Cutaneous Barriers and Skin Immunity: Differentiating A Connected Network

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The skin is the outermost barrier of the organism that ensures protection from external harm. Lately, our view of the skin has evolved from an inert mechanical barrier to an active organ that can sense danger signals and mount perfectly adapted defense measures in response to invading pathogens. This Review highlights the different levels of the cutaneous barrier (the microbiome, chemical, physical, and immune barriers), their characteristics, and functional, highly interconnected network of cells and mediators that allow balanced defense measures to protect the body and maintain barrier integrity.

From an Anatomical to a Functional Description of the Skin

The skin, measuring ~2 m^2 in an adult, is our largest organ, and provides our organism integrity and identity. It further allows exchange with our environment, while simultaneously mediating protection from it. The skin balances body temperature and moisture, protects from UV light, transmits sensations, and represents a tight barrier against myriad microbes, toxins, and other dangers. Historically, the skin was seen as an organ comprising an outermost layer, the epidermis, and a subjacent connective tissue, the dermis. Whereas the epidermis comprises different stages of differentiated keratinocytes building up a layer of cornified cells, the stratum corneum (SC), which creates a mechanical barrier against potentially harmful invaders, the dermis is a rich collection of collagen fibres, fibroblasts, and nerve endings. While these distinct anatomical layers are critical to our understanding of the organization of the skin, they accomplish many disparate functions. Currently, researchers divide the skin into four, carefully orchestrated, functional levels of the cutaneous barrier: the microbiome barrier, the chemical barrier, the physical barrier, and the immune barrier. These developed during evolution and function to both stabilize and restore cutaneous homeostasis and to mount measures of defense when needed. Alterations in each component of the skin barrier can cause pathogenic conditions, such as skin infections, sterile skin inflammation, allergic sensitization, or cutaneous tumor development. Consequently, the best possible understanding of the functioning of the different parts of the cutaneous barrier is a prerequisite to develop strategies to conserve the integrity of the skin and to support the recovery of disturbed barriers. In this Review, we highlight the peculiarities of each barrier compartment and their interconnection, and summarize recent insights into dysregulation and disease development based on skin barrier dysfunction.

The Cutaneous Barrier: Its Levels and Basic Functions

The microbiome barrier is the outermost layer of the cutaneous barriers (Figure 1). It comprises diverse microbial communities, which cover all surface areas of the skin. The composition of these microbial communities includes bacteria, fungi, and viruses, and is fairly stable. Culture-independent genomic approaches have shown that, in contrast to the gut microbiome, the skin microbiota is dominated by Actinobacteria with an abundance of Gram-positive bacteria, such as *Staphylococcus*, *Propionibacterium*, and *Corynebacterium* species. Stability is preserved through a multitude of communication pathways and several checks and balances that exist

Highlights

The skin acts as an active immune organ, where microbiome, chemical, physical, and immune barriers form an interactive network. Barrier disruption contributes to pathogenic skin conditions, such as infections, inflammation, allergy, or cancer.

The microbiome is a complex ecosystem where commensals keep pathogenic bacteria, such as *Staphylococcus aureus*, under control and instruct cutaneous immunity.

The chemical barrier maintains the moisture and acid mantle of the skin, which inhibit the growth of bacterial pathogens.

Keratinocytes form the physical barrier, preserving the structure of the skin by forming tight junctions and carrying out immune functions, such as the secretion of cytokines, antimicrobial peptides, and antigen presentation.

The immune barrier comprises innate and adaptive immune cells, which are either resident or recruited to the skin and sense danger signals, protect against pathogens, and mount memory responses.

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between the microbes, their communities, and skin cells [1]. Several studies have addressed how commensal bacteria within these communities control potentially pathogenic bacteria. For example, the serine protease Esp secreted by *Staphylococcus epidermidis* inhibits colonization by *Staphylococcus aureus* and blocks the formation of *S. aureus* biofilms [2]. Some *S. epidermidis or Staphylococcus lugdunensis* strains produce antibiotics to specifically prevent *S. aureus* survival [3,4]. In human keratinocytes, *S. epidermidis* induces the expression of antimicrobial peptides and/or proteins (AMPs) and activates pathways distinct from *S. aureus*, resulting in *S. epidermidis*-orchestrated innate immune alertness [5].

The microbial communities on the skin also constitute a living and ideal first response barrier to environmental factors. They act as a border post and transmit external signals to the functional immune network of the skin. The outcome of this threefold crosstalk between skin cells, the skin immune system, and skin microbiota determines the functionality of the microbiome barrier [6].

The definition of the chemical barrier of the skin is less sharp compared with other parts of the cutaneous barrier and is tightly connected to the physical barrier (see next paragraph). Commonly, the 'chemical barrier' comprises factors that contribute to the acidic surface pH and compounds that together comprise the 'natural moisturizing factor' (NMF) (Figure 1). Schade and Marchionini coined the term 'Säuremantel' of the skin to explain the safety belt of acidity that covers it [7]. The NMF collectively refers to these hygroscopic compounds and represents ~20–30% of the dry

