-resistant clinical isolates of *S. aureus* strains as well as *Propionibacterium* and *Pseudomonas*.

Synergy of the antimicrobial activity between sapienic acid and ethanol was unexpected. Since fatty acid and ethanol act synergistically, we suggest that the fatty acid must partition into the cell, and ethanol increases the cell membrane fluidity, or alternatively this could enhance partitioning of fatty acid into the membrane. Ethanol is a known permeability enhancer (62). It could also facilitate diffusion into the cytoplasm of bacteria. This finding could have important implications for dealing with antibiotic resistance. In addition to ethanol, there are a number of compounds known to increase membrane fluidity and to thereby permit more-rapid diffusion of compounds into and across the membrane. Such compounds have been identified for use in transdermal drug delivery (62). Known collectively as penetration enhancers, this group includes DMSO, some fatty acids, terpenes and azones, among others.

In a related experiment, MRSA was exposed to 15% ethanol for 2 min, followed by transfer to fresh medium containing C16:1 at a concentration ineffective by itself. This still resulted in complete killing. This result indicates that the action of ethanol is on the bacteria. Ethanol is not simply increasing availability of C16:1 monomer in the medium. The interaction of ethanol and C16:1 was discovered serendipitously when C16:1 in ethanol was added to culture medium with a resulting final concentration of
15% ethanol. Because this concentration of ethanol by itself did not inhibit growth of the test organism, it was used in further experiments to test the interaction of alcohol and fatty acid.

It has also been shown that free sphingosine bases are active not only against a similar range of gram-positive bacteria, but also against gram-negative bacteria and \textit{C. albicans} (6, 11). Table 1 gives MIC values for long-chain bases versus several strains of \textit{Staphylococcus aureus}, \textit{Escherichia coli}, and \textit{Candida albicans}. Sphingosine and phytosphingosine proved to be potent, and dihydrosphingosine, although active, was somewhat more variable in effectiveness among repeated experiments. \textit{C. albicans} was susceptible to sphingosine and phytosphingosine but somewhat resistant to dihydrosphingosine.

We have observed synergy in killing bacteria between the long-chain bases and the antimicrobial cathelicidin LL37. Future work will determine whether there is synergy between the lipids and other antimicrobial peptides as well as between different pairs of lipids.

When a fatty acid is dispersed in an aqueous medium, such as culture medium, it will exist as a mixture of micelles and monomeric units. The monomers are most likely the active component. The proportion of a given fatty acid in the monomeric state would be expected to increase with decreasing chain length, and this would be expected to result in increasing overall activity with decreasing chain length. This is in fact what we have observed, with essentially no activity with C20:0 and increasing activity through C12:0. However, solubility, or monomer concentration, is not the only factor determining activity, because activity decreases again with fatty acid chains shorter than C12:0. Bergsson et al. (63) have shown that lauric acid or its monoglyceride was very effective in killing three different gram-positive bacteria. These investigators suggested that the mechanism was disruption of the cell membrane. However, it has recently been shown by other investigators that lauric acid, at concentrations that do not inhibit growth, does inhibit production of lactamase and toxins by stationary-phase \textit{S. aureus} by interfering with signal transduction (64, 65). Similar interference with signal transduction by lauric acid has also been demonstrated with \textit{Enterococcus faecalis}.

