YALE JOURNAL OF BIOLOGY AND MEDICINE 93 (2020), pp.133-143.

REVIEW

Unraveling Immune-Epithelial Interactions in Skin Homeostasis and Injury

Kody Mansfield and Shruti Naik*

Department of Pathology, Department of Medicine, and Ronald O. Perelman Department of Dermatology, New York University School of Medicine, New York, NY

The skin serves as a front line of defense against harmful environmental elements and thus is vital for organismal survival. This barrier is comprised of a water-tight epithelial structure reinforced by an arsenal of immune cells. The epithelial and immune components of the skin are interdependent and actively dialogue to maintain health and combat infectious, injurious, and noxious stimuli. Here, we discuss the molecular mediators of this crosstalk that establish tissue homeostasis and their dynamic adaptations to various stress conditions. In particular, we focus on immune-epithelial interactions in homeostatic tissue regeneration, during natural cycling of the hair follicle, and following skin injury. We also highlight the epithelial derived factors that orchestrate immunity. A comprehensive and mechanistic understanding of dynamic interactions between cutaneous immune cells and the epithelium can be leveraged to develop novel therapies to treat of range of skin diseases and boost skin health.

INTRODUCTION

Skin, one of the largest organs of the body, provides a water-tight barrier that protects us from an array of environmental insults. Proper development, homeostatic maintenance, and rapid restoration following injury of this structural barrier is therefore vital for life. Hair follicle and epidermal stem and progenitor cells give rise to differentiated keratinocytes and maintain the skin's physical barrier throughout our lifetime. This physical barrier is also reinforced by an immunological arsenal of resident and re-circulating immune cells. Communication between cutaneous immune cells and epithelial cells is emerging as a key regulator of the fitness and function of the skin epithelial barrier. In this review, we cover this essential crosstalk between the epithelial and immune cell populations that orchestrates skin homeostasis, injury responses, and inflammation.

THE SKIN IS A STRUCTURAL AND IMMUNOLOGICAL BARRIER

Structural Layers of the Skin

The skin is composed of two main compartments that are separated by a basement membrane, the epidermis and the dermis (Figure 1). The epidermis is the outer most layer of the skin which interfaces with the environment, while the dermis lies beneath providing tensile strength though its rich collagen matrix. The epidermis is cell dense and comprised of stratified epithelial layers

*To whom all correspondence should be addressed: Shruti Naik, Department of Pathology, Department of Medicine, and Ronald O. Perelman Department of Dermatology, New York University School of Medicine, New York, NY; Email: Shruti.Naik@nyulangone.org.

Abbreviations: LCs, Langerhans cells; TRM, CD8+ resident memory T cells; ILCs, innate lymphoid cells; DETCs, Dendritic Epidermal γδ T cells; APCs, antigen presenting cells.

Keywords: skin immunity, epithelial stem cells, immune-epithelial interactions, tissue regeneration

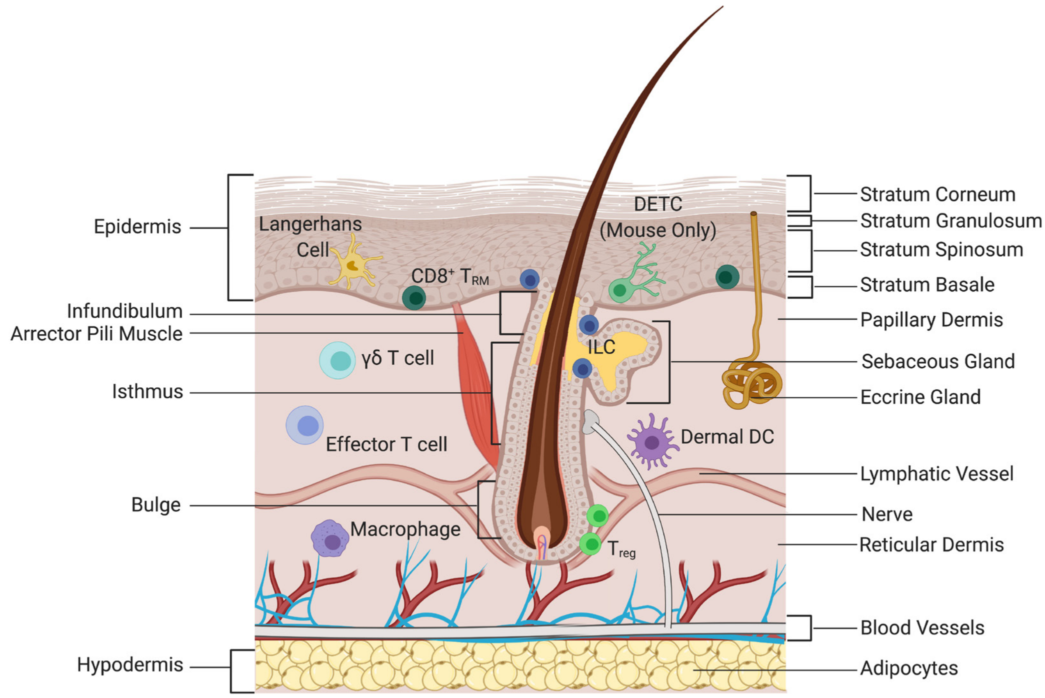

Figure 1. **Structural and cellular stratification of the skin**. Structural and cellular components of the skin illustrated relative to their *in vivo* localization within the tissue. Epidermal Structural Components: Stratum Corneum, Stratum Granulosum, Stratum Spinosum, Stratum Basale. Epidermal Cellular Components: Langerhans Cells, $CD8^+$ Tissue Resident Memory Cells ($CD8^+T_{RM}$), Dendritic Epidermal T Cell (DETC, mouse only). Appendage Structural Components: Pilosebaceous Unit (Hair Follicle, Sebaceous Gland, and Arrector Pili Muscle) and Eccrine Sweet Gland. Appendage Cellular Components: Infundibulum, Isthmus, Bulge, Nerve, Innate Lymphoid Cells (ILCs), T Regulatory Cells (T_{reg}). Dermis Structural Components: Papillary Dermis, Reticular Dermis, Lymphatic Vessel, Blood Vessels, Nerve Fibers. Dermis Cellular Components: γδ T Cell, Effector T Cell, Dermal Dendritic Cell (DC), Macrophage. Hypodermis Cellular Components: Adipocytes.

that provide physical, mechanical, and chemical protection while the dermis is largely comprised of extracellular matrix and sparsely dispersed dermal fibroblasts. Both the epidermis and dermis contain resident immune cell populations that engage in an active dialogue with structural cells of the tissue (*e.g.* epithelia) under steady state and stress conditions [1].