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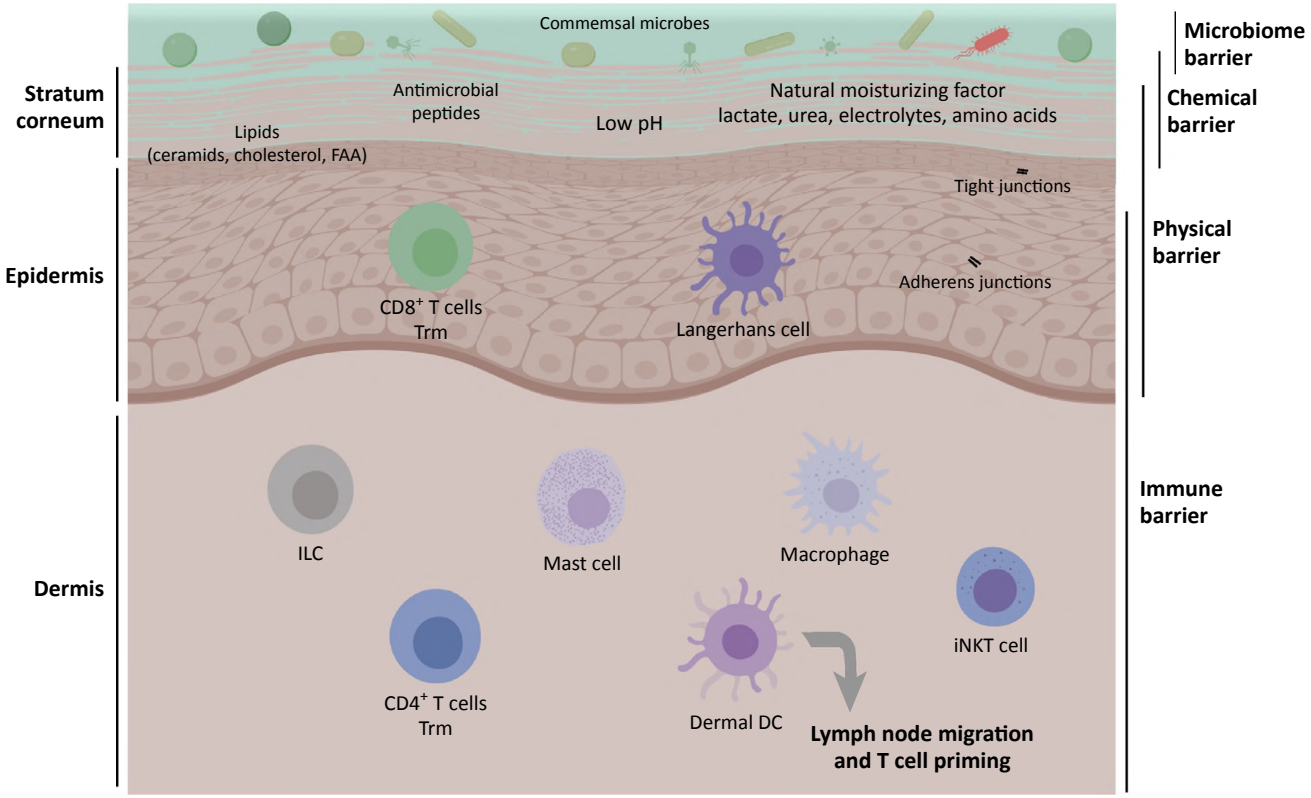

Figure 1. Levels and Components of the Cutaneous Barrier. Abbreviations: FAA, free fatty acids; ILC, innate lymphoid cell; iNKT, invariant natural killer cell; Trm, tissue-resident memory cell.

weight of corneocytes [8]. Much of the NMF comprises amino acids and their derivatives (pyrrolidone carboxylic acid and urocanic acid), resulting from the proteolysis of epidermal filaggrin (FLG) [9,10]. Changes in the NMF are thought to also alter the SC pH and SC lipids, indicating an interdependence between the chemical and the physical barrier functions [11]. Other components of the NMF found not only within, but also external to the corneocytes include lactates, urea, and electrolytes. Lactate and potassium also have an important role in maintaining the state of hydration and physical properties of the SC, such as its pH [8,12].

Important parts of the physical barrier are the SC and the system of tight junctions (TJ) and their regulation (Figure 1). The formation of SC is the consequence of keratinocytes maturing, moving up the epidermal layers to finally become corneocytes by terminal differentiation. These corneocytes are flattened and denucleated keratinocytes and their membranes are replaced by a 'cornified envelope' [13]. Keratinocytes of one layer below the SC, the stratum granulosum, contain: (i) granules with important proteins, such as FLG, loricrin, and keratin filaments; and (ii) laminar bodies (LB) with lipids, corneodesmosins, and kallikreins [10,14]. The contents of those granules fill the intercellular space of the SC, which is often referred to as 'mortar between bricks' [15]. Many of the proteins that contribute to the 'mortar' were understood once monogenetic diseases, such as peeling skin syndrome, skin fragility syndromes, or ichthyosis, were unraveled [14]. Adjacent keratinocytes of the stratum granulosum are further connected by so-called TJ proteins to form a barrier especially against water and solutes [14]. TJ proteins are mostly transmembraneous and include claudins, occludin, and zona occludens (ZO) proteins. TJ protein claudin-1 null mutations lead to neonatal ichthyosis sclerosing cholangitis (NISCH) syndrome, demonstrating the crucial role of this protein in determining the physical barrier of the skin [16]; suppression of claudin-1 expression is also involved in inflammatory skin diseases [17]. Claudin-1, claudin-4, occluding, and ZO-1 are highly effective in regulating the transport of intermediate-sized and large molecules as well as ions from inside to outside because these are all stopped at the TJ level of the stratum granulosum following dermal injection [18,19]. It is believed that this also holds true for outside-to-inside transport, although the evidence for this is less firm.

