Cytokine and Growth Factor Reviews 49 (2019) 70–84

Contents lists available at ScienceDirect

Cytokine and Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Architecture of antimicrobial skin defense

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ARTICLE INFO

Keywords:
Skin homeostasis
Skin infection
Antimicrobial peptides
Cathelicidin
Secretory leukocyte protease inhibitor
Chemerin

ABSTRACT

The skin is the largest and the most exposed organ in the body and its defense is regulated at several anatomical levels. Here, we explore how skin layers, including the epidermis, dermis, adipose tissue, and skin appendages, as well as cutaneous microbiota, contribute to the function of skin antimicrobial defense. We highlight recent studies that reveal the differential and complementary responses of skin layers to bacterial, viral, and fungal infection. In particular, we focus on key soluble mediators in the layered skin defense, such as antimicrobial peptides, as well as on lipid antimicrobials, cytokines, chemokines, and barrier-maintaining molecules. We include our own evaluative analyses of transcriptomic datasets of human skin to map the involvement of antimicrobial peptides in skin protection under both steady state and infectious conditions. Furthermore, we explore the versatility of the mechanisms underlying skin defense by highlighting the role of the immune and nervous systems in their interaction with cutaneous microbes, and by illustrating the multifunctionality of selected antimicrobial peptides in skin protection.

1. Introduction

The skin provides physical, chemical, microbial, and immunological barriers against various insults, such as infection. The protective abilities of the skin barrier vary across skin regions [1–3] and age [4], often creating profoundly distinct local microenvironments for microbial growth. This suggests that the skin has evolved a variety of strategies to clear penetrating microorganisms that operate at every anatomical level of this organ. Although the nature of the spatiotemporal dynamics of antimicrobial responses is not well defined, studies have shown that direct antimicrobial protection is shared by multiple skin layers.

Here, we provide an architectural layout of skin defense. We focus on the chemical landscape of the antimicrobial function of the skin and highlight some multitasking products in the context of skin responses to microbial threat. We point out various functional links and mechanisms that engage the immune and nervous system in the skin, which play key roles in reacting to and interpreting microbial stimuli, and in limiting microbial spread to internal tissues. We start with the exogenous top layer (collectively termed the “skin microbiota”) that is formed by skin microbial communities. Next, we explore the antimicrobial potential of the main skin compartments, such as the epidermis, dermis, and adipose tissue. In addition, we explore the barrier role of skin appendages, such as sweat glands, sebaceous glands, and hair follicles that are present in multiple layers (Fig. 1).

2. Microbiota

Skin-residing microorganisms form one of the largest communities of microbes in humans [5,6]. Unlike the gut, the skin has a relatively low microbial biomass, likely due to the dry, acidic, and nutrient-poor environment on the surface. However, the exposed surface area of human skin, which includes the skin appendages, provides the largest interface among all barrier sites for interaction with microbes [6]. Each person’s skin microbiota is unique; as indicated by the 2-year longitudinal adult skin sampling results, it is largely consistent over time [7]. Cutaneous microbiota mainly contains bacterial, fungal, and viral communities, among which bacteria are the best characterized component of the human microbiome [5].

Abbreviations: AD, atopic dermatitis; AMPs, antimicrobial proteins and peptides; BAT, brown adipose tissue; BD, β-defensin(s); CGRP, calcitonin gene-related peptide; dWAT, dermal white adipose tissue; NETs, neutrophil extracellular traps; scWAT, subcutaneous white adipose tissue; SLPI, secretory leukocyte protease inhibitor; QS, quorum sensing; TRPV1, transient receptor potential vanilloid 1; WAT, white adipose tissue

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https://doi.org/10.1016/j.cytogfr.2019.08.001
Received 5 June 2019; Accepted 14 August 2019
Available online 15 August 2019
1359-6101/

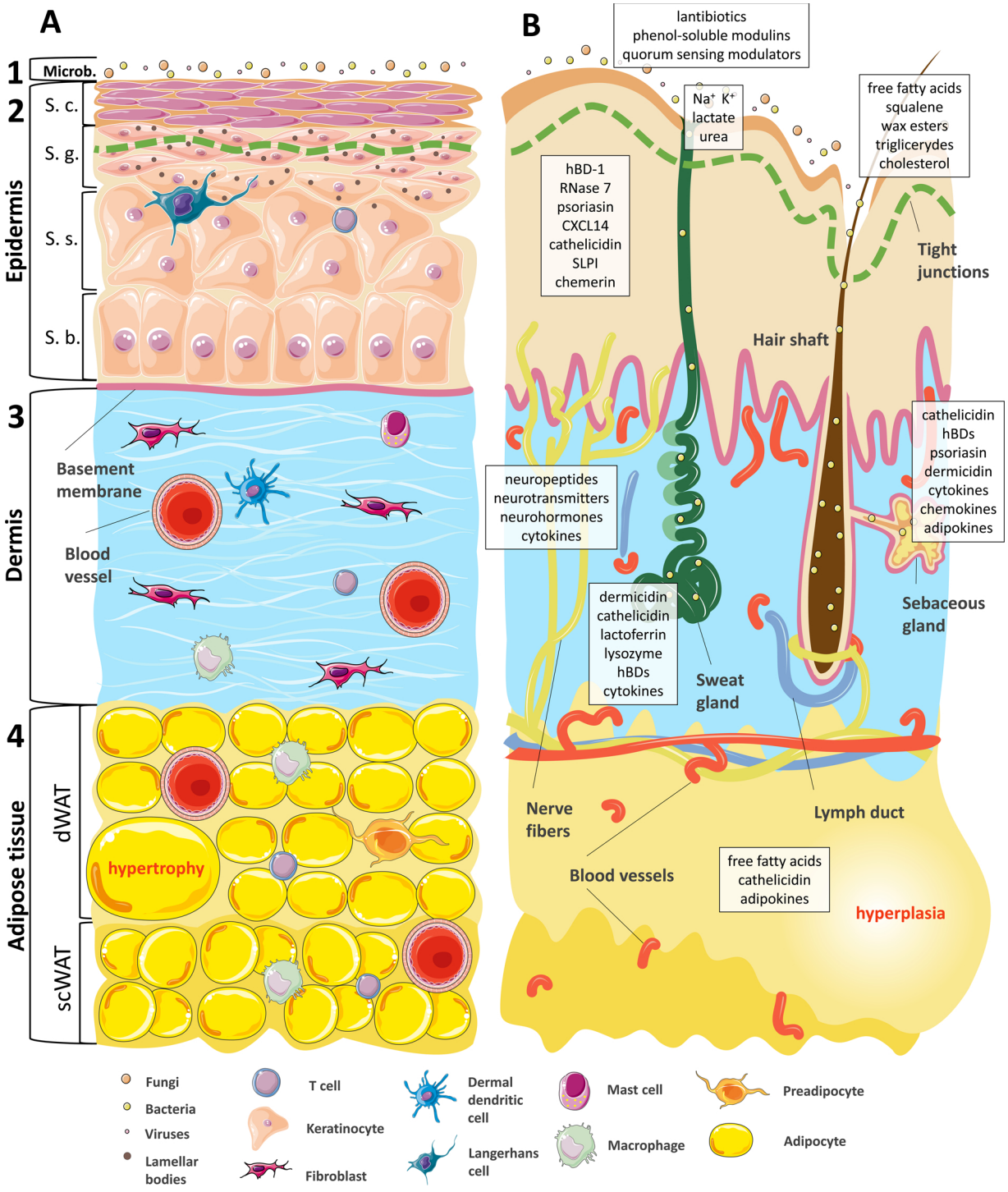

Fig. 1. The structural and functional overview of the skin layers.
A. Main structural and cellular elements in layered skin defense. **B**. Protective function of skin- associated structures and systems that traverse multiple skin layers. In order, from top to the bottom, the following layers are shown: **1**, Diverse microbes (bacteria, fungi and viruses) top the skin surface and penetrate associated structures (hair follicles, sebaceous glands and sweat glands). Microbes contribute to skin defense by secreting antimicrobial factors that shape the skin microbiota; **2**, Epidermis, with indicated strata (**S.c**. stratum corneum, **S. g.** stratum granulosum, **S. s.** stratum spinosum, **S. b**. stratum basale). Tight junctions, which together with the stratum corneum are responsible for barrier function, are depicted as a dotted line. Keratinocytes at different stages of differentiation are shown along with immune cells, such as Langerhans cells and T cells. These cells respond to microbes, and either constitutively, or in a regulated manner produce AMPs and/or antimicrobial lipids. **3**, Dermis, with extracellular matrix producing fibroblasts and representative skin residing or infiltrating leukocytes. The dermis is enriched with different types of leukocytes. Immune cells have an easy access into the dermis through the dermis-irrigating blood vessels. **4**, Adipose tissue consists of dermal white adipose tissue (dWAT) and subcutaneous white adipose tissue (scWAT) and contribute to skin defense through size expansion, production of AMPs (in which processes differentiating preadipocyte play a major role), and by providing a niche for lipid-dependent immune cells. (B) Skin appendages, immune barriers (leukocytes to be recruited from blood vessels and skin resident immune cells) and the cutaneous nervous system are present in multiple layers, and contribute to skin defense through a variety of means, including danger sensing, detaining of microbes, and production of AMPs. Representative antimicrobial or regulatory factors with important function in skin defense are shown in in the context of skin layers, framed in insets.

The microbiota exerts control over the flora composition and diversity, often acting in the host's benefit by eliminating pathogenic strains. Through various strategies, normal microbial skin residents (interchangeably referred to here as "common residents" or "commensal microbiota") may help defend the host from virulent microbes and the overexpansion of microbial burden. These include the displacement of more invasive strains via competition for nutrients and space, the production of antimicrobial factors that have antiproliferative function against virulent strains, the stimulation of skin resident cells for the secretion of antimicrobial factors that selectively restrict growth of invasive and expansive strains, and the engagement of host immunity to shape microbial communities.

Multiple studies have revealed the cellular and molecular mediators of microbial interactions that are relevant to host defense. The removal of potential competitors can occur through the production of antimicrobial agents that exhibit toxicity to other strains. Among the members of normal microbial skin residents are coagulase-negative *Staphylococci*, including *S. epidermidis*. *S. epidermidis*-derived protein toxins, such as phenol-soluble modulins, reduce the survival of the more harmful strains, such as *S. aureus* and *S. pyogenes*. These toxins cooperate with the host antimicrobial proteins and peptides (AMPs) and neutrophil extracellular traps (NETs) to inhibit the survival of cutaneous pathogens [8].

NETs are neutrophil DNA-based AMP-decorated structures that are implicated in the control of microbial expansion and promotion of inflammatory responses [9]. Thus, bacterial toxins can act in alliance with skin products to control microbial growth. Other bacterial products, such as lantibiotics that are derived from coagulase-negative *S. epidermidis* and *S. hominis,* were also found to protect the skin from *S. aureus* invasion [10]. The low frequency of *S. epidermidis* and *S. hominis* in human subjects with atopic dermatitis (AD) enabled the colonization of the compromised skin with *S. aureus*. Application of these *S. aureus*-controlling strains to the skin of AD patients inhibited the growth of *S. aureus.* Since *S. aureus* is well-known to aggravate AD symptoms, these findings illustrate how other bacteria can outcompete pathogens and promote skin health [10]. In addition, the blocking of quorum sensing (QS) is another strategy to limit skin infection from pathogenic strains. Coagulase-negative *S. caprae* secretes short autoinducing peptides which inhibit *S. aureus* accessory gene regulator (agr)-mediated QS. This specific quenching of QS reduces the progression of skin infection caused by methicillin-resistant *S. aureus* (MRSA) in a murine model [11].

In addition to releasing its own antimicrobial agents, skin-associated bacteria can also influence skin microbiome by stimulating the production of host AMPs. These include β-defensins (BD), cathelicidins (human hCAP18/LL37, or its mouse equivalent CRAMP), chemerin, and secretory leukocyte protease inhibitor (SLPI). For example, *Cutibacterium acnes* (formerly *Propionibacterium acnes*) and *Staphylococci* induce BD expression in human keratinocytes and sebocytes [12,13], whereas *S. aureus* upregulates antimicrobial chemerin levels in models of human epidermis and mouse skin [14]. The expression of cathelicidins is induced in the skin after injury, which often accompanies infection [15]. The skin microbiota of specific-pathogen-free (SPF) mice upregulates the gene encoding SLPI, which exhibits antibacterial, antifungal, and antiviral activity. This suggests that skin AMPs can be engaged in maintaining microbe interactions at the supra-kingdom level [16].

The impact of the commensals on enhancing skin defense through gene regulation is not restricted to stimulating the production of host-derived AMPs. Cutaneous gene expression profiles during skin colonization shows the upregulation of genes involved in the crosstalk between the microbiota and the immune system. The exposure to microbiota leads to the activation of genes encoding AMPs, as well as cytokine signaling proteins, pattern recognition receptors (including Toll-like receptors (TLRs)), and interferon regulatory factors [16]. Despite this extensive immune activation, the balance between microbes and host cells is maintained [17,18]. The tolerance of microbial skin residents by the immune system further suggests that the cutaneous microbiota has a notable role in skin protection against more invasive strains (see below-chapter 6).

3. Epidermis

The epidermis operates as the front line of host defense against the invasion of microbes. Based on the anatomy and function, the stratified human epidermis is subdivided into the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale (Fig. 1). Keratinocytes dominate in the epidermal structure, although they are phenotypically and functionally different in each stratum. Since keratinocytes express pattern recognition receptors, such as TLRs, they can trigger multiple programs in response to microbial stimulation that are key to maintaining host-microbial homeostasis [19].

