Published in final edited form as:

Pflugers Arch. 2017 April ; 469(3-4): 455–463. doi:10.1007/s00424-017-1953-7.

The Role of Macrophages in Skin Homeostasis

Diana A. Yanez¹, Richard K. Lacher¹, Aurobind Vidyarthi¹, and Oscar R. Colegio^{1,2,3,4,5}

¹Department of Dermatology, Yale University School of Medicine, 333 Cedar Street, New Haven CT 06520-8059

²Department of Pathology, Yale University School of Medicine, 333 Cedar Street, New Haven CT 06520-8059

³Department of Surgery, Yale University School of Medicine, 333 Cedar Street, New Haven CT 06520-8059

⁴Yale Cancer Center, Yale University School of Medicine, 333 Cedar Street, New Haven CT 06520-8059

⁵Yale-New Haven Transplantation Center, Yale University School of Medicine, 333 Cedar Street, New Haven CT 06520-8059

Abstract

The skin and its appendages comprise the largest and fastest-growing organ in the body. It performs multiple tasks and maintains homeostatic control, including the regulation of body temperature, protection from desiccation and from pathogen invasion. The skin can perform its functions with the assistance of different immune cell populations. Monocyte-derived cells are imperative for the completion of these tasks. The comprehensive role of macrophages and Langerhans cells in establishing and maintaining skin homeostasis remains incompletely defined. However, over the past decade, innovations in mouse genetics have allowed for advancements in the field. In this review, we explore different homeostatic roles of macrophages and Langerhans cells, including wound repair, follicle regeneration, salt balance, and cancer regression and progression, in the skin. The understanding of the precise functions of myeloid-derived cells in the skin under basal conditions can help develop specific therapies that aid in skin and hair follicle regeneration and cutaneous cancer prevention.

INTRODUCTION

The skin is the largest organ in the body and performs a variety of protective functions. It prevents desiccation by limiting unnecessary fluid loss, protects the organism against invading pathogens, and regulates body temperature [14]. It is subject to environmental stimuli and must react appropriately to insults through a variety of mechanisms, which despite compromise of structural integrity must maintain homeostasis. These functions are

promoted through continuous integration of environmental inputs and communication among immune, epithelial, stromal, and stem cells [4].

The skin is composed of an outer layer of stratified epithelium, the epidermis, an inner less cellular layer, the dermis, and a subcutaneous layer comprised primarily of adipose tissue, the hypodermis or panniculus [49]. Each layer is occupied by different immune cell populations, which shares the same precursor. The epidermis is populated by Langerhans cells (LCs), also known as the antigen presenting cells (APC) of the epidermis, the dermis contains tissue resident dermal macrophages (large vacuolar phagocytic cells) and dermal dendritic cells (also APCs), and the hypodermis contains macrophages and T cells [48]. They can be distinguished by their differential expression of markers. Langerhans cells, express CD1a, CD11b, CD11c, F4/80, CD207 (langerin) and major histocompatibility complex MHC class II, dermal dendritic cells (DCs) express CD1a^{low}, CD1c, CD11b^{hi}, CD206, CD209 and MHC class II, and macrophages express CD11b, F4/80, CD163, factor XIIIa, CD16, CD32 and CD64 [19, 40, 48]

Even though they share the same precursor, their mode of renewal is different post-embryonically. Dermal macrophages are equally derived during embryonic development by the yolk sac and fetal liver [16]. During adulthood, they are replenished by hematopoietic stem cells [61]. Langerhans cells also originate during embryonic development from the fetal liver and yolk sac, with the liver providing approximately 80% of Langerhans cells [16]. However, they seed in the epidermis early in development, and self-renew independently of blood monocytes throughout adulthood [16]. These cells are members of the mononuclear phagocyte system, a specialized network of phagocytes that is classically known for their participation in innate immunity and phagocytic functions [22]. Less is known about their role during cutaneous homeostasis. Nevertheless, growing evidence suggests that they play an essential role in the maintenance of skin homeostasis and integrity. They aid during wound healing, tissue repair, hair follicle regeneration, and the stress response. In this review we focus on the role of monocyte-derived macrophages and DCs in skin homeostasis by providing evidence obtained from studies in murine and human skin (Figure I).