Cells of the physical barrier further contribute to chemical barrier function by producing epidermal lipids. Here, keratinocytes deliver mainly triglycerides and cholesterols, whereas sebaceous glands secrete triglycerides, wax esters, and squalene containing sebum into the upper part of the hair follicle, thereby delivering those lipids directly onto the SC. Bacteria and yeasts then hydrolyze triglycerides into free fatty acids (FFA), contributing to the acidification (also see the previous paragraph) of the skin [20]. These intercellular lipids provide a tight and effective barrier that also regulates transepidermal water loss (TEWL). However, most of the water in the SC is inside the corneocytes and there is no free water between the lamellae.

The immune barrier represents the final part of the cutaneous barrier and comprises a variety of resident immune cells populating the epidermis and dermis (Figure 1). The cellular composition of the immune barrier contains innate sentinels, such as several types of resident antigen-presenting cell, innate lymphoid cells, innate-like cells, keratinocytes, and adaptive tissue-resident memory cells, which all work together to maintain the barrier integrity. This immune armada efficiently senses microbial danger signals via pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) and initiates an adequate immune response, subsequent tissue inflammation by recruitment of circulating counterparts, and further barrier disruption to clear the invasion. Besides this necessary but harmful action, resident immune cells further contribute to barrier repair and homeostasis. Given that cells of the immune barrier are distributed all over the skin, this barrier is highly interconnected with other levels of the cutaneous barrier; for example, it responds to signals derived from epithelial cells and secretes

signals that orchestrate epithelial behavior. Components of the immune barrier sense microbial signals of the microbiome barrier, are shaped by the condition of the physical barrier, directly respond to parts of the chemical barrier, and can orchestrate these not only by disturbing, but also by supporting the regeneration and recovery of the previous levels of the cutaneous barrier (Figure 2).

Crosstalk of the Microbiome Barrier and other Barrier Elements

The cutaneous microbial communities evolved together with the skin and their composition and functional interdependence are essential to the overall function of the skin and its barriers.