**LIPIDS AS PART OF THE INNATE IMMUNE SYSTEM**

The innate immune system has long been recognized (29, 66, 67). It is thought to be an ancient first line of defense present in all epithelia, which represents the boundary between organism and environment. A number of small cationic peptides have been identified as antimicrobial factors that serve as part of the innate immune system. Among the most extensively studied of these are the defensins and cathelicidins (66, 68). These peptides are synthesized by differentiating keratinocytes (68), and their production is upregulated in response to wounding or infection (66). In the absence of epidermal damage or a microbial challenge, their production is limited. It has been noted that under normal resting conditions, antimicrobial peptides in epidermis are produced near potential points of microbial entry, such as around follicles, but after physical damage to the skin barrier, there is a rapid increase in antimicrobial peptide production (57). In addition to direct antibacterial action, these peptides are also chemotactic and can attract leukocytes to sites of infection (29, 67). It has been suggested that the function of the innate immune system may not be to eliminate infective bacteria, but to limit their growth while signaling and activating the adaptive immune system. The migration of leukocytes to a site of infection, and subsequent phagocytosis by macrophages and neutrophiles and production of reactive oxygen species would complete the response. Consistent with this overall view are findings that in psoriatic scale, where the surface is colonized with \textit{S.}}
aureus, β-defensins and cathelicidin are expressed at relatively high levels (69). Leukocytes infiltrate the skin. Patients with atopic dermatitis are similarly colonized with S. aureus, but suffer a much higher incidence of skin infection compared with psoriatrics (30% vs. 7%) (70). It has recently been shown that production of both β-defensins and cathelicidin in atopics is minimal, compared with psoriatrics (69). Although immunohistochemical staining revealed the presence of these peptides in atopic epidermis, the levels did not differ significantly from those in normal control epidermis.

The defensins and cathelicidins are thought to act by disrupting the bacterial cell membrane (29, 67, 71), and they act synergistically in in vitro tests (69). The fact that they act synergistically suggests that they may act on the bacterial membrane by different mechanisms.

It is a contention of the present review that antimicrobial epidermal lipids are also a part of the innate immune system of the skin. Lauric acid, sapienic acid, and the sphingoid bases are all present at the skin surface, and all have documented antibacterial activity against various potential skin pathogens.

Whether the fatty acids derived from the sebaceous triglycerides are mainly liberated by bacterial lipases or by the epidermal acid lipase, they are thought to be at least partly responsible for the microbial defense of the skin (72). There are several reasons to think that the epidermal acid lipase has a role in hydrolysis of the sebaceous triglycerides. This could provide a mechanism for accelerating hydrolysis in the event of a challenge that could make the skin surface more vulnerable to colonization by bacteria other than the normal flora. Anything that damages the normal barrier provided by the stratum corneum could trigger a defensive response. Our published studies have demonstrated that an acid lipase is packaged into lamellar granules (43). This enzyme is secreted from the lamellar granules into the intercellular space at the boundary between the granular layer and the stratum corneum, where it may participate in hydrolysis of mono- and diglycerides derived from the phospholipids. However, when this lipase reaches the skin surface, it could also hydrolyze sebaceous triglycerides. Significantly, it has been shown that there is rapid extrusion of the contents of preformed lamellar granules, presumably including acid lipase, following various forms of damage to the stratum corneum (73, 74). Although there is no indication that bacterial lipases show any degree of selectivity, it is possible that the epidermal acid lipase shows a degree of selectivity for the more active lauric or sapienic acids. It seems likely that lipases from both the normal bacterial flora at the human skin surface as well as the epidermal lipase contribute to the liberation of lauric and sapienic acids at the skin surface. Lamellar granules are enriched in acid lipase (43), and extrusion of their contents is accelerated following injury (73, 74), suggesting that triglyceride hydrolysis may be part of the innate immune system of the skin.