The stratum corneum, which is poised directly at the interface with the external environment, comprises a structure of cornified (enucleated, dead) keratinocytes embedded in a lipid-enriched matrix. Flattened keratinocytes (corneocytes) and the cell-crosslinking lipid matrix are often described as the bricks and mortars of this layer. The stratum corneum, together with the tight junctions that seal neighboring keratinocytes in the underlying stratum granulosum (Fig. 1), forms an interdependent permeability and antimicrobial barrier [20]. The physical and chemical features of the stratum corneum and the tight junctions prevent trans-epidermal water loss and allow control over which molecules can pass through this barrier [21]. They also offer resistance against penetration of most microbes, engaging AMPs to fortify barrier integrity [20]. The presence of the tight junctions and the lack of accompanying stratum corneum in the epithelial lining of skin appendages (Fig. 1) make these structures a likely target for microbial expansion [22].

The lipid-enriched matrix that surrounds the flattened keratinocytes of the stratum corneum is largely delivered by lamellar bodies (Fig. 1). The stratum granulosum is enriched with these ellipsoid, secretory granules, which also sequester the preformed AMPs. Lamellar bodies cluster together at the interface between the stratum granulosum and stratum corneum. By fusing with cell membranes, lamellar bodies release their cargo (namely co-packed lipids and AMPs) into the extracellular space [21]. At the bottom of the stratified epidermis lays the stratum basale. During epidermal differentiation, keratinocytes progress inside-out from the mitotically-active basal cells through the spinous and granular cells of the stratum spinosum and stratum granulosum to the fully-differentiated squamous cells of the stratum corneum (Fig. 1). This carefully orchestrated program of keratinocyte proliferation and differentiation is crucial for proper function of the epidermis as a whole, and is often disrupted in inflammatory skin diseases, such as psoriasis and AD [23]. At homeostasis, corneocytes are continuously shed during the process of desquamation and replaced by keratinocytes derived from basal cells. This mechanism results in the loss of microorganisms colonizing the epidermis and prevents skin penetration by microbes [24].

The chemical barrier of epidermis is mainly composed of AMPs and lipids; both exhibit antimicrobial function, often against broad range of microbes. AMPs are structurally different peptides that share the ability to limit microbial growth [15]. Their secretion to extracellular spaces varies depending on skin sites (glabrous or enriched in appendageal structures) and conditions (healthy or infected).

Cysteine-rich defensins, such as human BD-1 (hBD-1), are constitutively expressed in the healthy epithelium. HBD-1, which in the reduced form has potent antimicrobial activity against bacteria and fungi, is detected in all layers of the epidermis, with a slightly lower presence in the stratum corneum [25,26]. Another constitutively expressed antimicrobial protein, RNase 7, is produced at high levels in the stratum corneum, and to a lesser extent in the stratum granulosum. This prevents the colonization of skin explants by *S. aureus,* and possibly

Table 1
Transcriptome analysis of AMPs in human skin.

Gene	Protein	Homeostasis (TPM)		Infection (DEGs)		Antimicrobial activities
		GTEx	HPA	Leish.	Acne	
BPI	Bactericidal permeability-increasing protein	n.d.	L	–	↑	Bactericidal activity limited to species of Gram-negative bacteria, [119].
CAMP	Cathelicidin antimicrobial peptide	n.d.	L	↑	↑	Shows antibacterial, antifungal, and antiviral activities, [15].
CTSG	Cathepsin G	M	M	↓	–	Exerts broad-spectrum antibacterial action against Gram-negative and -positive bacteria independent of its serine protease activity, [120].
CXCL14	C-X-C motif chemokine 14	H	M	↓	–	Strong antimicrobial activity toward *E. coli*, *S.* coag.neg. spp., *S. aureus*, *Propionibacterium* spp., *C. albicans*, [28].
DCD	Dermicidin	H	L	↓	–	Product of sweat glands. Highly effective against *E. coli*, *E. faecalis*, *S. aureus* and *C. albicans*, [45].
DEFB1	Beta-defensin 1	M	M	↓	↓	Oxidized form shows limited bactericidal activity. Reduced hBD-1 displays antimicrobial activity against numerous pathogens, [26].
ELANE	Neutrophil elastase	L	L	↓	–	Present in neutrophil azurophil granules. Shown to be directly antimicrobial against *E. coli*, [121].
GNLY	Granulysin	L	L	↑	↑	Present in CD8+ T-cell granules. Shown to kill extra- and, to a lesser extent, intracellular *M. tuberculosis*, [122].
LCN2	Neutrophil gelatinase-associated lipocalin	L	M	–	–	Bacteriostatic activity, [123].
LEAP2	Liver-expressed antimicrobial peptide 2	L	L	↓	–	Shows selective antimicrobial properties without clear Gram-positive/negative differences. Effective against *B. subtilis*, *M. luteus*, *N. cinereal* and *S. cerevisiae* whilst being non-effective against *E. coli* and *Pseudomonas fluorescens*, [124].
LTF	Lactotransferrin	L	M	↑	↑	Direct and indirect activity against bacteria, fungi, protozoa and viruses, [125].
LYZ	Lysozyme	L	M	↑	↑	Bacteriostatic and antimicrobial activity towards Gram-positive bacteria, [126].
PGLYRP3	Peptidoglycan recognition protein 3	M	M	↓	–	Bactericidal against several pathogenic and nonpathogenic Gram-positive bacteria. Bacteriostatic against Gram-negative bacteria and normal flora Gram-positive bacteria, [127].
PGLYRP4	Peptidoglycan recognition protein 4	M	M	–	–	Bactericidal against several pathogenic and nonpathogenic Gram-positive bacteria. Bacteriostatic against Gram-negative bacteria and normal flora Gram-positive bacteria, [127].
PI3	Elafin	M	M	↑	↑	Acts on *S. aureus*, *Pseudomonas aeruginosa* but not *E. coli*. Shows anti-viral and anti-fungal properties, [128].
PLA2G2A	Phospholipase A2 group IIA	M	n.d.	–	–	Kills Gram-positive bacteria (e.g. *L. monocytogenes* and *S. aureus*), [129].
RARRES2	Chemerin	M	L	↑	↑	Displays antibacterial activity against *E. coli*, *K. pneumoniae* and other bacteria or fungus *Candida* [109,117].
RNASE2	Non-secretory ribonuclease	L	L	↑	↑	Present in neutrophils and eosinophils. Shows antiviral properties, [130].
RNASE6	Ribonuclease K6	L	L	↑	↑	Shows potent antimicrobial activity against uropathogenic *Escherichia coli* (UPEC), *Enterococcus faecalis*, and *Staphylococcus saprophyticus*. Produced by urinary epithelial cells, [131].
RNASE7	Ribonuclease 7	M	M	–	–	Displays a broad-spectrum antimicrobial activity against many pathogenic microorganisms including *S. aureus*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *E. coli*, *E. faecium* and *C. albicans*, [27].
S100A7	**Protein S100A7**	M	M	↑	↑	Antibacterial agent restricting growth of *E. coli*, [63].
S100A8/ S100A9	Calprotectin	M	M	↑	↑	Effective against *S. aureus*, *C. albicans*, and *Aspergillus fumigates* [132].
SLPI	Antileukoproteinase	M	M	–	–	Exhibit a broad spectrum of antimicrobial activity, [101].

The list of genes in alphabetical order expressed in healthy skin (Homeostasis) or in skin infected with *C. acnes* or *Leishmania braziliensis* (Infection). TPM values (transcripts per million) were extracted from the Human Protein Atlas (HPA), [133] and the Genotype-Tissue Expression (GTEx) project [134]. Gene ontology (GO) identifiers were then added to each gene record using the UniProt database [135]. Then genes associated with at least one GO identifier linked to antimicrobial activity were selected using Microsoft Access query. Resulting genes were subdivided into 3 categories based on their TPM values [136] H: high- expression genes (TPM over 1000); M: medium-expression genes (TPM between 10 and 1000) and L: low-expression genes (TPM between 0.5 and 10). Each gene set was then additionally validated for false-positive/negative results using both Reactome [137] and manual analysis. Discrepancy for skin transcriptome between the HPA and GTEx can be explained by the differences in sampling procedures. The HPA is based on shave biopsies that include mainly the epidermis, while the GTEx consortium collected full-thickness skin biopsies that included the dermis, and the accompanying hair follicles and sweat glands [138]. The list of differentially expressed genes (DEGs) comparing the skin of healthy controls and patients infected with *L. braziliensis* (Leish.) was downloaded from the published data [139]. Then GO identifiers were added to each gene record. Genes linked to antimicrobial activity were then selected for false positive/negative results using Reactome [137] and manual analysis. To analyse DEGs comparing healthy skin and late acne lesion from the same donor, the RNA-seq raw data (SRX4282126 and SRX4282127) were downloaded from the Sequence Read Archive (NCBI), [140] using the Galaxy platform [141]. The reads were then quality-trimmed using Trim Galore! and aligned to the Human Genome (hg38) using HISAT2. For each gene, expression was then quantified at the transcript level using the featureCounts. DEGs were analysed using MS Excel. Afterwards the datasets were processed as described above. Genes discussed in this review in detail are shown in bold. ↑ - up-regulated DEGs, ↓ - down-regulated DEGs, n.d. - not detected.

other microbes (including Gram-positive, Gram-negative bacteria, and yeast), against which it exhibits a high antimicrobial activity in vitro [27]. Transcriptome analysis of human skin signature at steady state conditions identified hBD-1 and RNAse 7 as medium-expression genes (Table 1). The analysis revealed that one of the most abundant mRNA encoding for protein equipped with antimicrobial activity belongs to the molecule best known for its chemoattractant function, CXCL14. Immunocytochemistry staining of human healthy skin have also shown high expression levels of the chemokine CXCL14 in the epidermis, except in the stratum corneum [28]. CXCL14 contributes to the maintenance of local skin homeostasis by killing pathogens in the early stages of infection, since both the synthetic and cell culture supernatant-derived CXCL14 were found to exhibit potent growth inhibitory potential against Gram-positive and Gram-negative bacteria, as well as fungi [28].

Damaged or infected epithelium often increases the production of AMPs. For example, an AMP highly relevant to cutaneous defense, cathelicidin hCAP18/LL37 (encoded by *CAMP* gene; see below, chapter 8.1), is upregulated in the epidermis as a result of skin injury and infection [15]. Thus, AMPs can act as skin protective factors either constitutively, or in a regulated manner. Skin transcriptome analyses of constitutively expressed AMPs, as well as AMPs upregulated following skin infection with *C. acnes* or *Leishmania braziliensis,* are shown in Table 1.

In addition to proteins and peptides, another important component of the chemical barrier of the skin is antibacterial lipids, which are synthesized in the epidermis and transferred to the surface during cell differentiation. One of the main classes of lipids in the stratum corneum is ceramides, a heterogeneous and complex group consisting of a sphingoid base linked to a fatty acid [29]. Hydrolytic enzymes present in the epidermis, especially in the stratum corneum, cleave ceramides into free sphingoid bases; these bases exhibit antimicrobial activity against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria in vitro [30]. Furthermore, as a result of cell membrane damage free sphingosine is phosphorylated to produce sphingosine-1-phosphate (S1P). Normal human keratinocytes treated with *S. aureus* increase the production of S1P, expression of its receptor (S1PR2), and consequently the pro-inflammatory cytokines to signal the infection [31].

4. Dermis

The epidermis is separated from the underlying connective tissue, the dermis, by the basement membrane (Fig. 1). Fibroblasts, a major dermal cell type, produce collagen, elastin, and other extracellular matrix components to provide the skin with underlying structure. Dermal fibroblasts also participate in the skin antimicrobial defense, mainly by sensing and amplifying the danger signals, suggesting that they are important immunomodulatory cells. In response to microbial components, fibroblasts can produce cytokines (IL-6, IL-8, TNFα), growth factors (VEGF, TGFβ1, HB-EGF), and metalloproteinases (MMP-1), [32]. Inflammatory mediators secreted by fibroblasts have several functions in the skin protection; for example, they can support the adhesion and migration of immune cells.

The dermis is one of the major gateways for leukocytes in the skin. In physiological conditions, the dermis is populated with resident immune cells, such as macrophages, mast cells, and dermal DCs [2]. Immune cells can migrate through the organ using scaffolds made of collagen and elastin fibers that are created by fibroblasts [33]. In inflammatory settings, the dermis is often heavily infiltrated with leukocytes that are recruited from the circulation [22]. This dermal infiltration by immune cells, in which fibroblasts play an accessory role, may compensate for protective mechanisms that are less abundant that those of the epidermis.

In contrast to the epidermis, which is exposed to the outside world and can be seen as a dominant antimicrobial shield, the dermis is likely much less efficient in producing antimicrobial factors. Mast cells in the dermis, provide additional antimicrobial defense; they localize near the blood vessels, carrying large amounts of cathelicidins in their granules [34]. In the case of skin injury or viral infection, they can easily degranulate and resist the spread of pathogen. Cathelicidins, such as hCAP18/LL37, can also take part in the wound repair processes by stimulating keratinocyte proliferation and induction of angiogenesis [35,36], and by downregulating collagen production by fibroblasts [37]. During wound healing, keratinocytes also produce hBD2, which provides strong antimicrobial protection, and can additionally promote fibroblast migration [38].

Any damage made to the skin structure has to be immediately repaired in order to prevent further invasion of microorganisms. Wound healing is a complex process, often requiring different types of cells to communicate in order to clear pathogens, and to repopulate and reconstruct the injury site. Dermal fibroblasts and epidermal keratinocytes are a vital part of the process required to restore the skin barrier [39]. The barrier restoration may be facilitated by the contact of fibroblasts with microbes. For example, IL-8 and TNFα, released by fibroblasts in response to microbial components, stimulate angiogenesis [39,40]. Certain adipocyte types are necessary for fibroblast recruitment to the site of injury, as mice without adipocytes have wound healing defects [41]. Dermal adipocytes also provide strong antimicrobial response. When challenged with *S. aureus*, fibroblasts can differentiate into adipocytes in order to strengthen the defense against pathogens. The number of adipogenic fibroblasts in human skin decrease over lifetime [4], likely leading to, at least in part, greater age-related susceptibility to skin infections.

Altogether, dermal fibroblasts are an important component of the skin's protective response to pathogens, as they have to harmoniously communicate with keratinocytes, dermal adipocytes, and immune cells in order to maintain the skin's structure and function as a barrier against invading microorganisms.