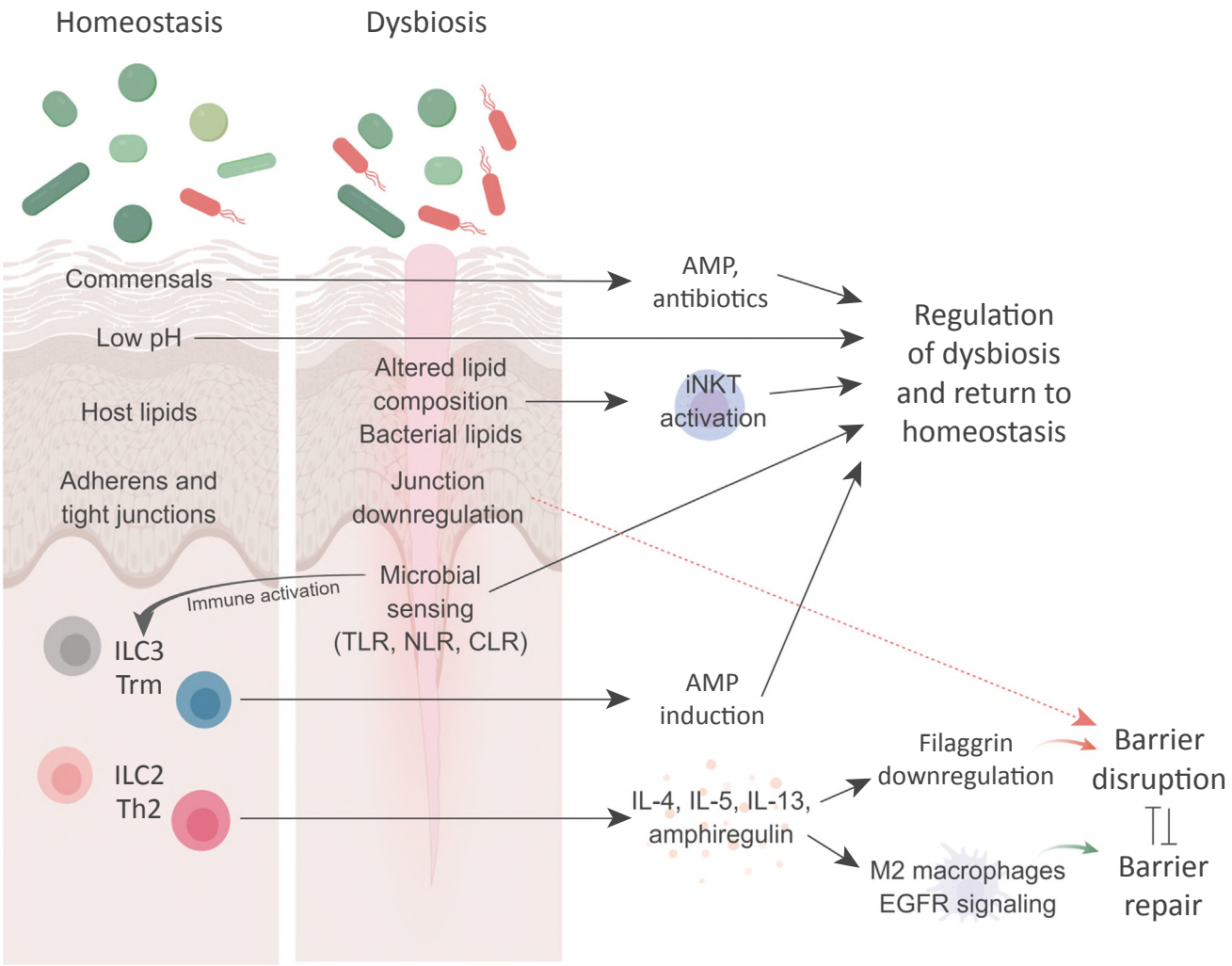

Figure 2. Homeostasis and Dysbiosis: A Delicate Balance between Microbial Diversity, Inflammation, and Barrier Repair. Under homeostatic conditions, commensals, epithelial cells, chemical barrier components, and immune cells quietly work hand in hand to maintain barrier integrity. In cases of dysbiosis, inflammatory pathways are activated that lead to barrier disruption and a vicious circle of inflammation and consecutively enhanced dysbiosis. However, this vicious circle can be stopped by immune cells because they induce antimicrobial peptides (AMPs) in epithelial cells and activate M2 macrophages and epidermal growth factor receptor (EGFR) signaling, which in turn mediate barrier repair. Abbreviations: CLR, C-type lectin receptor; IL, interleukin; ILC, innate lymphoid cell; iNKT, invariant natural killer cell; NLR, Nod-like receptor; Th, T helper cell; TLR, Toll-like receptor; Trm, tissue-resident memory cell.

Breakdown of the cutaneous microbial communities is associated with skin diseases, as shown for atopic dermatitis dominated by *S. aureus* [21], and contributes to disease persistence [22]. By contrast, the recovery of the cutaneous microbiome indicates resolution of disease [21]. The breakdown of these well-balanced microbial communities is often referred to as dysbiosis. Dysbiosis may either be a consequence of the dysfunction of other parts of the cutaneous barrier or its cause. While the 'hen and egg' problem in atopic dermatitis is not solved regarding dysbiosis and cutaneous inflammation, recent studies identified that *S. aureus* expansion precedes detectable skin inflammation [23] and that *S. epidermidis* strain diversity associates with less severe disease, whereas clonal *S. aureus* strains are found in more severely affected patients [24], suggesting that dysbiosis is one of the initiating events in this case. Experimental models showed that a missing skin microbiome in germ-free mice (lacking both a cutaneous and intestinal microbiome) resulted in impaired anti-infectious IL-17 responses. These anti-infectious immune responses are mediated by $CD8^+$ T cells (Tc17) and were shown to be effective against *Candida albicans* or *Leishmania spp*. [25]. In addition, a defect in, or complete loss of, cutaneous barrier integrity also allows invasion of bacteria into deeper layers of the skin [26]. By contrast, components of the cutaneous microbiome also shape pathways and players of regulatory immune responses and immune tolerance, as shown for exposure early in life to skin commensals and the marked expansion and influx of regulatory T cells (Tregs) into the skin (Figure 3) [27].

The 'control' of the microbial composition on the skin is also maintained by the upper most cellular layer of the skin, the keratinocytes and their products. Following encounters with danger signals or immune triggers, keratinocytes produce AMPs, such as human β-defensins, cathelicidins, and RNAses, to coregulate the composition of the microbial communities (Figure 1). In addition, these signals upregulate pattern recognition receptors, such as Toll-like receptors (TLRs), to allow keratinocytes to mount adequate responses to microbial signals [28–30]. Conversely, *S. aureus* was shown *in vitro* and in porcine models to decrease the density and expression of TJ proteins, such as claudin-1, ZO-1 (TJP-1), ZO-2 (TJP-2), occludin, and the adherens junction (AJ) protein E-cadherin, demonstrating that the composition of the microbial communities or its dysbiosis co-determine the setup of the physical barrier [31,32].

Barrier disruption at different levels results in microbial dysbiosis, with expanding pathogenic bacteria causing inflammation and inflammation-derived signals from the skin, resulting in further barrier disruption that sustains the growth of pathogenic bacteria, particularly *S. aureus* [33]. This is also mirrored by monogenic diseases, such as Netherton syndrome, which results from mutations in *SPINK5*, which encodes a serine peptidase inhibitor and whose loss of function results in defects of the physical and chemical barriers. Netherton syndrome and hyper IgE syndrome can also cause skin barrier disruption at the level of the immune barrier, with STAT1/STAT3 mutations resulting in defects of the type 17 immune response and leading to chronic skin inflammation that includes eczema and a shift in the microbiota towards *S. aureus* and *Acinetobacter* spp. [34–36]. This shift further impairs immune response because *Acinetobacter* actively repress T cell cytokine production (TNF-α, IFN-γ, and IL-22) upon *C. albicans* or *S. aureus* stimulation, further reducing the antimicrobial tissue defense [36]. Furthermore, commensal bacteria appear to directly shape adaptive immune responses. Recently, *S. epidermidis* was shown in mouse models to secrete peptides that are presented on nonclassical MHCI molecules to induce *S. epidermidis*-specific Tc1 and Tc17 cells [37]. These Tc17 cells express markers of tissue residency and a specific signature that allows induction of tissue repair after wounding. Thus, commensal bacteria not only prevent colonization with pathogenic bacteria by secreting antibiotics, but also regulate adaptive immune surveillance. (Further recent publications and reviews focusing on the interaction between the microbiome and immune system are detailed in Table 1.)