Role of macrophages in cutaneous wound healing

Wound healing is the process of restoring the skin to a state of homeostasis after injury. It is a dynamic and highly coordinated process. It is divided into three overlapping phases: Inflammation, tissue formation and maturation, which occur approximately 0–5, 5–10, and 10–60 days after insult, respectively [41]. Inflammation (1st stage) occurs immediately after injury to promote the recruitment of factors required for repair and provide a provisional wound matrix. Tissue formation (2nd stage) aids in the stabilization of vascular structures and induce fibroblast differentiation into activated contractile myofibroblasts [37]. Lastly, maturation (3rd stage) promotes wound closure and complete tissue regeneration [37].

There are two major types of myeloid-derived cell populations in the skin. Langerhans cells, which share features of dendritic cells and macrophages, are present in the epidermis [40]. Dermal macrophages and dermal dendritic cells are present in the dermis [19]. The role of Langerhans cells in skin repair has yet to be definitively determined. However, decades ago,

Leibovich and Ross [31] described a role for dermal macrophages during wound repair. They eliminated macrophages locally with antimacrophage serum from skin wounds in guinea pigs, and found that they have a critical role in debridement and promote proliferation of fibroblasts [31]. Neutrophils are also recruited to the wound after injury and they infiltrate into damaged tissue. However, depletion of neutrophils in mice did not alter dermal healing nor collagen deposition [10], suggesting that compared to macrophages, they do not have an essential role in dermal wound repair.

The role of macrophages during wound repair goes beyond wound clearance and induction of proliferation. Macrophages are present during all phases of wound repair, and they outnumber all other inflammatory cells [77]. To determine the relevance of macrophages during skin repair, Lucas, *et al.*, used Cre-inducible diphtheria toxin receptor cell ablation to temporally deplete dermal macrophages using the promoter of lysozyme M to drive Cre expression (LysMCre/iDTR) during the three different stages of wound healing [32]. Notably, complete depletion of peritoneal macrophages was obtained, while the levels of peritoneal neutrophils and B cells remained unaffected, suggesting this method specifically targeted macrophages, however the presence of markers that indicate ablation of other myeloid-derived cells (LCs and DCs) was not assessed. Depletion during the inflammatory phase showed a delay in the repair response, decreased proliferation of granulation tissue, reduced vascularization, diminished wound contraction, decreased myofibroblast differentiation and decreased expression of transforming growth factor (TGF β 1) and vascular endothelial growth factor-A (VEGF-A) [32]. Depletion of macrophages during the 2nd stage, or tissue formation, resulted in lower rate of wound closure, delayed maturation of granulation tissue with extreme hemorrhage, which appeared to be caused by injury to endothelial cells, and hindered transition to next phase [32]. Finally, depletion at late stage of wound maturation did not have a significant effect on wound repair [32]. Similar results have been observed by other groups. For instance, local ablation of skin macrophages caused a delay in re-epithelialization, compromised angiogenesis, decreased formation of granulation tissue and deposition of collagen, and altered cytokine protein levels with higher tumor necrosis factor (TNF)- α , and lower VEGF-A and TGF- β 1[42]. Also, macrophage depletion has resulted in compromised wound morphology, significantly increased healing time, reduced neovascularization and impaired closure [18].

The different functions of macrophages are reflected in their broad gene expression profile and scope of activation during the repair process. During the initial phase, a temporary wound matrix is formed to achieve hemostasis. Inflammation begins with the recruitment of neutrophils [55] and blood monocytes [56], which eventually differentiate into pro-inflammatory macrophages in response to factors in the microenvironment. They are CCR2⁺Ly6C⁺ and express pro-inflammatory cytokines (interleukin (IL)-6, IL-1b, TNF- α) [13] as well as other inflammation regulators such as inducible nitric oxide synthase (iNOS), Arginase (Arg)-1 and VEGF-A [76]. These cytokines and growth factors propagate the inflammatory cascade and recruit cells and factors required to coordinate repair [13]. This is followed by the clotting cascade, in which platelets promote vasoconstriction to prevent loss of blood from compromised vessels, resulting in the formation of a clot, which covers exposed tissue and contains fibrin molecules that form a provisional scaffold for recruited cells [55]. Macrophages phagocytose debris and necrotic tissue, and release reactive oxygen

species (ROS) to eliminate invading pathogens. Also, pro-inflammatory macrophages are capable of inhibiting fibroblasts or inducing them to produce matrix metalloproteinase (MMP) -1, which enhances extra-cellular matrix (ECM) degradation and inhibits fibrogenesis [79]. Macrophages also secrete serine proteases and elastases that degrade the ECM.