The major cell type of the epidermis is the keratinocyte. Keratinocytes are stratified into four distinct layers (Figure 1). The innermost layer is the stratum basale that is comprised of stem and progenitors attached to a basement membrane. These proliferative cells differentiate upwards into the stratum spinosum. In this layer, epithelial cells lose their mitotic potential and adopt a polygonal morphology, allowing them to more effectively form a structural barrier [2]. Spinous cells move up into the stratum granulosum where they produce granules and lamellar bodies generating a lipid filled barrier [3]. These cells begin to anucleate as they differentiate upwards into the outermost epidermal layer, the stratum corneum. This layer is comprised of flat-dead cells, corneocytes, void of all organelles, though rich in proteolytic resistant proteins, hydrophobic lipids, corneodesmosomes, and tight-junctions; all of which maintain the mechanical stability and form a plastic cling wrap like water-tight seal [4-9]. While the stratum corneum is primarily responsible for the biomechanical and hydrophobic properties of the barrier, the underlying layers of the epidermis also contribute to barrier function by maintaining tight junctions, adherens junctions, desmosomes, and gap junctions [10]. Synergistic functioning of these epidermal layers is paramount to limit the penetration of harmful, inflammation-inducing, infectious, and noxious agents.

The skin possesses appendages such as the pilosebaceous unit comprised of hair follicle, sebaceous gland, and arrector pili muscle, as well as eccrine and apocrine sweat glands (Figure 1). These "mini-organs" are essential for thermoregulation and organismal responses to stress via the secretion of surface lipids, regulation of water loss, and generation of protective hair. Keratinocytes of the hair follicle, like those of the interfollicular epidermis, are also organized into stratified layers differentiating perpendicular from the basement membrane with unique characteristics going down the hair follicle to the root. Moreover, the follicle itself can be segregated into distinct functional regions, based on the unique cellular and molecular features of their basal and suprabasal cells [11-13]. Going inward from the skin's surface, the hair follicle is fractionated into the infundibulum, isthmus, and hair bulge (Figure 1). Each of these regions of the follicle houses distinct progenitors, with unique homeostatic and injury responses, and engage in local cross talk with immune cells in their vicinity [14-17]. Hair follicle stem cells, residing in the bulge region of the follicle, not only generate hair in cyclical bouts, but also supply a cellular pool for the repair of the interfollicular epidermis following injury [18]. While both human and murine hair follicles undergo phases of quiescence, activation, and regression, some important species-specific distinctions remain. Unlike mice, human follicle cycling is asynchronous, as each follicle cycles independently of its neighbor [19]. Additionally, anagen, or the growth phase of the follicle, lasts years in humans as compared to weeks in mice [19]. Nevertheless, many important features of epithelial stem cell biology as well hair follicle biology have been unearthed using the murine follicle as a model.

The dermal compartment lies beneath the epidermis, and is structured into the papillary, reticular, and hypodermis (Figure 1). The papillary dermis is the region directly below the basement membrane and houses epidermal appendages (sweat glands and hair follicles), while the reticular dermis lies between the papillary dermis and the hypodermis (white adipose tissue) [20,21]. Papillary fibroblasts provide a supportive niche for the development and maintenance of the hair follicle while the reticular fibroblasts provide structural support by producing fibrillar extracellular matrix, and during wound repair, express smooth muscle actin [22,23]. In addition to papillary and reticular fibroblasts, sweat glands, and hair follicles, the dermis also contains structural cells of lymphatic vessels, blood vessels, periphery nerve cells, and immune cells. These cell types work in unison to ensure proper functioning of the skin barrier and immunological protection. However, an in-depth discussion of the constellation of interactions between these cellular populations is beyond the present scope and discussed in detail by Hsu and colleagues [24]. Here we focus on the essential dialogue between epithelial cells of the epidermis and hair follicle with cutaneous immune cells.

Stratification of the Skin Immunological Barrier

Healthy human and murine skin is populated by a diverse array of immune cells including dendritic cells, innate lymphoid cells, T lymphocytes, and macrophages [25]. Their recruitment, retention, and survival are tightly regulated and spatially restricted. This allows for precise targeting of cells to microenvironments within the skin where they exert their effects, and whose milieu meets the survival signals of each immune cell type (Figure 1).

Immune cells residing above the basement membrane, in the epidermis, include Langerhans cells (LCs), $CD8^+$ resident memory T cells (T_{RM}), innate lymphoid cells (ILCs), and in mouse Skin Dendritic Epidermal γδ T cells (DETCs) [17,25] (Figure 1). As prototypic antigen presenting cells (APCs), LCs continually surveil the epidermis, take up, and process antigens for presentation to T cells [26,27]. Of the epidermal resident cells derived from the lymphoid lineage, ILCs are highly responsive to local cytokine cues whereas the T cells rely on antigen presentation and cytokine signals from adjacent epithelia and/or innate immune cells for activation [17,28]. Even in the epidermis, there is regional segregation of ILCs that relates to their tissue modulatory function. For instance, the interfollicular epidermis is enriched for Type 3 ILCs (ILC3) while Type 2 ILCs (ILC2) localize to the sebaceous gland epithelium to limit sebocyte hyperplasia [17]. Phenotypically heterogeneous populations of $CD8^+$ T_{RM} reside in the interfollicular epidermis and rapidly respond to injury and infections [29,30]. T_{RMs} can persist and re-activate even in the absence of cognate antigen by heterogenous inflammatory cues [30]. In addition, mouse epidermis uniquely contains DETCs that take up residence in the neonatal epidermis and provide key signals for tissue repair [31].

Dermal T cells include effector and regulatory $CD4^+$ T cells (Th1, Th2, Th17, and T_{regs}) and interleukin (IL)-17A producing γδ T cells [32,33] (Figure 1). Dermal T cell populations, both effector and regulatory, are localized in close proximity of hair follicle [16]. The papillary dermis is also enriched for MHCII expressing dendritic cells [2,27] and under both steady state and inflammatory conditions, $CD4^+$ T effector populations are intimately associated with dermal dendritic cell populations [34,35].

Innate immune cells are also stratified to provide a tiered sentinel system with dendritic cells residing in the papillary dermis and macrophages in the lower dermal layers [36] (Figure 1). Both epidermal and dermal dendritic cells continually collect exogenous antigens through the epidermal barrier and engage T cells [35,37]. By contrast, resident macrophages are thought to localize to and survey post-capillary venules for systemic threats in the blood [38,39]. The proper development and maintenance of this organized network of immune cells is essential for optimal immune surveillance in the skin barrier. Below we discuss the key epithelial factors that direct the location and activity of immune cells.