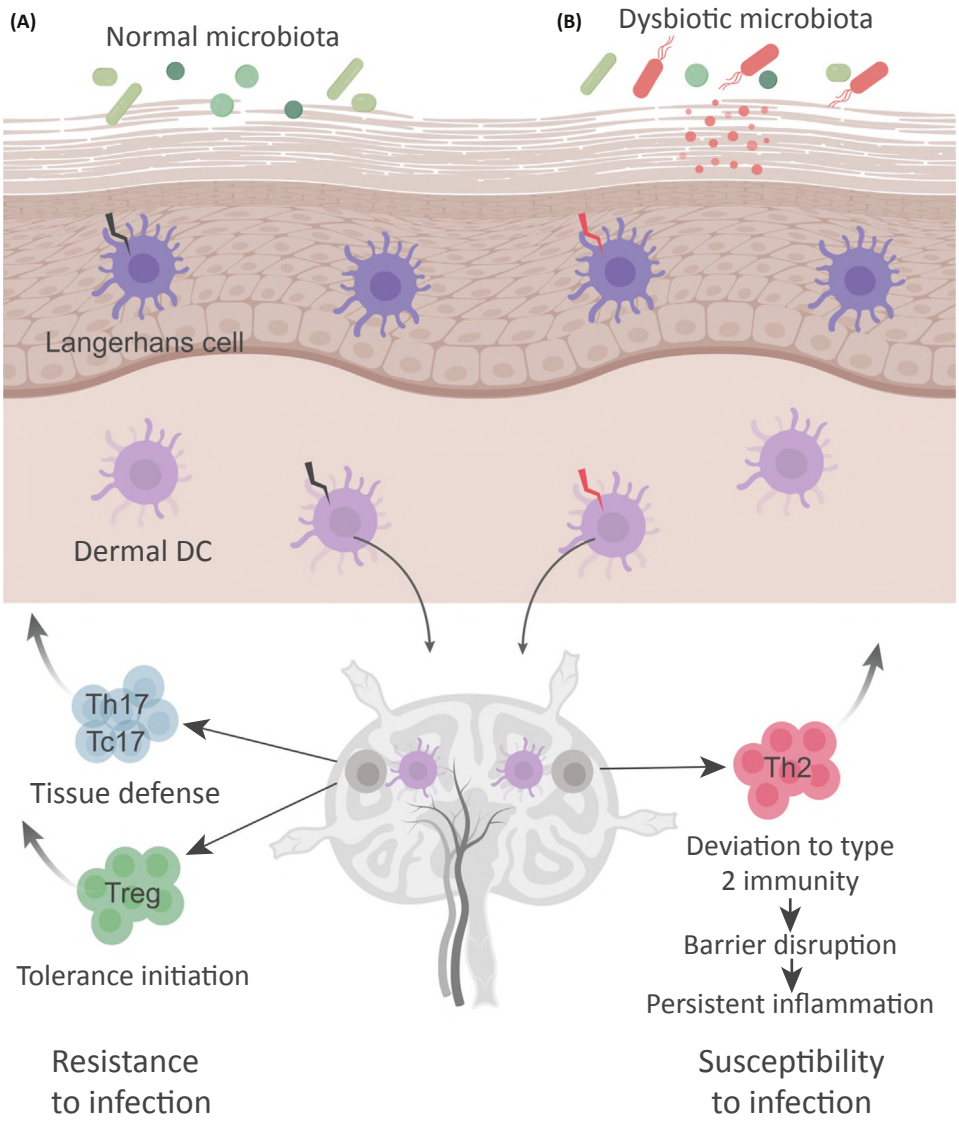

Figure 3. Shaping of the Immune System by the Skin Microbiome. A well-balanced cutaneous microbiome (A) has the potential to shape immune responses by establishing: (i) regulatory T cells (Tregs) and their immigration into the skin following 'early in life' exposure; and (ii) IL-17 producing $CD4^+$ and $CD8^+$ T cells (Th/Tc17) assuring effective immune defense against pathogens. By contrast, during dysbiosis (B), the skin microenvironment can lead to the priming of Th2 responses by migrating dermal dendritic cells (DCs), potentially fueling a cycle of inflammation, as outlined in Figure 2. Abbreviations: Tc, cytotoxic T cell; Th, T helper cell.