4.1. Sweat glands

Sweat glands are coiled tubular skin appendages and their main function is to control body temperature through water evaporation from the skin. There are two types of sweat glands in humans – apocrine – where the duct opens into the hair follicle (in humans, present only in the hair-rich regions), and eccrine – which releases sweat onto the skin surface, and is the main type of sweat gland in humans. Sweat secretions, in addition to dissipating heat, hydrate the skin surface with moisturizing agents, such as lactate, urea, sodium, and potassium salts [42], Fig. 1. This aids in maintaining the skin elasticity and integrity, which are essential for the skin defense system, in particular the mechanical barrier function.

Sweat glands consists of three types of cells: clear and dark secretory cells, and myoepithelial cells, which serve as mechanical support and surround the gland coil. Clear cells contain numerous mitochondria and are believed to have major secretory function [43]. In contrast, dark cells have fewer mitochondria, but assist clear cells in sweat production [44]. Additionally, dark cells play an important role in skin defense, producing antimicrobial dermicidin and storing it in electron-dense granules [45]. Dermicidin, which represents one of the most abundantly expressed cutaneous AMP in regions enriched with appendageal structures, (Table 1) is constitutively produced in eccrine glands. Following its release into sweat, dermicidin is proteolytically cleaved and transported through the eccrine duct, and as a cleaved peptide, reaches the surface of the skin [46]. It is processed into dermicidin-1 (DCD-1, 47 amino acids) and/or dermicidin-1 L (DCD-1 L, 48 amino acids). Both

isoforms have antibacterial properties, such as for *S. aureus* [47,48]. Dermicidin is not induced by inflammatory conditions, which may suggest a constitutive bactericidal role in controlling cutaneous microbes [45]. Eccrine glands also produce other antimicrobial factors, such as cathelicidins, lactoferrin, lysozyme, and hBDs [45,49–51].

In addition, sweat contains immunoglobulins (IgA) and cytokines (IL1α/β, IL-8, IL-31), which can modulate the immune response. Immunoglobulins and cytokines in sweat can also contribute to the inflammatory reactions, as they can stimulate keratinocytes as a "danger" signal, leading to production of chemokine CCL2 [52]. Thus, sweat glands are a vital part of both the direct and indirect antimicrobial skin defense systems.

4.2. Sebaceous glands

The sebaceous gland, together with the hair, the hair follicle, and the arrector pili muscle, form the pilosebaceous unit (Fig. 1). The sebaceous gland can be divided into three zones, which contain cells (sebocytes) at different stages of differentiation. The inner zone is composed of progenitor cells. During differentiation, they move towards the center of the gland, lose their mitotic activity, increase their size, and accumulate lipid droplets, forming the maturation zone. In the central zone, the terminally differentiated sebocytes disintegrate and release their content via holocrine secretion.

The most obvious function of the sebaceous gland is to excrete sebum. Sebum is a species-specific mixture of neutral lipids, most of which are synthesized *de novo* by sebocytes; it also provides hydrophobic protection against overwetting and heat insulation. Sebaceous and keratinocyte lipids form a slightly acidic (pH 4.5–6.0) film on the surface of the skin that provides a barrier against pathogens, thus contributing to the body's first line of defense. Human sebum contains squalene, wax esters, triglycerides, cholesterol, and cholesterol esters. On the skin surface, some of the triglycerides are converted to free fatty acids by bacterial and yeast lipases. Sebum also plays an important physiological role as a delivery system for antimicrobial peptides and antioxidants, such as vitamin E, to the skin surface [53].

Sebaceous lipids also possess antimicrobial activities, which are especially effective against Gram-positive bacteria. Sebum lipids can act on different cell types and enhance protective responses; for example, squalene (a unique component of sebum) is able to activate T cells [54]. In addition, keratinocytes treated with squalene peroxide exhibit enhanced secretory levels of prostaglandin E2 [55].

Another important group of antimicrobial lipids is free fatty acids, which arise from sebum triglycerides upon hydrolysis via lipases of commensal microbiota or via the epidermal acid lipase [56]. The most potent of these lipids, lauric acid and sapienic acid, are active against a broad range of Gram-positive bacteria. Another example is arachidonic acid, which is a substrate for the synthesis of leukotriene, an inflammatory mediator and a potent neutrophil attractant [57]. In addition, sebaleic acid can be converted by neutrophils; its metabolites possess chemotactic properties and can be involved in neutrophil infiltration into the skin [58]. Other free fatty acids, such as lauric, palmitic, and oleic acids, can also interact with sebocytes, leading to the upregulation of AMPs expression [59].

Despite lipid production, sebaceous glands take an active role in the innate skin defense via secretion of pro-inflammatory cytokines, chemokines, and AMPs upon activation of TLR1/2 and TLR4 [60]. Sebocytes express a broad range of AMPs, including cathelicidin [61], hBDs [62], S100A7 (also known as psoriasin), [63], and dermcidin [64], (Table 1). Sebocytes are capable of producing different (mostly pro-inflammatory) cytokines and lipid-derived inflammatory mediators. Among cytokines, the expression of IL-1α, IL-1β, IL-6, IL-8/CXCL-8, and TNFα was reported in both human sebaceous glands and cultured sebocytes [12,57]. Furthermore, sebocytes have also been identified as sources of various adipokines, such as adiponectin, IL- 6, leptin, serpin E1, resistin, and visfatin [65], which suggests their potential role in the regulation of multiple cellular skin constituents.

5. Skin adipose tissue

In the conventional context of skin antimicrobial protection, adipose tissue does not seem to be an important player, especially among other major skin compartments, such as the epidermis and dermis. However, a recent study has shifted focus on skin white adipose tissue (skin WAT) and its role in the defense against foreign pathogen [66].

There are different variants of fat tissue; brown adipose tissue (BAT), white adipose tissue (WAT), and "beige" adipose tissue. BAT is known for its thermogenic and oxidative properties, and its primary function is to dissipate stored energy in the form of heat [67]. WAT primarily functions by regulating energy homeostasis, as it controls the storage and release of lipids, aids in preventing heat loss, and provides padding for organs [22,67]. A "beige" phenotype exhibits both brown and white adipocytes characteristics [68]. Only WAT and beige fat deposits are found in skin; from these subsets, dermal WAT (dWAT) and subcutaneous adipose tissue (scWAT), (Fig. 1), will be further discussed due to their functional role in skin homeostasis.

The dWAT and scWAT share many similarities. The primary function of adipocytes in these WAT deposits is to regulate energy homeostasis via storage and release of lipids and reduce heat loss. WAT adipocytes regulate energy homeostasis through the storage of excess calories in lipid droplets, releasing free fatty acids in response to metabolic needs [69]. In addition, they modulate energy balance via secretion of adipokines and provide key thermogenic insulation and protection against cold stress [69].

The dWAT and scWAT are also very different. The former is found in the dermal layer alongside hair follicles and sebaceous glands [70]. The latter, however, is found underneath the dermal layer and is distributed all over the body. In humans, these two adipose depots are tough to distinguish by eye and appear to run continuously with each other, while their separation becomes more apparent when observed in mice samples due to the much more visible panniculus carnosus muscle [67,71]. This separation hints at the different roles dWAT and scWAT play physiologically [71].

The scWAT has been shown to exhibit a high capacity to adapt to an energy-consuming BAT-like "beige" phenotype [72]. This is notable because dWAT responds differently to cold exposure than other BAT (and by association, beige adipose tissue): dWAT becomes lipogenic, while BAT becomes lipolytic. There are also a few other notable differences between the dWAT and scWAT. First, dWAT fluctuates in size, while scWAT tissue mass is more stable [73]. Second, dWAT produces cathelicidins, while this function has not been observed in scWAT Third, dWAT is closely associated with hair follicles and undergoes size fluctuation in line with hair growth cycling [73].

This ability to change in size is the key mechanism through which fat tissue in skin provides antimicrobial defense. Notably, when mouse skin was infected with *S. aureus,* rapid proliferation of adipocyte precursors and dWAT expansion was observed [66]. This is due to the hypertrophy of pre-existing adipocytes and proliferation of adipocyte precursors. Hypertrophy leads to dermal adipocytes expressing and secreting cathelicidin, which targets *S. aureus.* Cathelicidin production depends on proper adipogenesis; disruption to homeostasis leads to the skin being more prone to infections.

Lastly, following subcutaneous infection in non-functional fat tissue due to impaired adipogenesis, an increase in local infection and potential systemic infection, such as bacteria in spleen, can be observed [66]. Treatment of experimental animals with inhibitors of

adipogenesis demonstrate a causal link between fat expansion and microbial protection. In all, these recent findings shine light on the multifunctionality of dWAT as the deepest layer within the skin that has antimicrobial barrier function both physically and functionally.

6. The immune system through the layers of various skin compartments

Whereas the skin relies on the protective characteristics of its own constituents and structures, such as the keratinocyte-dominated epidermis and their AMPs, it also has an “in-hand and on-call” immune system to alert and fight against infection. Immune cells can be found in any skin compartment, although their compositions and numbers vary, depending on depth and niche [2,3].

Starting from the outermost levels, the immune system is actively engaged in recognizing and responding to skin microbiota. In the stratum spinosum and stratum granulosum reside Langerhans cells (LCs), (Fig. 1). Since LCs are professional antigen-presenting cells, they are an important component of innate immunity in the epidermis. After recognition of pathogen-associated molecular patterns, LCs are able to migrate into the lymph nodes and prime T cells, although they also exhibit context-dependent immunomodulatory function. Under homeostatic conditions, LCs interact with skin resident regulatory T cells and induce their activation to develop immunotolerance; however, after activation through contact with microbial components, they are able to initiate inflammation by skin resident effector memory T cells stimulation [74].

The settings in which microbes can interact with skin barrier cells, such as the developmental stage of the host [18], or an intact or compromised skin, can have a dramatic influence on the outcome of the cross-talk between microbiota and the cutaneous immune system. Since microbiotas are an important contributor to skin homeostasis, they are usually found to peacefully co-exist with the host. The conversation between microbiota and the skin immunity is bidirectional. The microbiota can be influenced by the skin immune system [75], and immune fitness also depends on resident microorganisms that functionally tune skin responses [17,76].

The distribution and composition of cutaneous leukocytes, such as the presence of antigen-experienced T cells in the epidermis, indicate past activity of the underlying immune responses. Each contact with microbes leaves an immune signature imprinted in the skin. Even under homeostatic conditions, the skin is harbored by both pathogen and common bacteria -elicited lymphocytes [76]. This may be reflective of a prior skin infection with invasive strains, or of a skin barrier disruption and the subsequent increased exposure to commensal microbes. However, many immune cells are expected to exist under homeostatic conditions, with the continuous detection of noninvasive microbes across an intact skin barrier [76,77]. The mechanisms required for establishing tolerance to skin microbiota are preferentially active during neonatal life. During a defined developmental period, the accumulation of regulatory T cells in skin mediates tolerance to skin-associated microbes [18].

In adulthood, homeostatic encounters between the host and microbiota contribute to the maturation of cutaneous immunity. This occurs by altering the number and function of skin-resident immune cells, but without resultant tissue inflammation [17]. The partnership between noninvasive microbiota and the host has important consequences for immune defense against pathogens or skin adaptation to injuries. Skin microbiota has the capacity to promote T cell responses against cutaneous pathogens [76,77]. The interaction of the immune system and microbial skin inhabitants under homeostatic conditions leads to the induction of cytotoxic T cells and T helper cells specific for microbiota. These IL-17A-commited RORγT + cells (Tc17 and Th17, respectively) co-express GATA-3, which is the lineage-defining transcription factor for both Th2 and group 2 ILCs. At steady state, Tc17 and Th17 produce IL-17A and express mRNA, but not protein for type 2 cytokines, such as IL-5 and IL-13. Whereas production of IL17A enhances innate barrier immunity under homeostatic conditions, acquisition of type 2 effector program can promote tissue repair [77]. Tc17 and Th17 cells, due to GATA-3-controlled transcription of type 2 cytokines, are already “half-ready” to support tissue repair. Therefore, they can be rapidly triggered by tissue injury to implement full Th2 program and deliver IL-5 and IL-13 when needed. The plasticity of tissue-resident, microbiota-specific T cells allows them to participate in two critical strategies underlying skin antimicrobial protection: the promotion of IL-17-mediated antimicrobial defense at baseline and type 2 immunity-mediated tissue repair following skin injury.

Whereas noninvasive microbiota on the top of the skin surface typically do not perturb the host and educate immune system at steady state, once the barrier is breached, the immune system is the most important player in clearing infection. How and into which outcomes the immune system is called into action may also depend on the skin location, such as skin depth. Skin compartments differ in leukocyte profiles [2,3], suggesting that strength, kinetics and type of immunity may be influenced by the spatial aspects that the immune cell encounters with microbes. For example, hair follicles are immune privileged sites, enriched in regulatory T cells and resident bacteria. The colocalization of these populations may promote tolerance of the microorganisms and explain why microorganisms enter and persist in hair follicles [22].

To better understand the spatial differences in cutaneous immunity against microbes, one point that requires further studies is in which instances microbes can come in close contact with deep skin layers. Visual methods, such as TEM or fluorescence microscopy, usually do not demonstrate a substantial number of microbes below the surface keratinocytes (e.g. stratum corneum and the tight junctions). This suggests that microbes rarely, if at all, penetrate these structures, which is in line with the notion that the stratum corneum and the tight junctions can maintain an absolute barrier to bacterial entry. Alternatively, bacteria can be quickly eliminated, and therefore be difficult to detect. The exception may be appendageal structures, in which a high amount of bacteria reside.

On the other hand, diverse elements of the cutaneous microbiota, such as their DNA or LPS, are found in the dermis and dermal adipose tissue of healthy human skin [78]. In addition, in models of organotypic cultures of human keratinocytes, *S. aureus* entry past multiple keratinocyte layers was observed [79]. However, most experimental models exploring the interactions between layered skin barriers and microbes rely on intradermal injection of bacteria or their administration following mechanical barrier disruption, such as by tape-stripping. All these methods bypass the superficial barrier of the skin stratum corneum and the tight junctions. Therefore, it remains obscure how often and which microbes are encountered by cutaneous cells at different depths.