At least two different ceramidases have been detected in epidermis (56, 75). Given that no free phytosphingosine, α-hydroxyacids, or ω-hydroxyacids have been detected in epidermis or stratum corneum (unpublished observations), it would appear that the ceramides consisting of normal fatty acids amide-linked to sphingosines, dihydro sphingosines, and 6-hydroxy sphingosines would be the sources of free long-chain bases in human epidermis. In addition to the free ceramides in the intercellular spaces of the stratum corneum, the covalently bound ceramides on the outer surface of the cornified envelope may also be a source of these long-chain bases. In this case, when the amide linkage is hydrolyzed, the resulting ω-hydroxyacids remains attached to the cornified envelope. In atopic dermatitis, the level of ceramides in the stratum corneum is reduced, owing to a defect in sphingomyelin and glucosylceramide metabolism (57). This appears to result in lower levels of free sphingosine. The skin surface of atopic individuals is often colonized by S. aureus, and an inverse correlation has been demonstrated between the free sphingosine level and the quantitative carriage of S. aureus in this condition (60). It has been noted that the level of covalently bound ω-hydroxy ceramides is reduced in the stratum corneum of atopic individuals (76). This may reflect the action of a ceramidase on the covalently bound ω-hydroxyceramides to release free sphingoid bases and to leave behind ω-hydroxyacids attached to the cornified envelope. A limiting factor in ceramidase action within the stratum corneum would be the availability of water. It has recently been shown that essential fatty acid deficiency or ultraviolet light damage to the stratum corneum resulting in increases in transepidermal water loss (TEWL) was accompanied by reduced levels of covalently bound ω-hydroxyceramide in the stratum corneum (77). In essential fatty acid deficiency, the reduction in covalently bound ω-hydroxy ceramide levels appears to reflect hydrolysis of the amide linkage of the covalently bound ceramide as TEWL increases (unpublished observation). Paradoxically, in a study of antimicrobial activity of mouse epidermal lipids, it was noted that the total lipid extracted from essential fatty acid-deficient mice was more potent than the lipid extracted from normal control epidermis. However, the number of bacteria on the skin surface per unit area was greater on the essential fatty acid-deficient skin than on the control skin (35). Thus, the immune system does become compromised in essential fatty acid deficiency.

**SIGNIFICANCE**

It is generally thought that the normal bacterial flora on the skin surface have been selected through a combination of limited water availability and differential sensitivity to antimicrobial lipids at the skin surface (72). Because these natural antimicrobials have been selected by evolutionary forces, it seems relatively unlikely that resistant bacterial strains would arise. This is supported by a study in which incubation of 10^8 Helicobacter pylori cells of three different strains overnight with two times the MIC of lauric acid resulted in no resistant colonies (78). This raises the
possibility that these lipids could be incorporated into topical formulations for prophylaxis in individuals at risk of infection as well as for treating skin infections, including those caused by antibiotic-resistant organisms.

Although not considered part of the normal bacterial flora found on the human skin, *S. aureus* is frequently present on the skin surface and has significant potential for causing infections if the skin barrier function, normally provided by an intact stratum corneum, is abrogated or the surface milieu is otherwise altered (79–82). *S. pyogenes* is another common cause of potentially serious skin infections (83, 84), including necrotizing fasciitis (85). *Pseudomonas aeruginosa* is an opportunistic gram-negative bacterium that can infect skin wounds or hyper-hydrated regions of the skin (82, 86). Infections caused by these organisms are normally treated with antibiotics; however, the increasing occurrence of antibiotic-resistant bacterial strains makes it highly desirable to identify new antimicrobials. The long-chain bases and saponin acid could prove useful in this regard.

The self-disinfecting properties of the skin have long been recognized and attributed to lipids (30). It is a contention of the present review that like the cationic epithelial antimicrobial peptides, the antimicrobial lipids are part of an innate immune system. Natural endogenous antimicrobial lipids would have advantages over other exogenous materials. These endogenous antimicrobials would have been arrived at through the process of natural selection to provide protection against the most common potential pathogens. It could be anticipated that their mechanism of action is such that they do not readily give rise to resistant strains; otherwise, they would no longer be effective. Because they are normal constituents of the tissue, it would be expected that they would not be irritating or sensitizing, which is a potential problem with the use of topically applied exogenous materials. Identification of the most-effective antimicrobial lipids could lead to new strategies for treatment of or prophylaxis against skin infections.

Further work is clearly needed to determine the mechanisms by which the antimicrobial lipids are generated and act on microorganisms. The possible interactions between the antimicrobial lipids and peptides could also be a topic of future research.

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