A disintegrin and metalloproteinase 17 (ADAM17) deficiency also leads to eczematous dermatitis and pustular lesions with *S. aureus* infections [38]. ADAM17 is a transmembrane protease that cleaves a variety of membrane-bound proteins to release their soluble forms and has a major role in the shedding of TNF-α and epidermal growth factor receptor (EGFR) and is thereby involved in the signaling pathways of both factors [39]. In concordance with this, a mouse model with ADAM17 deficiency manifested a phenotype similar to the human monogenic disease, with the development of atopic dermatitis associated with barrier

Table 1. Recent Literature on Microbiome–Immune Interactions

Refs	Main finding
[93]	Contextual control of skin immunity and inflammation by *Corynebacterium*: independent from the metabolic and inflammatory state of the host, *Corynebacterium* activates a subset of δγ T cells in an IL-23 mediated way, highlighting the dependence of the immune-activating abilities of commensals on the overall status of the host
[94]	Commensal microbiota modulates gene expression in the skin: bacterial colonization regulates gene expression in the epithelium with induction of innate immune responses and modulation of epidermal differentiation
[37]	Nonclassical immunity controls microbiota impact on skin immunity and tissue repair: *Staphylococcus epidermidis* specifically activates adaptive T cells in a nonclassical MHCI-dependent manner with a distinct gene expression profile leading to tissue repair and wound healing
[95]	Epidermal lipid composition, barrier integrity, and eczematous inflammation are associated with skin microbiome configuration: epidermal lipid composition and barrier integrity strongly influence the composition of the microbiome
[96]	Skin commensal *Malassezia globosa*-secreted protease attenuates *Staphylococcus aureus* biofilm formation: the commensal yeast *Malassezia* secretes a protease that is able to cleave *S. aureus* protein A, preventing biofilm formation and colonization of the skin by *S. aureus*; a cross-Kingdom defense mechanism
[23]	Skin colonization by *S. aureus* precedes the clinical diagnosis of atopic dermatitis in infancy: presence of *S. aureus* on skin that had yet to be affected was shown to precede the development of atopic dermatitis lesions
[24]	*S. aureus* and *S. epidermidis* strain diversity underlies pediatric atopic dermatitis: high-resolution sequencing of pediatric atopic dermatitis revealed a greater predominance of *S. aureus* than *S. epidermidis* at the species level and clonal *S. aureus* at the strain level in severely affected patients
[3]	Antimicrobials from human skin commensal bacteria protect against *S. aureus* and are deficient in atopic dermatitis: coagulase-negative staphylococci produce AMPs that selectively kill *S. aureus* and synergize with LL-37
[27]	A wave of regulatory T cells into neonatal skin mediates tolerance of commensal microbes: during a short developmental window, Tregs migrate into neonatal skin to induce tolerance towards commensal microbiota, establishing a healthy relationship between the host immune system and bacteria
[33]	Dysbiosis and *S. aureus* colonization drives inflammation in atopic dermatitis: using an ADAM17-deficient mouse model, dysbiosis and consecutive skin inflammation were induced that could be reversed by applying strain-specific antibiotics against *S. aureus*, *Corynebacterium mastitidis*, or *Corynebacterium bovis*, highlighting the induction of modulations by these bacteria that lead to eczematous lesions
[25]	Commensal–DC interactions specify a unique protective skin immune signature: *S. epidermidis* induced Tc17 responses in skin that are not associated with inflammation
[97]	Comprehensive review on skin microbial communities, their composition in health and disease as well as their dynamics and interactions with the immune system

impairment and chronic inflammatory skin disease. Importantly, microbiome analysis of eczematous dermatitis of these ADAM17-deficient mice showed *S. aureus*-dominated dysbiosis [33]. Of note, a common adverse effect of the treatment of patients with cancer with EGFR inhibitors is the development of skin rashes with pustules [40], from which *S. aureus* can be isolated [41,42].

Therefore, microbiome barrier is an integrated part of the cutaneous barriers (Figure 2). The diversity of the components of the cutaneous barriers, their plasticity and flexibility, together with their enormous potential to regenerate, partly rely on a well-functioning microbiome barrier. Precise orchestration of cutaneous barrier functioning through regulation of the microbial composition is a promising approach to use to intervene with disease development.

Crosstalk of the Chemical Barrier and other Barrier Elements

The acidic pH of the skin (4–6) has a central role in the functioning of the SC and cutaneous barrier, because proteases and enzymes involved in the generation of SC lipids function in a pH-dependent manner and, for example, the formation of the lamellae requires an acidic pH

[43–45]. Neutralization of the SC pH alone results in aberrant permeability of the barrier and decreased physical barrier integrity [46]. Acidity of the SC and sweat is also important for antimicrobial activity. The diverse composition of the cutaneous microbiome is maintained by an acidic pH, because it inhibits pathogens, such as *S. aureus*, and favors coagulase-negative staphylococci and corynebacteria [47,48]. Furthermore, efficacy of AMPs depends on the acidic pH of the skin. This is shown, for example, for dermcidin, an AMP derived from sweat. This medication functions optimally at pH 5.5, while its activity is reduced to 60% at pH 6.5 [49]. This highlights the strong dependence of a healthy microbiome barrier on the maintenance of the chemical barrier (Figure 2).

Crosstalk of the Physical Barrier and other Barrier Elements

TJ proteins are regulated following contact with microbes during both homeostatic colonization and infection. While low microbial loads strengthen TJ function partly by triggering pathogen recognition receptors (PRR; e.g., TLR2 on keratinocytes [50]), more intense contact with microbes, such as during infection or in highly colonized and inflamed skin, results in the downregulation of TJ proteins, as shown in atopic dermatitis for claudin-1 [51]. Importantly, not only do infection and inflammation regulate TJ proteins, but TJ proteins also determine inflammation, as shown for the dose-dependent regulation of claudin-1 in animal models of atopic dermatitis [17]. Furthermore, keratinocyte- and sebocyte-derived lipids not only have moisturizing functions, but also actively influence immune reactions because they drive the differentiation of alternatively activated macrophages [52] and contribute to the survival of memory T cells in the skin [53]. Proteases and protease inhibitors also contribute to shaping the unique organization of lipid lamellae in terms of their ideal lipid composition and function [54].