Several patterns of skin colonization, which depend on microbes and host predisposition, can be predicted. Some virulent strains induce skin inflammation independent of host predisposition, whereas in the compromised host, normal constituents of the microbiota can cause infection. The microbe’s intrinsic mechanism is supported by the observation that different strains of *S. aureus* differ in their capacity to penetrate the epidermis. Notably, enhanced transit of *S. aureus* across the epidermal surface was noted in the compromised skin such as in patients with AD [79].

Even the incidental presence of microbes below the external

interfollicular epithelial surface suggests that deeper compartments cannot be defenseless at any given time. However, below the epidermis, the immune system is likely to play a major role in intercepting cutaneous bacteria, fungi, or viruses, in line with the circulating leukocytes having easy access to the dermal compartment (Fig. 1). The most effective strategy to detain microbes, provided by the immune barrier, may be necessary at this location. Leakage of microorganisms from the skin surface, or outside of appendageal structures into the dermal compartment, may signal the major problem, such as broken epidermal integrity and an increased likelihood of microbial dissemination. The immune barrier is best equipped to cut microbes off and sterilize tissues deep in the skin.

In addition to the dermis, skin-associated adipocyte tissue (skin WAT, which may consist of dWAT and/or scWAT) harbors a substantial number of immune cells, including T cells [80]. However, immune barrier in the dermis and skin WAT is likely to be fitted to accommodate different needs. Although skin WAT differs functionally from the other fat depots, such as mesenteric WAT [81], they both may share the ability to store memory T cells and quickly supply these cells in response to secondary antigen challenge or metabolic changes. When rechallenged with mucosal antigens, memory T cells in mesenteric WAT respond more rapidly and efficiently than T cells from the spleen or lamina propria [80]. This is attributed to the different metabolic profile of memory T cells from mesenteric WAT, which is characterized by higher rates of lipid uptake *ex vivo* and elevated mitochondrial function. Long life span and potent protective abilities of these cells may be supported by the lipid-rich environment of WAT [80]. Therefore, as a potential reservoir for memory T cells, skin WAT may contribute to skin defense by promoting protective memory responses to infection and by linking skin immunity with host metabolism [80].

7. Crosstalk between the nervous system and microbes in the context of skin defense

The response of the nervous system to external skin insults, which occurs faster (milliseconds) than that of the immune system (minutes, hours), plays a key coordinating role in the host defense [82].

The skin is highly innervated by several types of nerves, which generally are divided into autonomic and sensory nerves. Autonomic nerve fibers constitute only a minority of cutaneous nerve fibers and are restricted to the dermis. They regulate functions that are handled without conscious control (blood circulation, lymphatic function, and the regulation of skin appendages) [83]. A significant part of cutaneous innervation consists of neurons that process sensory information. Sensory nerves are responsible for detecting and transmitting the outside signals to the spinal cord, and then to the brain. Environmental insults initiate the response following the activation of three classes of receptors at sensory nerve endings in the skin: thermoreceptors for heat and cold, nociceptor for pain, and mechanoreceptors for mechanical changes. By evoking sensations, such as pain, the host can rapidly initiate protective behavioral responses.

Sensory fibers are located in the epidermis, dermis, as well as in the skin adipose tissue (Fig. 1). The epidermis, blood vessels, hair follicles, sebaceous and sweat glands are innervated by several subtypes of sensory nerves [83]. Sensory neurons that innervate the epidermis have long axons originating from cell bodies in the dorsal root ganglions (DRGs), close to the spinal cord. DRG neurons are pseudounipolar, meaning that their axons consist of two branches that connect the sensory endings in the skin with the synapses in the dorsal horn of the spinal cord. Cutaneous nerve fibers are partly uncovered from their Schwann-cell sheaths, forming bulbous enlargements called varicosities, which enable the nonsynaptic release of neurotransmitters and neuropeptides along the axon [84]. The close contact of peptidergic nerve fibers (nerves using peptides as neurotransmitters) and skin cells, such as dermal fibroblasts, keratinocytes, mast cells, or LCs, has been suggested to be the basis of functional interactions, as neuropeptides released from activated sensory neurons can bind to the specific receptors on skin cells [84].

Sensory neurons are able to directly respond to cutaneous microbes. Commensal and pathogenic microbiota are detected by sensory neurons through specific metabolites, cell-wall components, and toxins. The proper interplay between indigenous bacteria and the nervous system is essential for bacterial adaptation of the host. However, in the case of skin pathogens, the peripheral nervous system can be expected to rapidly signal a threat and possibly cooperate with the immune barrier in clearing infection. On the other hand, pathogens can manipulate the nervous system to influence sensory information. Recent observations indicate that the pain that accompanies infection can occur as the result of direct activation of nociceptors by pathogens. The production of inflammatory cytokines (that are characteristic of pain) by neurons follows the neural stimulation by bacterial cell products. For example, the activation of TLR4 on DRG neurons by *E. coli*-derived LPS causes an increase in TNFα and IL-1β levels [85].

Another bacterial product, the pore-forming toxin streptolysin S, which is secreted by *S. pyogenes,* directly activates the $TRPV1^+$ (transient receptor potential vanilloid 1, also called the “pain” or “capsaicin” receptor) neurons to evoke pain sensation [86]. N-formylated peptides and the pore-forming toxin α-haemolysin that are produced by *E. coli* and/or *S. aureus* can induce direct pain responses as well [87]. Contrary to the above findings, certain bacterial toxins, such as mycolactone (produced by *Mycobacterium ulcerans)* are effective analgesics to annul the pain of the lesions they cause, potentially as a means to evade detection [88]. Collectively, the interaction between cutaneous microorganisms and the nervous system can result in a different magnitude of sensory perception, and potentially lead to a different outcome, such as hypersensitivity or lack of pathogen sensing.

Emerging data emphasizes the importance of the cross-talk between the peripheral nervous system and immune system in orchestrating skin defense. These data also indicate that in response to cutaneous microorganisms, the nervous system is regulated by an immune barrier. Conversely, the nervous system may be integral to microbiota-mediated skin immune responses.

Immune cells infiltrating the infected skin and the wide range of inflammatory mediators released by these cells are involved in the indirect activation of the nervous system by pathogenic microbes. Cytokines (IL-1β, TNFα, IL-6) and other mediators (eg. prostaglandins, histamine) interact with sensory nerves via cognate receptors expressed on these neurons, causing pain sensitization [86].

Bacteria, including *S. aureus* [87] and *E. coli* [85], increase the excitability of sensory neurons. Such activated neurons release large amounts of neuropeptides that, in turn, regulate the immune function in the infection site. Neuropeptides, like calcitonin gene-related peptide (CGRP), diminish the production of TNFα by macrophages in response to *S. aureus* [87]. CGRP also suppresses the recruitment and bactericidal activity of neutrophils during *S. pyogenes* infection. Silencing nerve fibers by administration of botulinum neurotoxin A or a CGRP antagonist results in the inhibition of bacterial invasion [86]. The size of microbial infection and pain could be also decreased in parallel to an increase in the local neutrophil and monocyte infiltration by genetic or pharmacological ablation of nociceptors [86,87]. Thus, by acting on sensory neurons, microbes may weaken protective immunity against bacterial pathogens.

The neuro-immune communication after pathogen invasion could also modulate immunity in an opposite fashion. Local treatment of *C. albicans*-infected skin with CGRP increases the number of IL-17$^+$ dermal γδT cells and reduce the fungal burden. Furthermore, mice with TRPV1$^+$-ablated sensory nociceptive fibers develop increased *C. albicans* infection accompanied by decreased level of IL-23 and reduced number of IL-17$^+$ dermal γδT cells, while the administration of a CGRP antagonist results in the opposite effect [89]. Together, these data support the beneficial role of sensory neurons in driving antifungal immune responses.

Another level of interaction between microbes and the nervous system that involves skin barrier defects is illustrated by psychological stress experimental models. Psychological stress is an important factor that has been shown to change the composition of commensal microbiota, disrupting the natural antimicrobial barrier of the skin and increase the severity of cutaneous infection [90,91]. In a mouse model of cutaneous *S. pyogenes* infection, psychological stress (e.g. insomnia) in a glucocorticoid-dependent fashion altered the structure and function of the stratum corneum, downregulated the expression of AMPs, such as CRAMP and BD-3, and resulted in more severe bacterial lesions [90]. The negative regulation of the host defense by psychological stress is amplified by stimulating the nicotinic acetylcholine receptor nAChR, which responds to the neurotransmitter acetylcholine. Blockade of nicotinic receptors in mice subjected to stress reduced the size of Group A *Streptococcus* infection [92].

Dialog between the nervous system and microbes is not confined to the local microenvironment, as the nervous system can support the spreading of microbes beyond the skin. After invading into the skin, microbes can disseminate to various secondary organs throughout the body. One of the well-described diseases is Lyme borreliosis, caused by *Borrelia burgdorferi*. This pathogen can spread from the tick bite on the skin to the heart, joints, and peripheral and central nervous system. Although little is known about the mechanisms through which neurons promote the spread of bacteria, bacterial evasion of the central nervous system may take place via retrograde axonal transport (from axon to cell body) along microtubules. In animal studies, *L. monocytogenes* inoculation into peripheral tissues leads to focal infection in the corresponding areas of the central nervous system. It is hypothesized that pathogenic bacteria may be transported in the axons of both sensory and motor neurons [93].

Likewise, the axonal transport is used by viruses. After initial infection of the corneal cells, *Herpes simplex virus* spreads to innervating sensory nerve axon terminals and then travels by retrograde axonal transport to sensory or autonomic ganglion neuron cell bodies. In the neuron cell body, the virus replicates, and new virus is re-delivered via anterograde axonal transport to axon terminals in the peripheral nervous system [94].

Together, these findings add the nervous system to the list of key players in cutaneous defense. Its actions encompass direct interaction with skin microbes, shaping host immunity and controlling skin barrier function, while also providing the route for the spread of microbes.

8. Multifunctional molecules in the skin defensive responses

As discussed in previous sections, AMPs serve as the principal chemical shield against cutaneous pathogens; they are the products of different cells (keratinocytes, mast cells, and dWAT adipocytes) that reside in different skin layers. AMPs are often endowed with the defensive functions that are not directly associated with the inhibition of the microbial growth. These include chemokines, such as CXCL14, that are equipped with antimicrobial activity; among AMPs, they represent one of the most transcribed products in human skin at steady state (Table 1). Here, we provide three examples of multitasking AMPs to illustrate the diversity and flexibility of an AMP-based system in skin protection.

8.1. hCAP18/LL37 (encoded by CAMP)

The best characterized AMPs known for their multifunctional role in the skin defense are cathelicidins. Only a single gene for cathelicidin has been identified in humans and mice [15]. Human cathelicidin precursor, known as hCAP18 (18 kDa), consists of a highly conserved cathelin domain and a variable C-terminal region. hCAP18 is secreted as an inert 2-domain pro-protein and requires proteolytic processing to display antimicrobial activity. The most extensively-studied antimicrobial derivative of hCAP18 is LL37, a 37-aa C-terminal peptide. Whereas only low levels of hCAP18/LL37 can be found in skin under basic conditions, its expression and processing by serine protease, such as kallikrein 5, are significantly induced following skin inflammation [15]. LL37 exhibits potent and broad-spectrum bacterial, fungal, and viral killing activity in vitro. Mice deficient in CRAMP (a murine homolog of hCAP18/LL37) show more severe cutaneous streptococcal infection, highlighting the important role of cathelicidins in skin protection against bacteria [15].

The involvement of LL37 in skin defense is also integral to its ability to regulate inflammatory responses through nucleic acid-dependent signaling pathways. For example, LL37 enables DCs and keratinocytes to sense nucleic acids that are released into the extracellular milieu as a result of skin injury or inflammation [95–97]. The nucleic acid-based "danger signals" lead to the production of cytokines and chemokines that shape the immunological environment of the skin. These mediators include type 1 interferons, such as IFN-α and IFN-β, proinflammatory cytokines TNF-α or IL-6, and chemokines CXCL8 or CCL5.

Type 1 interferons are important in inhibiting viral replication, as they promote functional activation of DCs and support wound healing. Thus, the production of type 1 interferons in the skin environment can have a range of beneficial effects in the context of antimicrobial defense. The induction of type 1 interferons depends on specific receptors, such as DNA-responsive TLR9, or single stranded RNA-responsive TLR7 and TLR8, which are expressed at high levels in specific subsets of DCs in the skin [95,96]. The delivery of extracellular DNA or RNA to endosomal TLR9 or TLR7 and production of IFN-α in plasmacytoid dendritic cells (pDCs) are dependent on LL37 [95,96]. Likewise, coupling of LL37 to extracellular RNA results in the production of TNFα and IL-6 from conventional (myeloid) DCs via TLR8 [95].

In addition to DCs, keratinocytes also serve as a source of type 1 interferons when exposed to nucleic acids and LL37 during skin injury [97]. These cells are highly responsive to double stranded RNA (dsRNA). Keratinocytes rely on endosomal TLR3, or TLR-independent cytoplasmic helicases RIG1 and MDA5, and signaling adaptor protein MAVS to detect and respond to dsRNA. The activation of the MAVS-dependent signaling pathway is responsible for the production of IFN-β by dsRNA and LL37 in keratinocytes [97].

Collectively, LL37 plays an important role in cutaneous inflammatory responses as an element of nucleic acid-based regulation system. In the context of skin infection, and subsequent cell activation and damage, LL37 and nucleic acids can be expected to enhance DC function, and consequently T cell activation. This can occur through direct stimulation of DCs for production of IFN-α, followed by DC maturation and stimulation of adaptive immunity. Under a second scenario, the promotion of immune responses is initiated by keratinocytes, through which secreted mediators, most notably IFN-β, promote

functional activation and maturation of DCs [97]. These examples illustrate that LL37, when linked with exogenous nucleic acids, is generally proinflammatory, and overall beneficial to skin health while constantly challenged by microbes. However, LL37 can also contribute to breaking tolerance to self-nucleic acids and driving auto-inflammatory responses in the skin, as reported for human pathological conditions [96].