EPITHELIA INSTRUCT HOMEOSTATIC IMMUNITY AND INITIATION IMMUNE RESPONSES

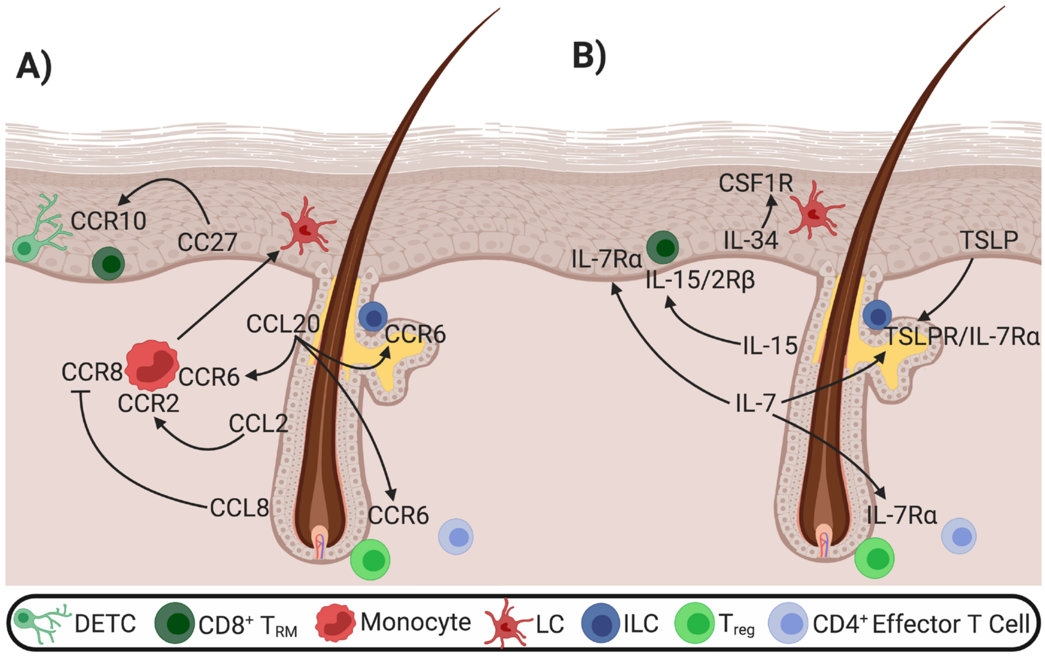

Figure 2. **Epithelial derived signals instruct local immunity**. **A**) Epithelial cells from the intrafollicular epidermis and distinct follicular regions produce chemokines to recruit immune cells to their vicinity. **B**) Epithelial cells supply key survival signals to maintain both innate and adaptive immune cells in the epithelial barrier.

Epithelial Signals Govern Immune Cell Localization and Survival in the Skin

Complex stratified immune networks in the skin are established via homing of the immune cells to distinct cutaneous microenvironments through tightly regulated and spatially restricted epithelial expression of chemokines and survival factors. Different fractions of the hair follicle have unique chemokine signatures [11] (Figure 2A). For instance, infundibular epithelia express CCL20, those of the isthmus CCL2, and of the bulge CCL8 [11]. Infundibular keratinocyte derived CCL20 binds to CCR6 as a skin homing signal for both effector and regulatory T cells, and ILCs to be recruited to their vicinity [17,40]. CCR10 expressing DETCs and T_{RMs} are recruited to the epidermis by CC27 expressing interfollicular basal cells. Under inflammatory conditions, epithelia express a number of inflammation specific chemokines, but also amplify their expression of CCL20 and CCL27 to future recruit T cells to their vicinity [41-43]. Thus, this axis may be valuable to target for limiting inflammatory diseases or augmenting anti-pathogen responses.

Bone marrow derived precursors replenish inflammation-depleted LCs in the epidermis through tightly regulated chemokine signals that involve both positive and negative spatial regulation. Expression of CCL20 and CCL2 by infundibular and isthmus epithelia directs recruitment of LC precursors to this region giving them access to the epidermis in inflammation. In this setting, hair follicle bulge epithelia express CCL8 to repel LC precursors from perifollicular regions [11]. While epithelial derived chemokines direct the localization of cells to distinct compartments within the skin, cellular retention and long-term tissue residency relies on expression of integrins that can interact with epithelial surface integrins and cadherins. ILCs and T cells express integrin αE that can bind to E-cadherin which enables interactions with adjacent epithelial cells and controls their residency in the epidermis [17,44].

Additionally, epithelial cells also supply essential survival signals to ensure the persistence of immune cells in their vicinity (Figure 2B). Epidermal resident ILCs utilize keratinocyte derived IL-7 and thymic stromal lymphopoietin (TSLP) in order to populate the epidermis where they are localize to sebaceous gland epithelia [17]. $CD8^{+}$ T_{RMs} require infundibular and isthmus derived IL-7 and IL-15 for survival in the epidermal niche [16], while $CD4^{+}$ T cells only required IL-7 but not IL-15 [16]. These epithelial derived immune survival factors are also co-opted in pathological settings, for instance epithelial derived IL-7/IL-15 was shown to be central for the pathology of Cutaneous T-Cell Lymphoma [16].

Epithelial production of IL-34 is essential for signaling through the colony-stimulating factor 1 receptor (CSF1R) for maintenance of LCs [45] (Figure 2B). These cells enter the skin at embryonic day (E)14.5 as monocyte derived precursors marked by their expression of CX3CR1 where their development and entry into the

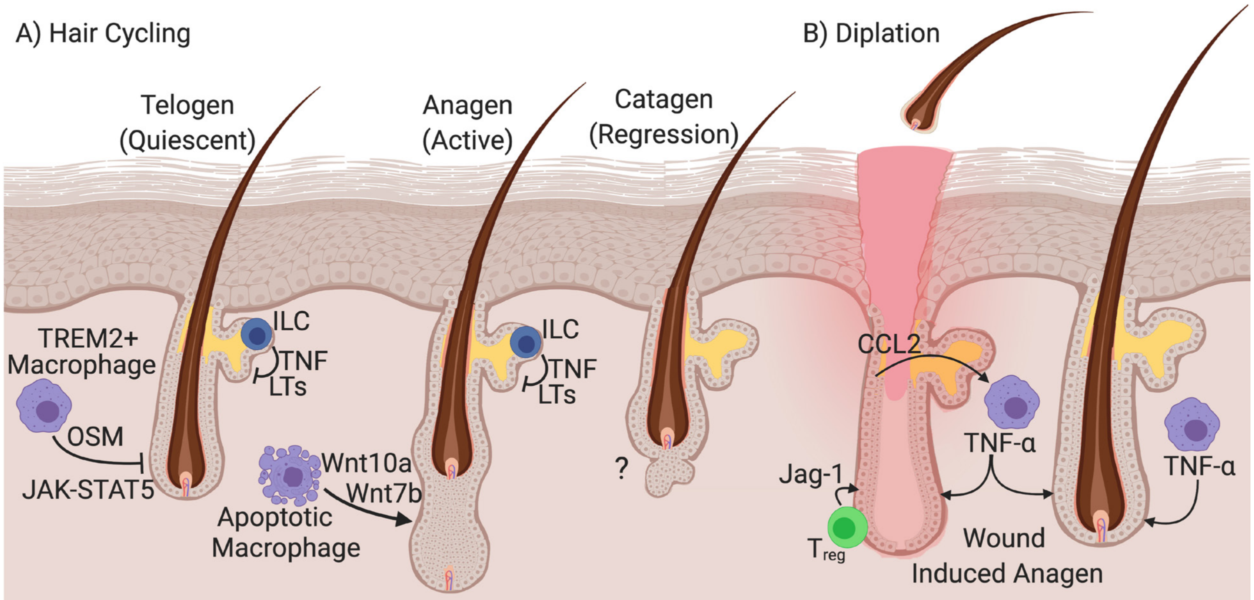

Figure 3. **Immune cells govern the transition from telogen to anagen during the natural hair cycle**. **A**) Phases of the natural hair cycle illustrated with local immune cell populations demonstrating the regulatory roles of epithelial-immune cell interactions during the progression of the hair cycle. Triggering Receptor Expressed on Myeloid cells 2 (TREM2), macrophages express Oncostatin M (OSM), to maintain telogen. Perifollicular macrophages undergo apoptosis and secrete anagen stimulating Wnt ligands Wnt7b and 10a. Innate Lymphoid Cell (ILC) localize to the sebaceous gland and regulate the growth of sebocytes in a TNF and Lymphotoxin (LT) dependent manner. **B**) Depilation induced hair injury that results in follicle activation and anagen entry or Wound Induced Hair Anagen (WIH-A).

epidermis is observed at (E)18.5 coinciding with expression levels of IL-34 greatly increasing as the embryo develops from (E)17.5, driving LC expansion [45,46]. The epidermal developmental programs responsible and teleological reasons for this embryonic expansion of LCs remains elusive. One possibility is that expansion of these immune sentinels at the epidermal interface, just prior to birth, may be necessary for sensing and responding to the plethora of terrestrial antigens that the new born is exposed to at birth.