In addition, these interactions between the microbiome and the immune barrier highlight the role of keratinocyte-derived constituents and corneocytes for the functioning of the skin barrier and demonstrate how sensing and appropriately reacting to alterations in the local microenvironment contribute to its proper composition (Figure 2).

Cutaneous Innate Immune Sensing: Handing over Information Outside In and Inside Out

Innate immune pathways, such as the NFκdB pathway, the inflammasome, or other cytokine-activated signal transductions, can operate in keratinocytes, epidermal immune cells, such as Langerhans cells or γδ T cells, as well as in dermal-resident innate immune cells [different dendritic cell (DC) subtypes, mast cells, macrophages, and innate lymphoid cells (ILCs)], resident adaptive immune cells (resident T cells) or recruited innate and adaptive immune cells. Keratinocytes participate in immune responses and represent an innate immune cell capable of initiating cascades of immune events relevant, for example, for inflammatory disease development, such as in atopic dermatitis, microbial defense, and wound healing [55–60]. Innate immune receptors constantly encountering signals from the external environment include, among others, PRRs, such as the TLR family, NOD-like receptors (NLR), or C-type lectin receptors (CLR). Importantly, the expression of these receptors is tightly regulated, especially in the cells of the outermost layer of the skin [61–64]. In addition, the engagement of more than one innate immune receptor may be necessary for activation, thus ensuring proper regulation of responses [64,65]. For example, in keratinocytes, proinflammatory conditioning, such as through TNF or IL-6, may be necessary to establish responsiveness to TLR ligands [66]. Consequently, this safety lock stays closed in response to commensal bacteria or mechanical stress, but, once it opens, relevant mediators are produced, among them inflammation-amplifying cytokines, such as TNF or IL-6, and AMPs regulating bacterial colonization (and more). Immune cells recruited downstream of innate induced keratinocyte activation and TNF, IL-6,and IL-17C production further amplify inflammation, as seen in psoriasiform and

atopic dermatitis-like cutaneous inflammation [67,68]. In the latter, innate signaling through TLR2–6 upregulates cutaneous IL-6 approximately 400-fold, leading to systemic inflammation and local accumulation of $Gr1^{+}CD11b^{+}$ myeloid-derived suppressor cells (MDSC) that can suppress T cell-mediated immune responses in the skin [68]. The recruitment of these cells into the skin may contribute to the resolution of inflammation, but in cases of exacerbated skin inflammation, relevant cutaneous immune suppression is established [68]. Another important immune function of keratinocytes is their production of immune mediators, such as TSLP, IL-25, or IL-33, which are critical orchestrators of type 2 cutaneous immune responses through the conditioning of DCs [55,56]. Conversely, NFκB and inflammasome activation in the skin drive type 17 immune responses, as regularly found in psoriasis. Direct PAMP sensing in innate immune cells, such as DCs, may lead to pro- and anti-inflammatory immune responses depending on the co-stimulation [64,69]. TLR2 sensing amplifies modulatory IL-10 active in sensing nonpathogenic bacteria; by contrast, in the presence of type 2 immune cytokines, such as IL-4, this IL-10 is shut off, leading to persistent inflammation. Given that the cutaneous cytokine profile may translate into T cell profiles, the cascade of immune events may also produce stable immune phenotypes [22,70]. Many examples demonstrate that innate immune cells, such as mast cells [71,72], macrophages [73], and ILCs [74,75], in the cutaneous microenvironment can govern the decision to resist or amplify inflammation. These influences are critical to balance local homeostasis and health with immune defense or inflammatory disease, the latter possibly with systemic consequences.

Physical Barrier Disruption as an Activator of Skin Immune Sentinels

Epithelial barrier integrity is a prerequisite to prevent penetration of potential harmful substances from the surrounding environment. Skin-resident immune cells are fine-tuned sensors of barrier breaches because they are either activated or induced to migrate by barrier disruption and altered lipid compositions, potentially leading to the initiation of immune responses in the draining lymph nodes. One essential molecule in this scenario is E-cadherin expressed on epithelial cells, because it inhibits the activation of ILC2 cells [76]. Upon barrier disruption, E-cadherin is downregulated and cytokines, such as TSLP, IL-33, and IL-25, are released. These mediators consecutively activate ILC2 cells to secrete IL-4, IL-5, IL-13, and amphiregulin, in turn leading to a plethora of downstream functions involved in defense and allergic responses. Another important but less well-understood indicator of tissue disruption is the local composition of lipids. Invariant natural killer cells (iNKTs) that express an invariant TCRα chain (Vα24-Jα18) combined with a TCRβ chain with limited specificity are activated by glycolipids presented by CD1d molecules [77]. iNKTs not only recognize lipids of bacterial origin, but can also be activated in response to changes in the lipid composition of the skin upon barrier disruption. Similar to Th cells and ILCs, there are different types of iNKT [78] and ensure efficient and relevant cytokine responses to defend against the infecting pathogen or to restore barrier integrity.