8.2. SLPI

Induction of nucleic acid sensing pathways that leads to the production of IFN-α is not exclusively mediated by LL37 in the skin. Other AMPs, such as SLPI [98,99] or hBD2 and hBD3 [100] were also found to enable the innate immune system to respond to nucleic acids.

Given its antimicrobial, antiprotease, and immunomodulatory activities [101], SLPI is a good example of a multifaceted protein that may guard the skin health via different means. SLPI is a ~12kDa protein consisting of two homologous whey acidic protein (WAP) domains. Whereas the N-terminal WAP1 domain is thought to be mostly responsible for bactericidal and antifungal properties of this protein, the C-terminal WAP2 domain is also important for the inhibitory function of SLPI against proteases, such as neutrophil-derived elastase (NE) and cathepsin G (CatG), [101].

SLPI also exhibits antiviral properties against some viruses, including HIV-1 and papillomaviruses [102,103]. In skin, SLPI is mainly expressed in keratinocytes and infiltrating neutrophils. Microorganisms regulate SLPI expression in keratinocytes, suggesting that SLPI can impact microbiota composition and burden in the epidermis. A sustained downregulation of SLPI by herpes simplex virus was proposed to render keratinocytes more susceptible to subsequent infection with human papillomavirus (HPV16), [103]. Thus, the presence of SLPI at the skin barrier has the potential to shape skin virome. However, it remains to be determined whether antiviral, bactericidal, or anti-fungal activity of SLPI is relevant for skin pathophysiology.

With respect to SLPI's broad role in skin defense, the impaired healing of cutaneous wounds in SLPI-deficient mice underscores the importance of SLPI in promoting the repair of injured skin [104]. A breach of the epithelium increases the risk of infection and potentially jeopardizes the life of the organism. Therefore, the wound is quickly sterilized by recruited leukocytes before it is closed by the epithelium. The lack of SLPI was accompanied by increased leukocyte infiltration and delayed re-epithelialization in mice subjected to the cutaneous injury model [104,105]. These data suggest that SLPI regulates two key stages of wound healing: the kinetics of inflammatory cell influx and the growth rate of new tissue. These effects of SLPI were subsequently attributed mainly to its ability to functionally control neutrophil elastase (NE).

NE is a serine protease stored in the primary granules of neutrophils. NE was proposed to target several substrates in the neutrophil-enriched wounds, including proepithelin [104,105]. NE-mediated processing of proepithelin generates fragments (epithelins) that exert contrasting effects on epithelial cells and neutrophils. Proepithelin acts as a growth factor for epithelial cells, but it inhibits neutrophil activation. On the other hand, epithelins inhibit the growth of epithelial cells, but stimulate neutrophils to secrete proinflammatory mediators. SLPI, as a factor that limits the NE-mediated conversion of proepithelin to epithelin, shifts the balance towards a less-excessive, more-timely controlled activation of neutrophils, as well as the promotion of epithelial regeneration. Proepithelin can replace SLPI in restoring normal wound healing in SLPI deficient mice, supporting the importance of this alliance between proepithelin and SLPI in the inhibitor-regulated repair of injured skin [105].

SLPI not only inhibits NE activity upon discharge of this protease from neutrophils, but also limits the deposition of NE into extracellular milieu together with other components of NETs. Chromatin remodeling that precedes NET release is linked to the NE-mediated histone processing. SLPI restricts NET release, at least partly through limiting core histone cleavage by NE [106]. Although SLPI-deficient neutrophils generate more NETs compared to normal neutrophils, SLPI does not completely prevent NET release. NETs deposited into the skin environment by immigrant neutrophils (under inflammatory conditions) contain SLPI, along with NE or CatG and DNA. SLPI/NE/DNA or SLPI/CatG/DNA structures stimulate the production of IFN-α from pDCs [99].

Although pDCs are scant in the skin at steady state, they infiltrate injured skin and produce type 1 interferons in response to nucleic acids [107]. Similar to chronic inflammatory conditions, nucleic acids-mediated signaling in pDCs can be triggered by antimicrobial peptides, such as LL37 in the context of wound healing, and lead to inflammatory responses and re-epithelization of injured skin [107]. However, LL37, which is sufficient, but not necessary to trigger production of type 1 interferons in pDCs in an injured skin, is unlikely to be solely responsible for promoting wound repair in a type 1 interferon-dependent manner. The presence of SLPI- and NE- or CatG-decorated NETs in the vicinity of pDCs in damaged skin (under inflammatory conditions) [98,99], suggests that these structures might provide stimuli for pDCs to produce IFN-α and support epithelial repair.

Thus, the net result of SLPI's defensive actions might be the coordination of innate responses with the healing of skin injury.

8.3. Chemerin (encoded by RARRES2)

LL37 and SLPI share functional similarities, and in some contexts, such as neutrophil-dominated cutaneous inflammation, may play a redundant role in facilitating the recognition of nucleic acids by DCs. Chemerin, a more recently characterized skin-operating multifunctional protein, shares structural similarities with hCAP18/LL37. Despite a low primary sequence homology between chemerin and cathelicidins, the conserved positioning of key cysteine residues suggests that chemerin is structurally related to cathelicidins [108]. This predicted structural similarity between chemerin and cathelicidins has provided the first hint that chemerin, which is abundant in the epidermis, may play a role in this layer as an antimicrobial factor [109].

Chemerin, a secreted 16 kDa protein, was originally identified as a chemoattractant for pDCs, and subsequently other leukocyte subsets [108]. Its chemotactic function is relevant for skin defense, since chemerin can contribute to the recruitment of pDCs to inflamed skin [108]. Chemerin is secreted as a precursor protein. The activation of chemerin involves proteolytic processing of the C-terminus and removal of inhibitory amino acids [109–112]. Keratinocytes are the main source of chemerin in healthy skin, as seen through immunostaining and qPCR. With chronic skin inflammation, such as in psoriasis, chemerin expression in epidermis declines, but also co-localizes with dermal fibroblasts [113]. Since chemerin circulates in low micromolar concentrations in blood as a precursor protein, it can be involved in skin defense, followed by exudation of plasma and activation by serine proteases of the coagulation, fibrinolytic, and inflammatory cascades [112]. Much less is known how locally produced chemerin can be activated, but skin-expressed serine or cysteine proteases of endogenous and bacterial origin can process chemerin to generate functional chemerin peptides [109,110,114].

In addition to its role in the chemotaxis of immune cells, chemerin was reported to regulate adipocyte differentiation and glucose uptake by adipocyte-like 3T3-L1 cells [115]. Chemerin involvement in

adipocyte biology is supported by its copious expression, and the presence of chemerin receptors in different fat depots, including subcutaneous fat, suggests that chemerin has either an autocrine or paracrine effect on adipocytes. Moreover, epidemiological studies have revealed that chemerin is a risk factor for metabolic syndrome. This syndrome involves abdominal obesity and high serum triglycerides, as well as elevated blood pressure, high fasting blood sugar, and low HDL (high density cholesterol) levels [116]. Since impaired adipogenesis increases the chance of infection [66], chemerin might have an impact on skin defense by regulating function or metabolism of subcutaneous fat in situ and/or other fat depots in a systemic manner.

Recombinant chemerin functions as an antibacterial agent. Chemerin deficiency results in higher counts of viable bacteria associated with the epidermis in an experimental model of skin infection. Chemerin is also a relevant AMP in human keratinocytes, since endogenous chemerin is largely responsible for the natural antimicrobial activity secreted by the human epidermal equivalents [14,117]. Given its location at the interface between the body and environment, the epidermis is constantly challenged by microorganisms and is directly exposed to a highly pro-oxidative environment. Although the latter suggests that the antimicrobial activity of epidermal proteins and peptides might be regulated by oxidative conditions, few examples or mechanisms are known. A chemerin-derived Val^{66}-Pro^{85} peptide (p4), which embodies the majority of chemerin's anti-microbial activity, is regulated by redox conditions; thus, p4 is maximally effective as an antimicrobial agent in oxidative niches, such as at the skin surface. This environment can strongly augment the antimicrobial activity of chemerin p4 by supporting the formation of disulfide bonds, leading to potent antimicrobial p4 dimers [118].

Together, these data suggest that chemerin can be involved in antimicrobial protection in several ways: as a chemoattractant ligand for different leukocytes (such as dendritic cells and macrophages), directly as an antimicrobial agent, and potentially as a metabolically active protein and contributor to adipocyte differentiation.

The provided examples of the mechanisms underlying the protective role of three AMPs illustrate the complexity of the cutaneous defense system. Skin homeostasis can be maintained by versatile AMP functions, some of which are specific (such as NE inhibition by SLPI), and some are shared among AMPs (such as the ability to sense nucleic acids and control wound healing by both LL37 and SLPI, or chemotaxis by either chemerin or LL37). Notably, the production of specific AMPs in multiple layers, such as LL37 by the epidermis and dermal fat, suggests that skin layers co-opt similar strategies to fight infection at different depths. Alternatively, the functional flexibility of the AMPs may enable the delivery of specific skills when selected from an array of the AMP assets, depending on the context. This could explain why the expression of certain AMPs, including chemerin, subsides in one layer (the epidermis) and appears in the next layer as a product of dermal fibroblasts [113]. Skin conditions, such as pH, ionic potential, oxygen accessibility (which are known to control bactericidal function and proteolytic processing of AMPs, may act as a physiological switch that helps regulate the functional transition of AMPs in a layer- or niche-dependent manner.

In conclusion, host interaction with skin-associated microbes and microbial invasion are under the control of highly coordinated skin responses that encompass all skin layers, the immune and nervous system, as well as cutaneous microbiota. The intricate communication between all these systems is provided by shared molecular signaling factors including cytokines, chemoattractants, neurotransmitters, and metabolites. At a molecular level, layered barriers against microbes rely on different molecular components, among which microbiota-derived antimicrobial factors, host-derived AMPs, and antimicrobial lipids act as crucial controllers of microbial burden and pathogenicity.

Declaration of interest

None.

Acknowledgments

This work was supported by grants from Polish National Science CenterUMO-2014/12/W/NZ6/00454 and UMO-2017/25/B/NZ6/01003 (to JC) and UMO-2013/10/E/NZ6/00745 (to MK). JJ is supported by the Polish-U.S. Fulbright Commission.

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References

[1] G. Beke, Z. Dajnoki, A. Kapitany, K. Gaspar, B. Medgyesi, S. Poliska, Z. Hendrik, Z. Peter, D. Torocsik, T. Biro, A. Szegedi, Immunotopographical differences of human skin, Front. Immunol. 9 (2018) 424.

[2] P.L. Tong, B. Roediger, N. Kolesnikoff, M. Biro, S.S. Tay, R. Jain, L.E. Shaw, M.A. Grimbaldeston, W. Weninger, The skin immune atlas: three-dimensional analysis of cutaneous leukocyte subsets by multiphoton microscopy, J. Invest. Dermatol. 135 (1) (2015) 84–93.

[3] X.N. Wang, N. McGovern, M. Gunawan, C. Richardson, M. Windebank, T.W. Siah, H.Y. Lim, K. Fink, J.L. Yao Li, L.G. Ng, F. Ginhoux, V. Angeli, M. Collin, M. Haniffa, A three-dimensional atlas of human dermal leukocytes, lymphatics, and blood vessels, J. Invest. Dermatol. 134 (4) (2014) 965–974.

[4] L.J. Zhang, S.X. Chen, C.F. Guerrero-Juarez, F. Li, Y. Tong, Y. Liang, M. Liggins, X. Chen, H. Chen, M. Li, T. Hata, Y. Zheng, M.V. Plikus, R.L. Gallo, Age-related loss of innate immune antimicrobial function of dermal fat is mediated by transforming growth factor Beta, Immunity 50 (1) (2019) 121–136 e5.

[5] A.L. Byrd, Y. Belkaid, J.A. Segre, The human skin microbiome, Nat. Rev. Microbiol. 16 (3) (2018) 143–155.

[6] R.L. Gallo, Human skin is the largest epithelial surface for interaction with microbes, J. Invest. Dermatol. 137 (6) (2017) 1213–1214.

[7] J. Oh, A.L. Byrd, M. Park, N.C.S. Program, H.H. Kong, J.A. Segre, Temporal stability of the human skin microbiome, Cell 165 (4) (2016) 854–866.

[8] A.L. Cogen, K. Yamasaki, J. Muto, K.M. Sanchez, L. Crotty Alexander, J. Tanios, Y. Lai, J.E. Kim, V. Nizet, R.L. Gallo, Staphylococcus epidermidis antimicrobial delta-toxin (phenol-soluble modulin-gamma) cooperates with host antimicrobial peptides to kill group A Streptococcus, PLoS One 5 (1) (2010) e8557.

[9] V. Brinkmann, A. Zychlinsky, Beneficial suicide: why neutrophils die to make NETs, Nat. Rev. Microbiol. 5 (8) (2007) 577–582.

[10] T. Nakatsuji, T.H. Chen, S. Narala, K.A. Chun, A.M. Two, T. Yun, F. Shafiq, P.F. Kotol, A. Bouslimani, A.V. Melnik, H. Latif, J.N. Kim, A. Lockhart, K. Artis, G. David, P. Taylor, J. Streib, P.C. Dorrestein, A. Grier, S.R. Gill, K. Zengler, T.R. Hata, D.Y. Leung, R.L. Gallo, Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis, Sci. Transl. Med. 9 (378) (2017).

[11] A.E. Paharik, C.P. Parlet, N. Chung, D.A. Todd, E.I. Rodriguez, M.J. Van Dyke, N.B. Cech, A.R. Horswill, Coagulase-negative staphylococcal strain prevents Staphylococcus aureus colonization and skin infection by blocking quorum sensing, Cell Host Microbe 22 (6) (2017) 746–756 e5.