Keratinocytes as Instigators of Inflammation

As a key interface with the terrestrial environment, the skin epithelium is constantly faced with injurious and inflammatory stimuli. Far from inert structural components, epithelial cells express a number of pattern recognition receptors (PRRS) and can directly sense and respond to stressors by producing antimicrobial peptides, cytokines, and chemokines, to actively engage immune cell function and facilitate inflammation.

Excess sun exposure leads to sun burns with significant inflammation. Ultraviolet (UV) B radiation (wavelength 280-315nm) is thought to be responsible for the inflammatory and carcinogenic effects of sun exposure [47] (Figure 3A). UVB exposure can induce keratinocyte production of immune mediators by influencing epidermal calcium levels and consequently the release of IL-1β and neutrophil infiltration [48]. Furthermore, UVB damaged keratinocytes upregulate the expression of a noncoding RNA, U1 RNA, and during necrosis, release double stranded RNAs (dsRNAs) [49,50]. These dsRNAs are sensed by Toll like receptor 3 (TLR3) in adjacent keratinocytes, inducing the production of inflammatory cytokines, ATP transporters, and proteins for lipid biosynthesis and metabolism. This pathway is co-opted during injury insult where release of dsRNA in damaged keratinocytes facilitates wound induced hair follicle neogenesis [51]. Paradoxically, UVB exposure is also known to cause immune suppression by dampening antigen presentation and augmenting anti-inflammatory factors such as IL-10 [52]. Whether immunosuppression results from direct UVB sensing by immune cells versus epithelial mediated responses to UVB remains an open question.

Apart from sun exposure, a number of topical irritants have been used to highlight the central role of keratinocytes in skin inflammation. In mice, topical application of a vitamin D analog, calcipotriol, engages Vitamin D receptor (VDR) driving keratinocyte expression of TSLP able to be presented by dendritic cells leading to Type 2 immune response and atopic inflammation, similar to atopic dermatitis [53] (Figure 3B). Keratinocytes subjected to 2-4,dinitrofluorobenzene (DNFB), a topical irritant, produce and activate IL-1β and IL-18 via NLRP3 in a caspase-1/ASC dependent manor [54]. IL-18 signals to LCs to activate their migratory programs to potentiate contact hypersensitivity [55]. Another hapten, 12-O-tetradecanoylphorbol- 13-acetate (TPA), induces MAPK and PI3K/Akt-dependent NF-κB signaling in keratinocytes resulting in the overexpression of inflammatory cytokines inducible nitric oxide synthase (iNOS) and cyclooxygenase- 2 (COX-2) [56] (Figure 3B).

The skin is home to a myriad of commensal microbes and is also a major portal of pathogen entry. The epithelium is thus tasked with maintaining tolerance to commensals while limiting the penetrance of microbes [57] (Figure 3C). *In vitro* studies have revealed that keratinocytes can directly sense and respond to microbial ligands such as *Escherichia coli* derived Lipopolysaccharide (LPS) and *Staphylococcus aureus* derived peptidoglycan (PNG) and lipoteichoic acid (LTA), to induce production of proinflammatory cytokines and chemokines [58,59] (Figure 3C). However, microbial stimuli can also dampen inflammation in a context specific manner. For instance, LTA from certain commensal *Staphylococcus epidermidis* strains was able to suppress the expression of inflammatory cytokines following skin injury [60]. Commensals also influence the epithelial barrier circuitously by augmenting homeostatic effector T cells, which secrete cytokines such as IL-17A. This baseline T cell activity is essential to upregulate expression of anti-microbial peptides in healthy epithelium and ultimately serves to limit the establishment infections by barrier penetration of cutaneous pathogens [35].

In addition to exogenous signals, keratinocyte specific deletion of cell-cell interaction molecules and transcriptional regulatory machinery have highlighted that epithelial perturbations are sufficient to drive cutaneous inflammation. This notion was first demonstrated in a landmark study by Wagner and colleagues. Deletion, specifically in epidermal cells, of the AP-1 family transcriptional regulators cJun/JUNB led to the rapid onset of skin inflammation in adult mice [61]. Indeed, cJun/JUNB are expressed at lower levels in psoriatic epithelium and cJun/JUNB loci were identified by genome wide association studies with increases in psoriasis susceptibility [61]. Deletion, in distinct keratinocyte subsets, of the cell-cell interaction proteins E-cadherin or p120 is sufficient to activate the inflammatory transcription factor Nuclear-Factor Kappa B (NF-κB) and a cascade of downstream inflammatory responses [62,63]. Altogether, keratinocytes have proven themselves as key instigators of inflammation through intrinsic stress sensing and direction of immune activity.

IMMUNE CELL REGULATION OF EPITHELIAL TURNOVER AND INJURY RESPONSE

Immune Cells Direct Hair Follicle Homeostasis and Injury

The epidermis is maintained by distinct subsets of stem or progenitor cells each with their unique "micro-niche." Immune cells have surfaced as prominent effectors of epidermal stem cell niches, dynamically regulating their activation and differentiation. While the interfollicular epidermis is continually regenerating, the hair follicle undergoes cyclical bouts of rest (telogen), growth (anagen), and regression (catagen), all mediated by the Hair follicle stem cell (HFSCs) [3] (Figure 4A). The immune derived signals that may regulate the homeostatic turnover of the interfollicular epidermis remain unexplored. However, cross-talk between HFSCs and immune cells has been an area of active investigation as perifollicular immune cells have been shown to potently influence the follicle cycle [64]. Two cell types in particular, macrophages and T_{regs}, have emerged as key instructors of hair follicle behavior.