The skin is populated not only by cells of the innate branch of immunity, but also by cells belonging to adaptive immunity. Indeed, the skin contains 1×10^{6} resident memory T cells (Trm)/cm^{2}, representing 2×10^{10} Trm cells in total residing in human skin, twice as many as circulate in blood [79,80]. Trm persist in the skin for long periods of time, probably throughout life, and are present in both the dermis and epidermis. Whereas CD69 expression and its interaction with E-selectin blocks the egress of Trm from the dermis and epidermis by sequestering the sphingosine-1-phosphate receptor (S1PR) [81], epidermal Trm co-express CD103, which binds to E-cadherin on keratinocytes and maintains their position at the outermost barrier. However, Trm not only sense physical barrier disruption, but also recognize changes in the microbiome barrier. Various studies using infection models showed that the

residency of T cells establishes after an initial infection and remains highest at the site of first encounter of the pathogen. In skin, Trm specific for herpes simplex [82], varicella zoster [83] and vaccinia virus [84], as wells as *Leishmania* [85], have been identified, highlighting that Trm in skin protect the barrier from pathogens that are commonly encountered in this organ. Interestingly, the newly developed memory is not only skin specific, but also spreads to other barrier organs to provide an overall surface protection (Figure 3). The potential of Trm to provide lifelong protection is addressed, for example, in the development of vaccines, but the mechanisms behind this longevity are not well understood. Pan *et al.* also recently showed that the chemical barrier impacts immune memory in skin. Here, FFA have been shown to not only support the functionality of Trm, but also to prolong their survival and, therefore, provide tissue-specific signals that are critical for maintaining protection [53]. How the lipid composition during barrier disruption is altered and how this could influence vaccination strategies aiming at efficient induction of long-term memory should be investigated further.

Immune Cells and the Restoration of Barrier Integrity

Inflammatory processes are beneficial for the host in terms of eradicating pathogens, but almost always occur alongside tissue damage and temporary loss of tissue functionality. To restore barrier integrity, two prerequisites have to be fulfilled: initiation of tissue healing and restoration of the microbiome barrier. Wound-healing processes in skin are mediated by various cells of innate and adaptive origin that work hand in hand to repair barrier breaches (Figure 2). For instance, Notch1 is activated in epithelial cells via its ligands, Jagged 1 and 2, leading to the induction of TNF-α and chemokines and, in turn, to the recruitment of IL-17F and IL-22 producing Rorγt+ ILC3 [86]. IL-22 in turn leads to wound healing by induction of the proliferation and migration of keratinocytes [87,88], myofibroblast differentiation, and extracellular matrix deposition [89]. The role of IL-17F in this scenario is not well understood and it is thought that it is not involved directly in wound closure, but instead keeps the local microbiota in check via the induction of AMPs in keratinocytes. Another important mediator of tissue repair is IL-33, which is induced upon barrier disruption in epithelial cells. IL-33 activates resident ILC2 cells by binding to the ST2 receptor to induce the secretion of IL-13, IL-5, and amphiregulin. In turn, IL-13 and IL-5 enhance the differentiation of M2 macrophages [90], whereas amhiregulin enhances the proliferation of epithelial cells via interaction with EGFR [91].

Whereas wound-healing responses are largely well understood, the restoration of the microbiome barrier is not and, therefore, is currently under intensive investigation. Interactions between the immunological barrier (IL-17 and IL-22) and the physical barrier (keratinocytes) lead to the induction of AMPs and the eradication of pathogenic bacteria, viruses, and fungi. However, how these AMPs selectively affect pathogenic invaders and not the commensal flora has not yet been elucidated. It is assumed that commensal bacteria are resistant to the effects of host AMPs [92] and that they produce their own set of bacterial AMPs to defend against pathogens and to create an advantage for commensals to colonize microbial niches on the skin [3].

Concluding Remarks

A well-balanced cutaneous barrier is a prerequisite to the maintenance of body integrity and health. We now know that the cutaneous barrier is a multifaceted structure comprising four functional barriers: the microbiome, chemical, physical, and immune barriers. Despite having their own characteristics and compositions, these parts of the cutaneous barrier are highly interconnected. This complex network is instrumental in the ability of the skin to fulfil its major tasks: the maintenance of the integrity of the body, which includes protection from external harm, and rapid restoration of the barrier and immune homeostasis in response to

Outstanding Questions

Will the use of metagenomics sequencing or novel culture methods be critical to functionally understand the skin microbiome? Are species- or strain-specific functions, often poorly resolved by 16S, critical for a proper microbiome barrier?

Will the study of biofluid samples enable the investigation of the interplay between the chemical barrier, bacteria, immune cells, and the physical barrier at a molecular level?

Can the use of transgenic mouse models with modified CD1a, CD1b, CD1c, and CD1e (present in humans but lacking in mice) expression enhance our understanding on the role of CD1-restricted T cells?

To what extent do commensal bacteria regulate not only microbial colonization of the skin, but also innate and adaptive immune responses?

Does knowledge of the interplay between the different barriers in the skin and the identification of master switches in this communication open new avenues of clinical intervention?

Do interventions aiming to restore homeostasis potentially harbor the risk of introducing new, as yet unknown, imbalances?

disturbances. However, if one barrier compartment is dysbalanced, this might lead to a vicious circle of inflammation and the development of skin disease. A major challenge for future treatment approaches will be to understand the interdependence of these four parts of the cutaneous barrier and to apply specific regimens that rebalance it (see Outstanding Questions).

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