[12] I. Nagy, A. Pivarcsi, K. Kis, A. Koreck, L. Bodai, A. McDowell, H. Seltmann, S. Patrick, C.C. Zouboulis, L. Kemeny, Propionibacterium acnes and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes, Microbes Infect. 8 (8) (2006) 2195–2205.

[13] R. Ommori, N. Ouji, F. Mizuno, E. Kita, Y. Ikada, H. Asada, Selective induction of antimicrobial peptides from keratinocytes by staphylococcal bacteria, Microb. Pathog. 56 (2013) 35–39.

[14] M. Banas, A. Zegar, M. Kwitniewski, K. Zabieglo, J. Marczynska, M. Kapinska-Mrowiecka, M. LaJevic, B.A. Zabel, J. Cichy, The expression and regulation of chemerin in the epidermis, PLoS One 10 (2) (2015) e0117830.

[15] L.J. Zhang, R.L. Gallo, Antimicrobial peptides, Curr. Biol. 26 (1) (2016) R14–9.

[16] J.S. Meisel, G. Sfyroera, C. Bartow-McKenney, C. Gimblet, J. Bugayev, J. Horwinski, B. Kim, J.R. Brestoff, A.S. Tyldsley, Q. Zheng, B.P. Hodkinson, D. Artis, E.A. Grice, Commensal microbiota modulate gene expression in the skin, Microbiome 6 (1) (2018) 20.

[17] S. Naik, N. Bouladoux, J.L. Linehan, S.J. Han, O.J. Harrison, C. Wilhelm, S. Conlan, S. Himmelfarb, A.L. Byrd, C. Deming, M. Quinones, J.M. Brenchley, H.H. Kong, R. Tussiwand, K.M. Murphy, M. Merad, J.A. Segre, Y. Belkaid, Commensal-dendritic-cell interaction specifies a unique protective skin immune signature, Nature 520 (7545) (2015) 104–108.

[18] T.C. Scharschmidt, K.S. Vasquez, H.A. Truong, S.V. Gearty, M.L. Pauli, A. Nosbaum, I.K. Gratz, M. Otto, J.J. Moon, J. Liese, A.K. Abbas, M.A. Fischbach, M.D. Rosenblum, A wave of regulatory t cells into neonatal skin mediates

tolerance to commensal microbes, Immunity 43 (5) (2015) 1011–1021.
[19] M.C. Lebre, A.M. van der Aar, L. van Baarsen, T.M. van Capel, J.H. Schuitemaker, M.L. Kapsenberg, E.C. de Jong, Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9, J. Invest. Dermatol. 127 (2) (2007) 331–341.
[20] K.M. Aberg, M.Q. Man, R.L. Gallo, T. Ganz, D. Crumrine, B.E. Brown, E.H. Choi, D.K. Kim, J.M. Schroder, K.R. Feingold, P.M. Elias, Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers, J. Invest. Dermatol. 128 (4) (2008) 917–925.
[21] J.M. Brandner, M. Zorn-Kruppa, T. Yoshida, I. Moll, L.A. Beck, A. De Benedetto, Epidermal tight junctions in health and disease, Tissue Barriers 3 (1-2) (2015) e974451.
[22] K. Kabashima, T. Honda, F. Ginhoux, G. Egawa, The immunological anatomy of the skin, Nat. Rev. Immunol. 19 (1) (2019) 19–30.
[23] P. Di Meglio, G.K. Perera, F.O. Nestle, The multitasking organ: recent insights into skin immune function, Immunity 35 (6) (2011) 857–869.
[24] K.A.U. Gonzales, E. Fuchs, Skin and its regenerative powers: an alliance between stem cells and their niche, Dev. Cell 43 (4) (2017) 387–401.
[25] M.R. Kesting, M. Stoeckelhuber, F. Holzle, T. Mucke, K. Neumann, K. Woermann, F. Jacobsen, L. Steinstraesser, K.D. Wolff, D.J. Loeffelbein, N.H. Rohleder, Expression of antimicrobial peptides in cutaneous infections after skin surgery, Br. J. Dermatol. 163 (1) (2010) 121–127.
[26] B.O. Schroeder, Z. Wu, S. Nuding, S. Groscurth, M. Marcinowski, J. Beisner, J. Buchner, M. Schaller, E.F. Stange, J. Wehkamp, Reduction of disulphide bonds unmasks potent antimicrobial activity of human beta-defensin 1, Nature 469 (7330) (2011) 419–423.
[27] J. Harder, J.M. Schroder, 7 RNase, A novel innate immune defense antimicrobial protein of healthy human skin, J. Biol. Chem. 277 (48) (2002) 46779–46784.
[28] C. Maerki, S. Meuter, M. Liebi, K. Muhlemann, M.J. Frederick, N. Yawalkar, B. Moser, M. Wolf, Potent and broad-spectrum antimicrobial activity of CXCL14 suggests an immediate role in skin infections, J. Immunol. 182 (1) (2009) 507–514.
[29] J. Becam, T. Walter, A. Burgert, J. Schlegel, M. Sauer, J. Seibel, A. Schubert-Unkmeir, Antibacterial activity of ceramide and ceramide analogs against pathogenic Neisseria, Sci. Rep. 7 (1) (2017) 17627.
[30] T.K. Lin, D. Crumrine, L.D. Ackerman, J.L. Santiago, T. Roelandt, Y. Uchida, M. Hupe, G. Fabrias, J.L. Abad, R.H. Rice, P.M. Elias, Cellular changes that accompany shedding of human corneocytes, J. Invest. Dermatol. 132 (10) (2012) 2430–2439.
[31] S. Igawa, J.E. Choi, Z. Wang, Y.L. Chang, C.C. Wu, T. Werbel, A. Ishida-Yamamoto, A. Di Nardo, Human keratinocytes use sphingosine 1-Phosphate and its receptors to communicate Staphylococcus aureus invasion and activate host defense, J. Invest. Dermatol. (2019).
[32] K.R. Kirker, G.A. James, P. Fleckman, J.E. Olerud, P.S. Stewart, Differential effects of planktonic and biofilm MRSA on human fibroblasts, Wound Repair Regen. 20 (2) (2012) 253–261.
[33] K. Wolf, R. Muller, S. Borgmann, E.B. Brocker, P. Friedl, Amoeboid shape change and contact guidance: T-lymphocyte crawling through fibrillar collagen is independent of matrix remodeling by MMPs and other proteases, Blood 102 (9) (2003) 3262–3269.
[34] Z. Wang, Y. Lai, J.J. Bernard, D.T. Macleod, A.L. Cogen, B. Moss, A. Di Nardo, Skin mast cells protect mice against vaccinia virus by triggering mast cell receptor S1PR2 and releasing antimicrobial peptides, J. Immunol. 188 (1) (2012) 345–357.
[35] J.D. Heilborn, M.F. Nilsson, G. Kratz, G. Weber, O. Sorensen, N. Borregaard, M. Stahle-Backdahl, The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium, J. Invest. Dermatol. 120 (3) (2003) 379–389.
[36] R. Koczulla, G. von Degenfeld, C. Kupatt, F. Krotz, S. Zahler, T. Gloe, K. Issbrucker, P. Unterberger, M. Zaiou, C. Lebherz, A. Karl, P. Raake, A. Pfosser, P. Boekstegers, U. Welsch, P.S. Hiemstra, C. Vogelmeier, R.L. Gallo, M. Clauss, R. Bals, An angiogenic role for the human peptide antibiotic LL-37/hCAP-18, J. Clin. Invest. 111 (11) (2003) 1665–1672.
[37] H.J. Park, D.H. Cho, H.J. Kim, J.Y. Lee, B.K. Cho, S.I. Bang, S.Y. Song, K. Yamasaki, A. Di Nardo, R.L. Gallo, Collagen synthesis is suppressed in dermal fibroblasts by the human antimicrobial peptide LL-37, J. Invest. Dermatol. 129 (4) (2009) 843–850.
[38] J.W.J. van Kilsdonk, P.A.M. Jansen, E.H. van den Bogaard, C. Bos, M. Bergers, P. Zeeuwen, J. Schalkwijk, The effects of human beta-defensins on skin cells in vitro, Dermatology 233 (2–3) (2017) 155–163.
[39] M. Brauchle, K. Angermeyer, G. Hubner, S. Werner, Large induction of keratinocyte growth factor expression by serum growth factors and pro-inflammatory cytokines in cultured fibroblasts, Oncogene 9 (11) (1994) 3199–3204.
[40] R.M. Strieter, M.D. Burdick, B.N. Gomperts, J.A. Belperio, M.P. Keane, CXC chemokines in angiogenesis, Cytokine Growth Factor Rev. 16 (6) (2005) 593–609.
[41] B.A. Schmidt, V. Horsley, Intradermal adipocytes mediate fibroblast recruitment during skin wound healing, Development 140 (7) (2013) 1517–1527.
[42] A. Watabe, T. Sugawara, K. Kikuchi, K. Yamasaki, S. Sakai, S. Aiba, Sweat constitutes several natural moisturizing factors, lactate, urea, sodium, and potassium, J. Dermatol. Sci. 72 (2) (2013) 177–182.
[43] D.L. Bovell, A. MacDonald, B.A. Meyer, A.D. Corbett, W.M. MacLaren, S.L. Holmes, M. Harker, The secretory clear cell of the eccrine sweat gland as the probable source of excess sweat production in hyperhidrosis, Exp. Dermatol. 20 (12) (2011) 1017–1020.
[44] C.Y. Cui, D. Schlessinger, Eccrine sweat gland development and sweat secretion, Exp. Dermatol. 24 (9) (2015) 644–650.
[45] B. Schittek, R. Hipfel, B. Sauer, J. Bauer, H. Kalbacher, S. Stevanovic, M. Schirle, K. Schroeder, N. Blin, F. Meier, G. Rassner, C. Garbe, Dermcidin: a novel human antibiotic peptide secreted by sweat glands, Nat. Immunol. 2 (12) (2001) 1133–1137.
[46] S. Rieg, C. Garbe, B. Sauer, H. Kalbacher, B. Schittek, Dermcidin is constitutively produced by eccrine sweat glands and is not induced in epidermal cells under inflammatory skin conditions, Br. J. Dermatol. 151 (3) (2004) 534–539.
[47] T. Flad, R. Bogumil, J. Tolson, B. Schittek, C. Garbe, M. Deeg, C.A. Mueller, H. Kalbacher, Detection of dermcidin-derived peptides in sweat by ProteinChip technology, J. Immunol. Methods 270 (1) (2002) 53–62.
[48] S. Rieg, H. Steffen, S. Seeber, A. Humeny, H. Kalbacher, K. Dietz, C. Garbe, B. Schittek, Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo, J. Immunol. 174 (12) (2005) 8003–8010.
[49] R.L. Gallo, L.V. Hooper, Epithelial antimicrobial defence of the skin and intestine, Nat. Rev. Immunol. 12 (7) (2012) 503–516.
[50] M. Papini, S. Simonetti, S. Franceschini, L. Scaringi, M. Binazzi, Lysozyme distribution in healthy human skin, Arch. Dermatol. Res. 272 (1–2) (1982) 167–170.
[51] J.H. Park, G.T. Park, I.H. Cho, S.M. Sim, J.M. Yang, D.Y. Lee, An antimicrobial protein, lactoferrin exists in the sweat: proteomic analysis of sweat, Exp. Dermatol. 20 (4) (2011) 369–371.
[52] X. Dai, H. Okazaki, Y. Hanakawa, M. Murakami, M. Tohyama, Y. Shirakata, K. Sayama, Eccrine sweat contains IL-1alpha, IL-1beta and IL-31 and activates epidermal keratinocytes as a danger signal, PLoS One 8 (7) (2013) e67666.
[53] J.J. Thiele, S.U. Weber, L. Packer, Sebaceous gland secretion is a major physiologic route of vitamin E delivery to skin, J. Invest. Dermatol. 113 (6) (1999) 1006–1010.
[54] A. de Jong, T.Y. Cheng, S. Huang, S. Gras, R.W. Birkinshaw, A.G. Kasmar, I. Van Rhijn, V. Pena-Cruz, D.T. Ruan, J.D. Altman, J. Rossjohn, D.B. Moody, CD1a-autoreactive T cells recognize natural skin oils that function as headless antigens, Nat. Immunol. 15 (2) (2014) 177–185.
[55] A. Ryu, K. Arakane, C. Koide, H. Arai, T. Nagano, Squalene as a target molecule in skin hyperpigmentation caused by singlet oxygen, Biol. Pharm. Bull. 32 (9) (2009) 1504–1509.
[56] C. De Luca, G. Valacchi, Surface lipids as multifunctional mediators of skin responses to environmental stimuli, Mediators Inflamm. 2010 (2010) 321494.
[57] T. Alestas, R. Ganceviciene, S. Fimmel, K. Muller-Decker, C.C. Zouboulis, Enzymes involved in the biosynthesis of leukotriene B4 and prostaglandin E2 are active in sebaceous glands, J. Mol. Med. 84 (1) (2006) 75–87.
[58] C. Cossette, P. Patel, J.R. Anumolu, S. Sivendran, G.J. Lee, S. Gravel, F.D. Graham, A. Lesimple, O.A. Mamer, J. Rokach, W.S. Powell, Human neutrophils convert the sebum-derived polyunsaturated fatty acid Sebaleic acid to a potent granulocyte chemoattractant, J. Biol. Chem. 283 (17) (2008) 11234–11243.
[59] T. Nakatsuji, M.C. Kao, L. Zhang, C.C. Zouboulis, R.L. Gallo, C.M. Huang, Sebum free fatty acids enhance the innate immune defense of human sebocytes by upregulating beta-defensin-2 expression, J. Invest. Dermatol. 130 (4) (2010) 985–994.
[60] D. Torocsik, D. Kovacs, S. Poliska, Z. Szentkereszty-Kovacs, M. Lovaszi, K. Hegyi, A. Szegedi, C.C. Zouboulis, M. Stahle, Genome wide analysis of TLR1/2- and TLR4-activated SZ95 sebocytes reveals a complex immune-competence and identifies serum amyloid A as a marker for activated sebaceous glands, PLoS One 13 (6) (2018) e0198323.
[61] D.Y. Lee, K. Yamasaki, J. Rudsil, C.C. Zouboulis, G.T. Park, J.M. Yang, R.L. Gallo, Sebocytes express functional cathelicidin antimicrobial peptides and can act to kill propionibacterium acnes, J. Invest. Dermatol. 128 (7) (2008) 1863–1866.
[62] C.M. Chronnell, L.R. Ghali, R.S. Ali, A.G. Quinn, D.B. Holland, J.J. Bull, W.J. Cunliffe, I.A. McKay, M.P. Philpott, S. Muller-Rover, Human beta defensin-1 and -2 expression in human pilosebaceous units: upregulation in acne vulgaris lesions, J. Invest. Dermatol. 117 (5) (2001) 1120–1125.
[63] R. Glaser, J. Harder, H. Lange, J. Bartels, E. Christophers, J.M. Schroder, Antimicrobial psoriasin (S100A7) protects human skin from Escherichia coli infection, Nat. Immunol. 6 (1) (2005) 57–64.
[64] M. Dahlhoff, C.C. Zouboulis, M.R. Schneider, Expression of dermcidin in sebocytes supports a role for sebum in the constitutive innate defense of human skin, J. Dermatol. Sci. 81 (2) (2016) 124–126.
[65] D. Kovacs, M. Lovaszi, S. Poliska, A. Olah, T. Biro, I. Veres, C.C. Zouboulis, M. Stahle, R. Ruhl, E. Remenyik, D. Torocsik, Sebocytes differentially express and secrete adipokines, Exp. Dermatol. 25 (3) (2016) 194–199.
[66] L.J. Zhang, C.F. Guerrero-Juarez, T. Hata, S.P. Bapat, R. Ramos, M.V. Plikus, R.L. Gallo, Innate immunity. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection, Science 347 (6217) (2015) 67–71.
[67] R.K. Zwick, C.F. Guerrero-Juarez, V. Horsley, M.V. Plikus, Anatomical, Physiological, and Functional Diversity of Adipose Tissue, Cell Metab. 27 (1) (2018) 68–83.
[68] W. Wang, P. Seale, Control of brown and beige fat development, Nat. Rev. Mol. Cell Biol. 17 (11) (2016) 691–702.
[69] S.S. Choe, J.Y. Huh, I.J. Hwang, J.I. Kim, J.B. Kim, Adipose tissue remodeling: its role in energy metabolism and metabolic disorders, Front. Endocrinol. (Lausanne) 7 (2016) 30.