Macrophages influence the hair cycle in a number of distinct ways (Figure 4A). During anagen, the HFSCs proliferate and differentiate to drive follicular growth. The metabolic requirements of this tremendous undertaking are beginning to unfold and macrophage-supplied iron has recently been implicated in follicle maintenance [65]. Iron metabolism is important for multiple cellular functions and absence of cellular iron is linked to G1/S phase mitotic arrest [66]. Macrophage specific deletion of an iron exporter, ferreportin, results in a profound disruption of the hair follicle cycle and hair loss [65]. Loss of the macrophage supplied tissue iron results in reduced epithelial proliferation. Apart from nutrient support, macrophage derived signals dictate the balance of epithelial quiescence and activation through soluble factors [67,68]. During telogen, a subset of perifollicular TREM2^{+} macrophages secrete Oncostatin M (OSM) which binds to its receptors OSMRβ and gp160 on HFSCs, inducing a JAK-STAT5 signaling cascade to maintain quiescence [67] (Figure 4A). The downstream targets of STAT5 in HFSCs that maintain cell quiescence remain opaque and future studies examining how STAT5 synergizes with other known quiescence factors such as NFATc1 are necessary to decode the complex molecular regulation of this cellular state [69]. During homeostatic shift from telogen to anagen, follicle-associated macrophages undergo apoptosis and release the HFSC-activating Wnt ligands Wnt7b and Wnt10a [68] (Figure 4A). Taken together, these data indicate that macrophages provide temporal signals to promote quiescence and activation. However, tissue macrophages are known to have distinct develop-

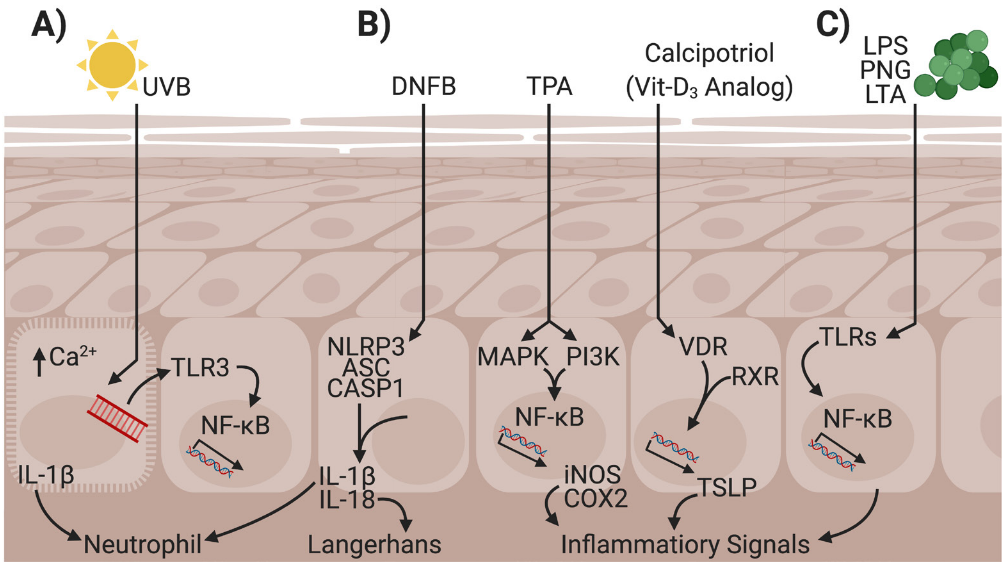

Figure 4. **Keratinocytes can directly sense and respond to inflammatory and microbial stressors**. **A**) Ultraviolet B Waves (UVB) leads to neutrophil recruitment and inflammatory signaling. **B**) Keratinocyte responses to chemical hapten insult and the consequent inflammatory signaling. 2-4,dinitrofluorobenzene (DNFB), 12-O-tetradecanoylphorbol-13-acetate (TPA), inducible nitric oxide synthase (iNOS), and cyclooxygenase- 2 (COX-2) C) Bacterial sensing through expression of pattern recognition receptors such as TLR4 results in activation of the inflammatory transcription factor NF-kB and a downstream inflammatory cascade. Lipopolysaccharide (LPS), Peptidoglycan (PNG), Lipoteichoic Acid (LTA).

mental origins and are functionally heterogenous. It is unclear whether the OSM producing TREM2$^+$ macrophages are the same population that undergo apoptosis and secrete Wnt ligands or if Wnt7b/10a releasing macrophages are a distinct subset. Another unaddressed facet of the macrophage-HFSC dialogue is how their activity is temporally regulated. The signals that drive OSM expression in Trem2$^+$ macrophages or results in perifollicular macrophage death in late telogen are still to be uncovered. Concomitant with perifollicular macrophage activity, T_{regs} begin to accumulate around the follicle in late telogen [70], though how follicle associated T_{regs} regulate the natural hair cycle remains to be addressed, few studies have begun addressing these interaction in a murine model of induced anagen through chemical depilation or plucking.

Following chemical depilation of the resting hair follicle, which results in injury or wound-induced activation of anagen (WIH-A), perifollicular T_{regs} facilitate HFSC proliferation by localizing to the follicle and producing a Notch ligand Jagged 1 (Jag-1) [70]. However, in experiments where T_{regs} populations are depleted, Notch response gene *Hey1*, indicative of HFSC proliferation, was increased rather than decreased [63]. Thus, it is tempting to speculate that T_{regs} support of homeostatic hair follicle cycling may be through other known T_{regs} factors such as amphiregulin signaling to Epidermal Growth Factor Receptor (EGFR) [63]. Notch signaling in the epidermis is also regulated by ILCs [17]. In the sebaceous gland, ILC2s produce TNF and lymphotoxins LTα3 and LTα1β2 resulting in the downregulating of a Notch ligand JAG-2 and Notch-regulated transcription factor PBX1 in sebocytes [17] (Figure 3). In the absence of ILCs, unregulated sebocytes hyperproliferate and augment lipids production, which in turn alters the composition of surface commensal bacteria [17]. How expression the Notch ligand Jagged is regulated in skin lymphocyte populations remains unclear. Both ILC2 and skin T_{regs} express high levels of the transcription factor GATA3 [71] and have been demonstrated as important niche factors to drive epithelial differentiation. Notch ligands, robustly expressed by the epithelia, are known to directly regulate GATA3 expression in Th2 cells [72]. Thus, it is tempting to speculate that epidermal Notch ligands may signal into ILCs and T_{regs} to induce or reinforce GATA3 expression, and adaptation to the epidermal microenvironment.

Hair plucking represents a micro-injury scenario, that results in epithelial apoptosis in the damaged follicle and the subsequent triggering of hair cycling (Figure 4B). Remarkably, adjacent unplucked follicles also begin to cycle and grow hair. Chen and colleagues uncovered that damaged follicles express the chemokine CCL2 to recruit TNF-α-producing macrophages to their vicinity. Recruited macrophages disseminate the damage signal to adjacent follicles via TNF-α and induce hair growth around the damaged follicle [73]. The mechanism by which TNF-α induced cycling of adjacent follicles remains elu-

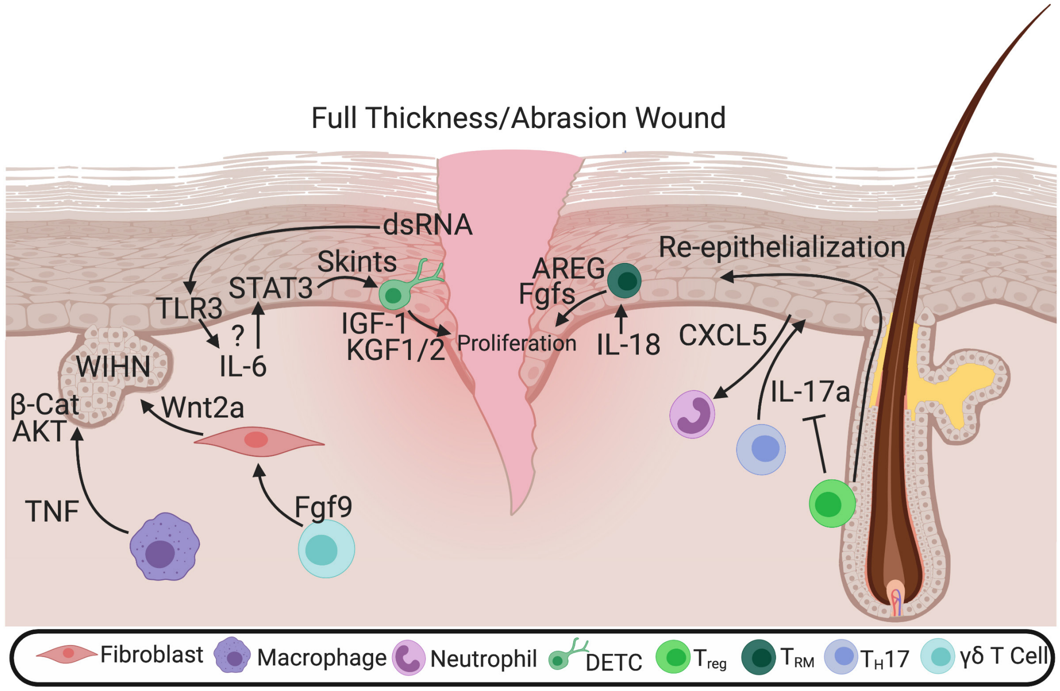

Figure 5. **Epithelial-immune interactions underlie injury responses**. The schematic illustrates immune-epithelial interactions in full thickness wound healing in which damage is caused to the intrafollicular epidermis and hair follicle resulting in induced Hair follicle Neogenesis (WIHN).

sive; however, synchronizing hair growth around injury may be a key mechanism by which the skin shields the damaged area during repair.

Immune Cells Activate Keratinocytes to Facilitate Wound Healing

Rapid repair of the skin epithelium following injury or inflammation is paramount for organismal survival. Stress-stimulated regeneration engages a distinct repertoire of immune-epithelial interactions and homeostatic epithelial turnover.

While hair follicles themselves must recover after injury, they also provide a source of stem cells for repair of the interfollicular epidermis [18]. Crosstalk between epithelia and both effector and regulatory T cells comes into play to ensure rapid re-epithelialization of the barrier (Figure 5). During wounding, T_{regs} stimulate HFSC proliferation and upward mobility to the interfollicular epidermis [74]. In mice depleted of T_{regs}, Th17 cells accumulate and signal to keratinocytes through IL-17A to produce CXCL5 for the recruitment of neutrophils. The IL-17A-CXCL5-neutrophil axis results in a delay in repair of the interfollicular epidermis [74]. TNF-α-producing macrophages promote HFSC function and wound induced hair follicle neogenesis by activating β-catenin in an AKT dependent manor. Precisely how TNF-α activates β-catenin, and if this activation is mediated by direct TNF-α signaling or through secondary mediators, remains to be determined [75]. Additionally, after wounding, dermal γδ T cells also signal to promote hair follicle neogenesis (WIHN), the generation of new follicles [76] (Figure 5). γδ T cell derived Fgf9 signals to dermal fibroblast inducing the production of Wnt2a that in turn signals into keratinocytes to generate new hair.

In addition to the dermal T_{regs}, epidermal T_{RMS} also communicate with keratinocytes in wound repair [77-79] (Figure 5). Commensal specific IL-17A expressing $CD8^+$ T (Tc17) cells accumulate around the wound's edge and dynamically upregulate expression of GATA3 in response to IL-18 released from the damaged epithelium [77,80]. These commensal specific cells supply cytokines and growth factors at the wound edge to promote repair. During healthy repair epidermal αβ and γδ T cells produce epithelial growth factors such as production of insulin growth factor 1, however T cells isolated from chronic nonhealing wounds failed to produce these ligands [78]. Thus, impaired immunity may be a key underlying feature of non-healing wounds. Indeed, in aged animals, DETCs fail to accumulate around the wounds edge and promote efficient repair. This impairment results from a loss of Stat3 dependent keratinocyte expression of the DETC activating ligands Skint3/9 at the wound's edge, inducible via recombinant IL-6 stimulation [31]. After wounding, damaged keratinocytes release dsRNA, which signal through TLR3 in adjacent undamaged keratinocytes and induce production IL-6 [51]. Whether the dsRNA-TLR3-IL-6 axis is operative in activation of DETCs at the edge of the wound, or other DAMPS/PAMPS play a role, remains to be examined. Thus, the immune-epithelial crosstalk may be used as a lever to kickstart healing. Further studies defining the immune basis of healthy and impaired healing will open doors to immune-based therapies for wound repair.

CONCLUSION

The skin epithelium is tasked with shielding our internal organs from environmental assaults and relies on a dynamic crosstalk with immune cells for optimal barrier function. In addition to classical anti-pathogen functions, the immune-epithelial crosstalk has also emerged as an essential regulator of epithelial regeneration in homeostasis and following injury. The epithelium recruits and maintains resident immune cells, which in turn supply key regenerative signals to maintain the skin's integrity. As our understanding of the molecular mediators of the immune-epithelial crosstalk improves, including relevant microbial and damage sensors and effectors, it may lead to the generation of novel therapeutics that boost skin health and mitigate disease.

Acknowledgments: We thank our friends and colleagues in skin biology whose work inspired us to write this review and apologize to those whose work we could not include due to space constrains. Figures generated using Biorender. This work was supported by NIAID (1K22AI135099-01 S.N.)