[70] C.M. Alexander, I. Kasza, C.L. Yen, S.B. Reeder, D. Hernando, R.L. Gallo, C.A. Jahoda, V. Horsley, O.A. MacDougald, Dermal white adipose tissue: a new component of the thermogenic response, J. Lipid Res. 56 (11) (2015) 2061–2069.
[71] R.R. Driskell, C.A. Jahoda, C.M. Chuong, F.M. Watt, V. Horsley, Defining dermal adipose tissue, Exp. Dermatol. 23 (9) (2014) 629–631.
[72] K. Ikeda, P. Maretich, S. Kajimura, The common and distinct features of Brown and beige adipocytes, Trends Endocrinol. Metab. 29 (3) (2018) 191–200.
[73] K. Wojciechowicz, K. Gledhill, C.A. Ambler, C.B. Manning, C.A. Jahoda, Development of the mouse dermal adipose layer occurs independently of subcutaneous adipose tissue and is marked by restricted early expression of FABP4, PLoS One 8 (3) (2013) e59811.
[74] H.C. West, C.L. Bennett, Redefining the role of langerhans cells As immune regulators within the skin, Front. Immunol. 8 (2017) 1941.
[75] O. Tirosh, S. Conlan, C. Deming, S.Q. Lee-Lin, X. Huang, N.C.S. Program, H.C. Su, A.F. Freeman, J.A. Segre, H.H. Kong, Expanded skin virome in DOCK8-deficient patients, Nat. Med. 24 (12) (2018) 1815–1821.
[76] Y.E. Chen, M.A. Fischbach, Y. Belkaid, Skin microbiota-host interactions, Nature 553 (7689) (2018) 427–436.
[77] O.J. Harrison, J.L. Linehan, H.Y. Shih, N. Bouladoux, S.J. Han, M. Smelkinson, S.K. Sen, A.L. Byrd, M. Enamorado, C. Yao, S. Tamoutounour, F. Van Laethem, C. Hurabielle, N. Collins, A. Paun, R. Salcedo, J.J. O'Shea, Y. Belkaid, Commensal-specific T cell plasticity promotes rapid tissue adaptation to injury, Science 363 (6422) (2019).
[78] T. Nakatsuji, H.I. Chiang, S.B. Jiang, H. Nagarajan, K. Zengler, R.L. Gallo, The microbiome extends to subepidermal compartments of normal skin, Nat. Commun. 4 (2013) 1431.
[79] T. Nakatsuji, T.H. Chen, A.M. Two, K.A. Chun, S. Narala, R.S. Geha, T.R. Hata, R.L. Gallo, Staphylococcus aureus exploits epidermal barrier defects in atopic dermatitis to trigger cytokine expression, J. Invest. Dermatol. 136 (11) (2016) 2192–2200.
[80] S.J. Han, A. Glatman Zaretsky, V. Andrade-Oliveira, N. Collins, A. Dzutsev, J. Shaik, D. Morais da Fonseca, O.J. Harrison, S. Tamoutounour, A.L. Byrd, M. Smelkinson, N. Bouladoux, J.B. Bliska, J.M. Brenchley, I.E. Brodsky, Y. Belkaid, White adipose tissue is a reservoir for memory t cells and promotes protective memory responses to infection, Immunity 47 (6) (2017) 1154–1168 e6.
[81] S.X. Chen, L.J. Zhang, R.L. Gallo, Dermal white adipose tissue: a newly recognized layer of skin innate defense, J. Invest. Dermatol. 139 (5) (2019) 1002–1009.
[82] S.B. McMahon, F. La Russa, D.L. Bennett, Crosstalk between the nociceptive and immune systems in host defence and disease, Nat. Rev. Neurosci. 16 (7) (2015) 389–402.
[83] D. Roosterman, T. Goerge, S.W. Schneider, N.W. Bunnett, M. Steinhoff, Neuronal control of skin function: the skin as a neuroimmunoendocrine organ, Physiol. Rev. 86 (4) (2006) 1309–1379.
[84] D. Roggenkamp, S. Kopnick, F. Stab, H. Wenck, M. Schmelz, G. Neufang, Epidermal nerve fibers modulate keratinocyte growth via neuropeptide signaling in an innervated skin model, J. Invest. Dermatol. 133 (6) (2013) 1620–1628.
[85] F. Ochoa-Cortes, T. Ramos-Lomas, M. Miranda-Morales, I. Spreadbury, C. Ibeakanma, C. Barajas-Lopez, S. Vanner, Bacterial cell products signal to mouse colonic nociceptive dorsal root ganglia neurons, Am. J. Physiol. Gastrointest. Liver Physiol. 299 (3) (2010) G723–32.
[86] F.A. Pinho-Ribeiro, W.A. Verri Jr, I.M. Chiu, Nociceptor sensory neuron-immune interactions in pain and inflammation, Trends Immunol. 38 (1) (2017) 5–19.
[87] I.M. Chiu, B.A. Heesters, N. Ghasemlou, C.A. Von Hehn, F. Zhao, J. Tran, B. Wainger, A. Strominger, S. Muralidharan, A.R. Horswill, J. Bubeck Wardenburg, S.W. Hwang, M.C. Carroll, C.J. Woolf, Bacteria activate sensory neurons that modulate pain and inflammation, Nature 501 (7465) (2013) 52–57.
[88] E. Marion, O.R. Song, T. Christophe, J. Babonneau, D. Fenistein, J. Eyer, F. Letournel, D. Henrion, N. Clere, V. Paille, N.C. Guerineau, J.P. Saint Andre, P. Gersbach, K.H. Altmann, T.P. Stinear, Y. Comoglio, G. Sandoz, L. Preisser, Y. Delneste, E. Yeramian, L. Marsollier, P. Brodin, Mycobacterial toxin induces analgesia in buruli ulcer by targeting the angiotensin pathways, Cell 157 (7) (2014) 1565–1576.
[89] S.W. Kashem, M.S. Riedl, C. Yao, C.N. Honda, L. Vulchanova, D.H. Kaplan, Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity, Immunity 43 (3) (2015) 515–526.
[90] K.M. Aberg, K.A. Radek, E.H. Choi, D.K. Kim, M. Demerjian, M. Hupe, J. Kerbleski, R.L. Gallo, T. Ganz, T. Mauro, K.R. Feingold, P.M. Elias, Psychological stress downregulates epidermal antimicrobial peptide expression and increases severity of cutaneous infections in mice, J. Clin. Invest. 117 (11) (2007) 3339–3349.
[91] S.J. Choe, D. Kim, E.J. Kim, J.S. Ahn, E.J. Choi, E.D. Son, T.R. Lee, E.H. Choi, Psychological stress deteriorates skin barrier function by activating 11beta-Hydroxysteroid dehydrogenase 1 and the HPA Axis, Sci. Rep. 8 (1) (2018) 6334.
[92] K.A. Radek, B. Lopez-Garcia, M. Hupe, I.R. Niesman, P.M. Elias, L. Taupenot, S.K. Mahata, D.T. O'Connor, R.L. Gallo, The neuroendocrine peptide catestatin is a cutaneous antimicrobial and induced in the skin after injury, J. Invest. Dermatol. 128 (6) (2008) 1525–1534.
[93] E.A. Antal, E.M. Loberg, P. Bracht, K.K. Melby, J. Maehlen, Evidence for intraaxonal spread of Listeria monocytogenes from the periphery to the central nervous system, Brain Pathol. 11 (4) (2001) 432–438.
[94] J.M. Draper, G. Huang, G.S. Stephenson, A.S. Bertke, D.A. Cortez, J.H. LaVail, Delivery of herpes simplex virus to retinal ganglion cell axon is dependent on viral protein Us9, Invest. Ophthalmol. Vis. Sci. 54 (2) (2013) 962–967.
[95] D. Ganguly, G. Chamilos, R. Lande, J. Gregorio, S. Meller, V. Facchinetti, B. Homey, F.J. Barrat, T. Zal, M. Gilliet, Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8, J. Exp. Med. 206 (9) (2009) 1983–1994.
[96] R. Lande, J. Gregorio, V. Facchinetti, B. Chatterjee, Y.H. Wang, B. Homey, W. Cao, Y.H. Wang, B. Su, F.O. Nestle, T. Zal, I. Mellman, J.M. Schroder, Y.J. Liu, M. Gilliet, Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide, Nature 449 (7162) (2007) 564–569.
[97] L.J. Zhang, G.L. Sen, N.L. Ward, A. Johnston, K. Chun, Y. Chen, C. Adase, J.A. Sanford, N. Gao, M. Chensee, E. Sato, Y. Fritz, J. Baliwag, M.R. Williams, T. Hata, R.L. Gallo, Antimicrobial peptide LL37 and MAVS signaling drive interferon-beta production by epidermal keratinocytes during skin injury, Immunity 45 (1) (2016) 119–130.
[98] J. Skrzeczynska-Moncznik, A. Wlodarczyk, M. Banas, M. Kwitniewski, K. Zabieglo, M. Kapinska-Mrowiecka, A. Dubin, J. Cichy, DNA structures decorated with cathepsin G/secretory leukocyte proteinase inhibitor stimulate IFNI production by plasmacytoid dendritic cells, Am J Clin Exp Immunol Cell Biol 2 (2) (2013) 186–194.
[99] J. Skrzeczynska-Moncznik, A. Wlodarczyk, K. Zabieglo, M. Kapinska-Mrowiecka, E. Marewicz, A. Dubin, J. Potempa, J. Cichy, Secretory leukocyte proteinase inhibitor-competent DNA deposits are potent stimulators of plasmacytoid dendritic cells: implication for psoriasis, J. Immunol. 189 (4) (2012) 1611–1617.
[100] P. Tewary, G. de la Rosa, N. Sharma, L.G. Rodriguez, S.G. Tarasov, O.M. Howard, H. Shirota, F. Steinhagen, D.M. Klinman, D. Yang, Oppenheim, beta-Defensin 2 and 3 promote the uptake of self or CpG DNA, enhance IFN-alpha production by human plasmacytoid dendritic cells, and promote inflammation, J. Immunol. 191 (2) (2013) 865–874.
[101] M. Majchrzak-Gorecka, P. Majewski, B. Grygier, K. Murzyn, J. Cichy, Secretory leukocyte protease inhibitor (SLPI), a multifunctional protein in the host defense response, Cytokine Growth Factor Rev. 28 (2016) 79–93.
[102] T.B. McNeely, M. Dealy, D.J. Dripps, J.M. Orenstein, S.P. Eisenberg, S.M. Wahl, Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro, J. Clin. Invest. 96 (1) (1995) 456–464.
[103] J.G. Skeate, T.B. Porras, A.W. Woodham, J.K. Jang, J.R. Taylor, H.E. Brand, T.J. Kelly, J.U. Jung, D.M. Da Silva, W. Yuan, W.M. Kast, Herpes simplex virus downregulation of secretory leukocyte protease inhibitor enhances human papillomavirus type 16 infection, J. Gen. Virol. 97 (2) (2016) 422–434.
[104] G.S. Ashcroft, K. Lei, W. Jin, G. Longenecker, A.B. Kulkarni, T. Greenwell-Wild, H. Hale-Donze, G. McGrady, X.Y. Song, S.M. Wahl, Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing, Nat. Med. 6 (10) (2000) 1147–1153.
[105] J. Zhu, C. Nathan, W. Jin, D. Sim, G.S. Ashcroft, S.M. Wahl, L. Lacomis, H. Erdjument-Bromage, P. Tempst, C.D. Wright, A. Ding, Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair, Cell 111 (6) (2002) 867–878.
[106] K. Zabieglo, P. Majewski, M. Majchrzak-Gorecka, A. Wlodarczyk, B. Grygier, A. Zegar, M. Kapinska-Mrowiecka, A. Naskalska, K. Pyrc, A. Dubin, S.M. Wahl, J. Cichy, The inhibitory effect of secretory leukocyte protease inhibitor (SLPI) on formation of neutrophil extracellular traps, J. Leukoc. Biol. 98 (1) (2015) 99–106.
[107] J. Gregorio, S. Meller, C. Conrad, A. Di Nardo, B. Homey, A. Lauerma, N. Arai, R.L. Gallo, J. Digiovanni, M. Gilliet, Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons, J. Exp. Med. 207 (13) (2010) 2921–2930.
[108] B.A. Zabel, M. Kwitniewski, M. Banas, K. Zabieglo, K. Murzyn, J. Cichy, Chemerin regulation and role in host defense, Am. J. Clin. Exp. Immunol. 3 (1) (2014) 1–19.
[109] P. Kulig, T. Kantyka, B.A. Zabel, M. Banas, A. Chyra, A. Stefanska, H. Tu, S.J. Allen, T.M. Handel, A. Kozik, J. Potempa, E.C. Butcher, J. Cichy, Regulation of chemerin chemoattractant and antibacterial activity by human cysteine cathepsins, J. Immunol. 187 (3) (2011) 1403–1410.
[110] P. Kulig, B.A. Zabel, G. Dubin, S.J. Allen, T. Ohyama, J. Potempa, T.M. Handel, E.C. Butcher, J. Cichy, Staphylococcus aureus-derived staphopain B, a potent cysteine protease activator of plasma chemerin, J. Immunol. 178 (6) (2007) 3713–3720.
[111] V. Wittamer, J.D. Franssen, M. Vulcano, J.F. Mirjolet, E. Le Poul, I. Migeotte, S. Brezillon, R. Tyldesley, C. Blanpain, M. Detheux, A. Mantovani, S. Sozzani, G. Vassart, M. Parmentier, D. Communi, Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids, J. Exp. Med. 198 (7) (2003) 977–985.
[112] B.A. Zabel, S.J. Allen, P. Kulig, J.A. Allen, J. Cichy, T.M. Handel, E.C. Butcher, Chemerin activation by serine proteases of the coagulation, fibrinolytic, and inflammatory cascades, J. Biol. Chem. 280 (41) (2005) 34661–34666.
[113] C. Albanesi, C. Scarponi, S. Pallotta, R. Daniele, D. Bosisio, S. Madonna, P. Fortugno, S. Gonzalvo-Feo, J.D. Franssen, M. Parmentier, O. De Pita, G. Girolomoni, S. Sozzani, Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment, J. Exp. Med. 206 (1) (2009) 249–258.
[114] S. Schultz, A. Saalbach, J.T. Heiker, R. Meier, T. Zellmann, J.C. Simon, A.G. Beck-Sickinger, Proteolytic activation of prochemerin by kallikrein 7 breaks an ionic

linkage and results in C-terminal rearrangement, Biochem. J. 452 (2) (2013) 271–280.
[115] J.L. Rourke, H.J. Dranse, C.J. Sinal, Towards an integrative approach to understanding the role of chemerin in human health and disease, Obes. Rev. 14 (3) (2012) 245–262.
[116] S.H. Chu, M.K. Lee, K.Y. Ahn, J.A. Im, M.S. Park, D.C. Lee, J.Y. Jeon, J.W. Lee, Chemerin and adiponectin contribute reciprocally to metabolic syndrome, PLoS One 7 (4) (2012) e34710.
[117] M. Banas, K. Zabieglo, G. Kasetty, M. Kapinska-Mrowiecka, J. Borowczyk, J. Drukala, K. Murzyn, B.A. Zabel, E.C. Butcher, J.M. Schroeder, A. Schmidtchen, J. Cichy, Chemerin is an antimicrobial agent in human epidermis, PLoS One 8 (3) (2013) e58709.
[118] U. Godlewska, B. Bilska, A. Zegar, P. Brzoza, A. Borek, K. Murzyn, O. Bochenska, A. Morytko, P. Kuleta, A. Kozik, E. Pyza, A. Osyczka, B.A. Zabel, J. Cichy, The antimicrobial activity of chemerin-derived peptide p4 requires oxidative conditions, J. Biol. Chem. 294 (4) (2019) 1267–1278.
[119] J. Weiss, K. Muello, M. Victor, P. Elsbach, The role of lipopolysaccharides in the action of the bactericidal/permeability-increasing neutrophil protein on the bacterial envelope, J. Immunol. 132 (6) (1984) 3109–3115.
[120] W.M. Shafer, J. Pohl, V.C. Onunka, N. Bangalore, J. Travis, Human lysosomal cathepsin G and granzyme B share a functionally conserved broad spectrum antibacterial peptide, J. Biol. Chem. 266 (1) (1991) 112–116.
[121] A. Belaaouaj, K.S. Kim, S.D. Shapiro, Degradation of outer membrane protein A in Escherichia coli killing by neutrophil elastase, Science 289 (5482) (2000) 1185–1188.
[122] S. Stenger, D.A. Hanson, R. Teitelbaum, P. Dewan, K.R. Niazi, C.J. Froelich, T. Ganz, S. Thoma-Uszynski, A. Melian, C. Bogdan, S.A. Porcelli, B.R. Bloom, A.M. Krensky, R.L. Modlin, An antimicrobial activity of cytolytic T cells mediated by granulysin, Science 282 (5386) (1998) 121–125.
[123] R.R. Shields-Cutler, J.R. Crowley, C.D. Miller, A.E. Stapleton, W. Cui, J.P. Henderson, Human metabolome-derived cofactors are required for the antibacterial activity of Siderocalin in urine, J. Biol. Chem. 291 (50) (2016) 25901–25910.
[124] A. Krause, R. Sillard, B. Kleemeier, E. Kluver, E. Maronde, J.R. Conejo-Garcia, W.G. Forssmann, P. Schulz-Knappe, M.C. Nehls, F. Wattler, S. Wattler, K. Adermann, Isolation and biochemical characterization of LEAP-2, a novel blood peptide expressed in the liver, Protein Sci. 12 (1) (2003) 143–152.
[125] N. Orsi, The antimicrobial activity of lactoferrin: current status and perspectives, Biometals 17 (3) (2004) 189–196.
[126] P. Huang, W.S. Li, J. Xie, X.M. Yang, D.K. Jiang, S. Jiang, L. Yu, Characterization and expression of HLysG2, a basic goose-type lysozyme from the human eye and testis, Mol. Immunol. 48 (4) (2011) 524–531.
[127] X. Lu, M. Wang, J. Qi, H. Wang, X. Li, D. Gupta, R. Dziarski, Peptidoglycan recognition proteins are a new class of human bactericidal proteins, J. Biol. Chem. 281 (9) (2006) 5895–5907.
[128] T. Moreau, K. Baranger, S. Dade, S. Dallet-Choisy, N. Guyot, M.L. Zani, Multifaceted roles of human elafin and secretory leukocyte proteinase inhibitor (SLPI), two serine protease inhibitors of the chelonianin family, Biochimie 90 (2) (2008) 284–295.
[129] R.S. Koduri, J.O. Gronroos, V.J. Laine, C. Le Calvez, G. Lambeau, T.J. Nevalainen, M.H. Gelb, Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases A(2), J. Biol. Chem. 277 (8) (2002) 5849–5857.
[130] D. Yang, H.F. Rosenberg, Q. Chen, K.D. Dyer, K. Kurosaka, J.J. Oppenheim, Eosinophil-derived neurotoxin (EDN), an antimicrobial protein with chemotactic activities for dendritic cells, Blood 102 (9) (2003) 3396–3403.
[131] B. Becknell, T.E. Eichler, S. Beceiro, B. Li, R.S. Easterling, A.R. Carpenter, C.L. James, K.M. McHugh, D.S. Hains, S. Partida-Sanchez, J.D. Spencer, Ribonucleases 6 and 7 have antimicrobial function in the human and murine urinary tract, Kidney Int. 87 (1) (2015) 151–161.
[132] S.M. Damo, T.E. Kehl-Fie, N. Sugitani, M.E. Holt, S. Rathi, W.J. Murphy, Y. Zhang, C. Betz, L. Hench, G. Fritz, E.P. Skaar, W.J. Chazin, Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens, Proc Natl Acad Sci U S A 110 (10) (2013) 3841–3846.
[133] M. Uhlen, L. Fagerberg, B.M. Hallstrom, C. Lindskog, P. Oksvold, A. Mardinoglu, A. Sivertsson, C. Kampf, E. Sjostedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C.A. Szigyarto, J. Odeberg, D. Djureinovic, J.O. Takanen, S. Hober, T. Alm, P.H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J.M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, F. Ponten, Proteomics. Tissue-based map of the human proteome, Science 347 (6220) (2015) 1260419.
[134] J.C. Keen, H.M. Moore, The genotype-tissue expression (GTEx) project: linking clinical data with molecular analysis to advance personalized medicine, J. Pers. Med. 5 (1) (2015) 22–29.
[135] C. UniProt, UniProt: a hub for protein information, Nucleic Acids Res. 43 (2015) D204–12 (Database issue).
[136] R. Petryszak, M. Keays, Y.A. Tang, N.A. Fonseca, E. Barrera, T. Burdett, A. Fullgrabe, A.M. Fuentes, S. Jupp, S. Koskinen, O. Mannion, L. Huerta, K. Megy, C. Snow, E. Williams, M. Barzine, E. Hastings, H. Weisser, J. Wright, P. Jaiswal, W. Huber, J. Choudhary, H.E. Parkinson, A. Brazma, Expression Atlas update–an integrated database of gene and protein expression in humans, animals and plants, Nucleic Acids Res. 44 (D1) (2016) D746–D752.
[137] A. Fabregat, S. Jupe, L. Matthews, K. Sidiropoulos, M. Gillespie, P. Garapati, R. Haw, B. Jassal, F. Korninger, B. May, M. Milacic, C.D. Roca, K. Rothfels, C. Sevilla, V. Shamovsky, S. Shorser, T. Varusai, G. Viteri, J. Weiser, G. Wu, L. Stein, H. Hermjakob, P. D'Eustachio, The reactome pathway knowledgebase, Nucleic Acids Res. 46 (D1) (2018) D649–D655.
[138] M. Uhlen, B.M. Hallstrom, C. Lindskog, A. Mardinoglu, F. Ponten, J. Nielsen, Transcriptomics resources of human tissues and organs, Mol. Syst. Biol. 12 (4) (2016) 862.
[139] S.M. Christensen, L.A. Dillon, L.P. Carvalho, S. Passos, F.O. Novais, V.K. Hughitt, D.P. Beiting, E.M. Carvalho, P. Scott, N.M. El-Sayed, D.M. Mosser, Meta-transcriptome profiling of the human-leishmania braziliensis cutaneous lesion, PLoS Negl. Trop. Dis. 10 (9) (2016) e0004992.
[140] R. Leinonen, H. Sugawara, M. Shumway, C. International nucleotide sequence database, the sequence read archive, Nucleic Acids Res. 39 (2011) D19–21 (Database issue).
[141] E. Afgan, D. Baker, B. Batut, M. van den Beek, D. Bouvier, M. Cech, J. Chilton, D. Clements, N. Coraor, B.A. Gruning, A. Guerler, J. Hillman-Jackson, S. Hiltemann, V. Jalili, H. Rasche, N. Soranzo, J. Goecks, J. Taylor, A. Nekrutenko, D. Blankenberg, The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update, Nucleic Acids Res. 46 (W1) (2018) W537–W544.

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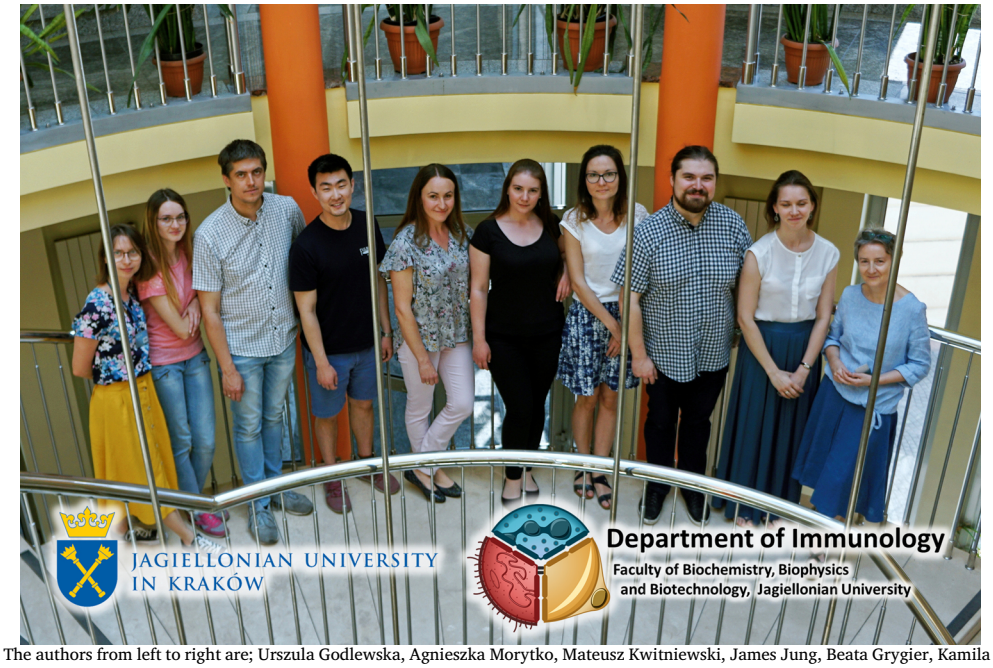

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