REFERENCES

1. Kobayashi T, Naik S, Nagao K. Choreographing immunity in the skin epithelial barrier. Immunity. 2019;50(3):552–65.
2. Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. Nat Rev Immunol. 2009;9(10):679.
3. Fuchs E. Skin stem cells: rising to the surface. J Cell Biol. 2008;180(2):273–84.
4. Koch PJ, Franke WW. Desmosomal cadherins: another growing multigene family of adhesion molecules. Curr Opin Cell Biol. 1994;6(5):682–7.
5. Al-Amoudi A, Dubochet J, Norlén L. Nanostructure of the epidermal extracellular space as observed by cryo-electron microscopy of vitreous sections of human skin. J Invest Dermatol. 2005;124(4):764–77.
6. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol. 2005;6(4):328.
7. Oren A, Ganz T, Liu L, Meerloo T. In human epidermis, β-defensin 2 is packaged in lamellar bodies. Exp Mol Pathol. 2003;74(2):180–2.
8. Downing DT, Stewart ME, Wertz PW, Colton SW, Abraham W, Strauss JS. Skin lipids: an update. J Invest Dermatol. 1987:88.
9. de Guzman Strong C, Wertz PW, Wang C, Yang F, Meltzer PS, Andl T, et al. Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3. J Cell Biol. 2006;175(4):661–70.
10. Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol. 2008;17(12):1063–72.
11. Nagao K, Kobayashi T, Moro K, Ohyama M, Adachi T, Kitashima DY, et al. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. Nat Immunol. 2012;13(8):744.
12. Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, et al. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science. 2010;327(5971):1385–9.
13. Jensen KB, Collins CA, Nascimento E, Tan DW, Frye M, Itami S, et al. Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell Stem Cell. 2009;4(5):427–39.
14. Jaks V, Kasper M, Toftgård R. The hair follicle—a stem cell zoo. Exp Cell Res. 2010;316(8):1422–8.
15. Christoph T, Müller-Röver S, Audring H, Tobin D, Hermes B, Cotsarelis G, et al. The human hair follicle immune system: cellular composition and immune privilege. Br J Dermatol. 2000;142(5):862–73.
16. Adachi T, Kobayashi T, Sugihara E, Yamada T, Ikuta K, Pittaluga S, et al. Hair follicle–derived IL-7 and IL-15 mediate skin-resident memory T cell homeostasis and lymphoma. Nat Med. 2015;21(11):1272.
17. Kobayashi T, Voisin B, Kennedy EA, Jo J-H, Shih H-Y, Truong A, et al. Homeostatic control of sebaceous glands by innate lymphoid cells regulates commensal bacteria equilibrium. Cell. 2019;176(5):982-97. e16.
18. Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, et al. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat Med. 2005;11(12):1351.
19. Oh JW, Kloepper J, Langan EA, Kim Y, Yeo J, Kim MJ, et al. A guide to studying human hair follicle cycling in vivo. J Invest Dermatol. 2016;136(1):34–44.
20. Harper RA, Grove G. Human skin fibroblasts derived from papillary and reticular dermis: differences in growth potential in vitro. Science. 1979;204(4392):526–7.
21. Driskell RR, Jahoda CA, Chuong CM, Watt FM, Horsley V. Defining dermal adipose tissue. Exp Dermatol. 2014;23(9):629–31.
22. Sennett R, Rendl M, editors. Mesenchymal–epithelial interactions during hair follicle morphogenesis and cycling. Seminars in cell & developmental biology. Elsevier; 2012.
23. Philippeos C, Telerman SB, Oulès B, Pisco AO, Shaw TJ, Elgueta R, et al. Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations. J Invest Dermatol. 2018;138(4):811–25.
24. Hsu YC, Li L, Fuchs E. Emerging interactions between skin stem cells and their niches. Nat Med. 2014;20(8):847.
25. Pasparakis M, Haase I, Nestle FO. Mechanisms regulating skin immunity and inflammation. Nat Rev Immunol.

2014;14(5):289.
26. Hunger RE, Sieling PA, Ochoa MT, Sugaya M, Burdick AE, Rea TH, et al. Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells. J Clin Invest. 2004;113(5):701–8.
27. Romani N, Ebner S, Tripp CH, Flacher V, Koch F, Stoitzner P. Epidermal Langerhans cells—changing views on their function in vivo. Immunol Lett. 2006;106(2):119–25.
28. Black AP, Ardern-Jones MR, Kasprowicz V, Bowness P, Jones L, Bailey AS, et al. Human keratinocyte induction of rapid effector function in antigen-specific memory CD4+ and CD8+ T cells. Eur J Immunol. 2007;37(6):1485–93.
29. Foster CA, Yokozeki H, Rappersberger K, Koning F, Volc-Platzer B, Rieger A, et al. Human epidermal T cells predominantly belong to the lineage expressing alpha/beta T cell receptor. J Exp Med. 1990;171(4):997–1013.
30. Mackay LK, Stock AT, Ma JZ, Jones CM, Kent SJ, Mueller SN, et al. Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. Proc Natl Acad Sci USA. 2012;109(18):7037–42.
31. Keyes BE, Liu S, Asare A, Naik S, Levorse J, Polak L, et al. Impaired epidermal to dendritic T cell signaling slows wound repair in aged skin. Cell. 2016;167(5):1323-38. e14.
32. Ouwendijk WJ, Laing KJ, Verjans GM, Koelle DM. T-cell immunity to human alphaherpesviruses. Curr Opin Virol. 2013;3(4):452–60.
33. van Velzen M, Jing L, Osterhaus AD, Sette A, Koelle DM, Verjans GM. Local CD4 and CD8 T-cell reactivity to HSV-1 antigens documents broad viral protein expression and immune competence in latently infected human trigeminal ganglia. PLoS Pathog. 2013;9(8):e1003547.
34. Vander Lugt B, Khan AA, Hackney JA, Agrawal S, Lesch J, Zhou M, et al. Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. Nat Immunol. 2014;15(2):161.
35. Naik S, Bouladoux N, Linehan JL, Han SJ, Harrison OJ, Wilhelm C, et al. Commensal–dendritic-cell interaction specifies a unique protective skin immune signature. Nature. 2015;520(7545):104.
36. Clausen BE, Stoitzner P. Functional specialization of skin dendritic cell subsets in regulating T cell responses. Front Immunol. 2015;6:534.
37. Ouchi T, Kubo A, Yokouchi M, Adachi T, Kobayashi T, Kitashima DY, et al. Langerhans cell antigen capture through tight junctions confers preemptive immunity in experimental staphylococcal scalded skin syndrome. J Exp Med. 2011;208(13):2607–13.
38. Abtin A, Jain R, Mitchell AJ, Roediger B, Brzoska AJ, Tikoo S, et al. Perivascular macrophages mediate neutrophil recruitment during bacterial skin infection. Nat Immunol. 2014;15(1):45.
39. Silva HM, Báfica A, Rodrigues-Luiz GF, Chi J, Santos PdEA, Reis BS, et al. Vasculature-associated fat macrophages readily adapt to inflammatory and metabolic challenges. J Exp Med. 2019;216(4):786–806.
40. Scharschmidt TC, Vasquez KS, Pauli ML, Leitner EG, Chu K, Truong H-A, et al. Commensal microbes and hair follicle morphogenesis coordinately drive Treg migration into neonatal skin. Cell Host Microbe. 2017;21(4):467-77. e5.
41. Morales J, Homey B, Vicari AP, Hudak S, Oldham E, Hedrick J, et al. CTACK, a skin-associated chemokine that preferentially attracts skin-homing memory T cells. Proc Natl Acad Sci USA. 1999;96(25):14470–5.
42. Jin Y, Xia M, Sun A, Saylor CM, Xiong N. CCR10 is important for the development of skin-specific γδT cells by regulating their migration and location. J Immunol. 2010;185(10):5723–31.
43. Huang V, Lonsdorf AS, Fang L, Kakinuma T, Lee VC, Cha E, et al. Cutting edge: rapid accumulation of epidermal CCL27 in skin-draining lymph nodes following topical application of a contact sensitizer recruits CCR10-expressing T cells. J Immunol. 2008;180(10):6462–6.
44. Agace WW, Higgins JM, Sadasivan B, Brenner MB, Parker CM. T-lymphocyte–epithelial-cell interactions: integrin αE (CD103) β7, LEEP-CAM and chemokines. Curr Opin Cell Biol. 2000;12(5):563–8.
45. Greter M, Lelios I, Pelczar P, Hoeffel G, Price J, Leboeuf M, et al. Stroma-derived interleukin-34 controls the development and maintenance of langerhans cells and the maintenance of microglia. Immunity. 2012;37(6):1050–60.
46. Chorro L, Sarde A, Li M, Woollard KJ, Chambon P, Malissen B, et al. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. J Exp Med. 2009;206(13):3089–100.
47. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B. 2001;63(1-3):8–18.
48. Feldmeyer L, Keller M, Niklaus G, Hohl D, Werner S, Beer HD. The inflammasome mediates UVB-induced activation and secretion of interleukin-1β by keratinocytes. Curr Biol. 2007;17(13):1140–5.
49. Bernard JJ, Cowing-Zitron C, Nakatsuji T, Muehleisen B, Muto J, Borkowski AW, et al. Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. Nat Med. 2012;18(8):1286.
50. Borkowski AW, Gallo RL. UVB radiation illuminates the role of TLR3 in the epidermis. J Invest Dermatol. 2014;134(9):2315–20.
51. Nelson AM, Reddy SK, Ratliff TS, Hossain MZ, Katseff AS, Zhu AS, et al. dsRNA released by tissue damage activates TLR3 to drive skin regeneration. Cell Stem Cell. 2015;17(2):139–51.
52. Schwarz T. Mechanisms of UV-induced immunosuppression. Keio J Med. 2005;54(4):165–71.
53. Li M, Hener P, Zhang Z, Kato S, Metzger D, Chambon P. Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. Proc Natl Acad Sci USA. 2006;103(31):11736–41.
54. Honda T, Egawa G, Grabbe S, Kabashima K. Update of Immune Events in the Murine Contact Hypersensitivity Model: Toward the Understanding of Allergic Contact Dermatitis. J Invest Dermatol. 2013;133(2):303–15.
55. Antonopoulos C, Cumberbatch M, Mee JB, Dearman RJ, Wei XQ, Liew FY, et al. IL-18 is a key proximal mediator of contact hypersensitivity and allergen-induced Langerhans cell migration in murine epidermis. J Leukoc Biol.

2008;83(2):361–7.
56. Liu W, Huang S, Li Y, Li Y, Li D, Wu P, et al. Glycyrrhizic acid from licorice down-regulates inflammatory responses via blocking MAPK and PI3K/Akt-dependent NF-κB signalling pathways in TPA-induced skin inflammation. MedChemComm. 2018;9(9):1502–10.
57. Belkaid Y, Segre JA. Dialogue between skin microbiota and immunity. Science. 2014;346(6212):954–9.
58. Song PI, Neparidze N, Armstrong CA, Ansel JC, Park YM, Abraham T, et al. Human keratinocytes express functional CD14 and toll-like receptor 4. J Invest Dermatol. 2002;119(2):424–32.
59. Mempel M, Voelcker V, Köllisch G, Plank C, Rad R, Gerhard M, et al. Toll-like receptor expression in human keratinocytes: nuclear factor κB controlled gene activation by Staphylococcus aureus is Toll-like receptor 2 but not Toll-like receptor 4 or platelet activating factor receptor dependent. J Invest Dermatol. 2003;121(6):1389–96.
60. Lai Y, Di Nardo A, Nakatsuji T, Leichtle A, Yang Y, Cogen AL, et al. Commensal bacteria regulate Toll-like receptor 3–dependent inflammation after skin injury. Nat Med. 2009;15(12):1377.
61. Zenz R, Eferl R, Kenner L, Florin L, Hummerich L, Mehic D, et al. Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. Nature. 2005;437(7057):369.
62. Perez-Moreno M, Davis MA, Wong E, Pasolli HA, Reynolds AB, Fuchs E. p120-catenin mediates inflammatory responses in the skin. Cell. 2006;124(3):631–44.
63. Lay K, Yuan S, Gur-Cohen S, Miao Y, Han T, Naik S, et al. Stem cells repurpose proliferation to contain a breach in their niche barrier. eLife. 2018;7:e41661.
64. Naik S, Larsen SB, Cowley CJ, Fuchs E. Two to tango: dialog between immunity and stem cells in health and disease. Cell. 2018;175(4):908–20.
65. Recalcati S, Gammella E, Buratti P, Doni A, Anselmo A, Locati M, et al. Macrophage ferroportin is essential for stromal cell proliferation in wound healing. Haematologica. 2019;104(1):47-58.
66. Cairo G, Bernuzzi F, Recalcati S. A precious metal: Iron, an essential nutrient for all cells. Genes Nutr. 2006;1(1):25–39.
67. Wang EC, Dai Z, Ferrante AW, Drake CG, Christiano AM. A Subset of TREM2+ Dermal Macrophages Secretes Oncostatin M to Maintain Hair Follicle Stem Cell Quiescence and Inhibit Hair Growth. Cell Stem Cell. 2019;24(4):654-69. e6.
68. Castellana D, Paus R, Perez-Moreno M. Macrophages contribute to the cyclic activation of adult hair follicle stem cells. PLoS Biol. 2014;12(12):e1002002.
69. Horsley V, Aliprantis AO, Polak L, Glimcher LH, Fuchs E. NFATc1 balances quiescence and proliferation of skin stem cells. Cell. 2008;132(2):299–310.
70. Ali N, Zirak B, Rodriguez RS, Pauli ML, Truong H-A, Lai K, et al. Regulatory T cells in skin facilitate epithelial stem cell differentiation. Cell. 2017;169(6):1119-29. e11.
71. Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, Ribeiro CH, et al. GATA3 controls Foxp3+ regulatory T cell fate during inflammation in mice. J Clin Invest. 2011;121(11):4503–15.
72. Fang TC, Yashiro-Ohtani Y, Del Bianco C, Knoblock DM, Blacklow SC, Pear WS. Notch directly regulates Gata3 expression during T helper 2 cell differentiation. Immunity. 2007;27(1):100–10.
73. Chen CC, Wang L, Plikus MV, Jiang TX, Murray PJ, Ramos R, et al. Organ-level quorum sensing directs regeneration in hair stem cell populations. Cell. 2015;161(2):277–90.
74. Mathur AN, Zirak B, Boothby IC, Tan M, Cohen JN, Mauro TM, et al. Treg-Cell Control of a CXCL5-IL-17 Inflammatory Axis Promotes Hair-Follicle-Stem-Cell Differentiation During Skin-Barrier Repair. Immunity. 2019;50(3):655-67. e4.
75. Wang X, Chen H, Tian R, Zhang Y, Drutskaya MS, Wang C, et al. Macrophages induce AKT/β-catenin-dependent Lgr5+ stem cell activation and hair follicle regeneration through TNF. Nat Commun. 2017;8:14091.
76. Gay D, Kwon O, Zhang Z, Spata M, Plikus MV, Holler PD, et al. Fgf9 from dermal γδ T cells induces hair follicle neogenesis after wounding. Nat Med. 2013;19(7):916.
77. Linehan JL, Harrison OJ, Han S-J, Byrd AL, Vujkovic-Cvijin I, Villarino AV, et al. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. Cell. 2018;172(4):784-96. e18.
78. Toulon A, Breton L, Taylor KR, Tenenhaus M, Bhavsar D, Lanigan C, et al. A role for human skin–resident T cells in wound healing. J Exp Med. 2009;206(4):743–50.
79. Havran WL, Jameson JM. Epidermal T cells and wound healing. J Immunol. 2010;184(10):5423–8.
80. Harrison OJ, Linehan JL, Shih HY, Bouladoux N, Han SJ, Smelkinson M, et al. Commensal-specific T cell plasticity promotes rapid tissue adaptation to injury. Science. 2019;363(6422):eaat6280.