Next, the 2nd phase stage of wound healing, tissue formation, begins. The goal of this phase is to replace the temporary wound matrix through re-epithelialization, formation of granulation tissue, and perfusion of nutrients [55]. The primary mediators of this stage are anti-inflammatory macrophages. This alternatively-activated macrophage phenotype, promotes the expression of cytokine IL-10 and upregulation of scavenging receptors for tissue remodeling, including CD206 and CD163 [76]. Moreover, neutrophils stimulate macrophage secretion of TGF β 1, which is required for fibroblast differentiation and wound contraction and dampens the inflammatory response [54]. Macrophages phagocytose apoptotic neutrophils preventing the release of their toxic contents further dampening the inflammatory response [62]. Moreover, phagocytosis of apoptotic cells by macrophages leads to the production of VEGF-A [17], which induces vascularization and tissue regeneration [77]. This process is mediated through CX3-chemokine receptor (CR)-1⁺ macrophages. Reduced vascularization, collagen deposition, TGF β and VEGFA levels were observed in CX3CR1-deficient mice [23]. Additionally, macrophage-induced fibroblast differentiation, along with their production of additional factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), TGF- α , further trigger angiogenesis and re-epithelialization[13] [55]. Lastly, differentiated fibroblasts (myofibroblasts) induce fibrosis and are the primary source of ECM, including collagen [33]. ECM forms framework for cell attachment, promotes growth and differentiation [55] through its ability to bind and release growth factors [60].

The 3rd and final stage of repair is tissue maturation and remodeling. The goal in this stage is to fully regenerate the tissue and complete the wound closure process. During this phase, angiogenesis stops and the vasculature undergoes regression [41]. ECM remodeling switches to the production of type I collagen arranged in a parallel orientation, replacing loosely arranged type III collagen, and myofibroblasts mediate wound closure through attachment to collagen [55]. This stage is also mediated by macrophages, however, they are not essential, which indicates that other cells execute similar functions in their absence [32]. Macrophages phagocytose residual ECM and debris through MMP-10-mediated collagen degradation [36] [57]. Madsen, *et al.*, found that alternatively activated anti-inflammatory macrophages internalize higher quantities of collagen when compared to other cells. However, fibroblasts internalize one quarter as much collagen as anti-inflammatory macrophages, and may compensate in the absence of macrophages[36] (Figure I).

Role of macrophages during hair follicle development

The mammalian hair follicles are essential to skin homeostasis. They have multiple functions, including creating a hair shaft, supplying a sensory apparatus for shaft movement detection, regulating body temperature, and offering a reservoir for Langerhans cells (LCs) and melanocytes [65]. The hair follicle develops from epithelial stem cells that are

stimulated by specialized cells located below the epidermis [1]. Epithelial stem cells differentiate into three-layered rods: the outermost rod is called the outer root sheath (ORS), the middle rod is the inner root sheath (IRS) and the innermost rod is the hair shaft [65]. The ORS is surrounded by the basal lamina, a reservoir of ECM and growth factors that separates the hair follicle from the dermis [1].

The hair follicle is maintained by continuous cycles of generation and degeneration, which are highly controlled by molecular signals that induce cell proliferation, differentiation, and death. Wnt, bone morphogenetic protein (BMP), sonic hedgehog (SHH), and Notch are a few of the signaling pathways that have been involved in regulating the hair cycle [30]. The hair cycle is divided into three stages, which are telogen, anagen, and catagen. Telogen is a quiescent period where the dermal papilla rests under the bulge stem cell compartment [14]. The dermal papilla is a group of mesenchymal cells that control hair follicle development and serve as a stem cell reservoir [11]. After receiving the necessary signals, the follicle enters the active state of hair growth, anagen. During this stage, the stem cell progeny, located below the bulge in the secondary hair germ, undergo rapid proliferation and reorganization [59] that restores the lower follicle and reconstructs the hair shaft [65]. Catagen then follows. In this stage, epithelial cells undergo apoptosis and the follicle undergoes regression, which induces the dermal papilla to migrate under the bulge and start the dormancy period beginning a new cycle [59].

The immune cells within the hair follicle epithelium have been referred to as the hair follicle immune system [52]. Langerhans cells represent the major cell population, along with T lymphocytes, in the skin of newborn mice [53]. They migrate from the epidermis to the ORS during hair follicle generation, and their numbers increase significantly as the hair cycle progresses [53]. They are also concentrated in the entrance of the hair follicle (infundibulum) and the follicular bulge [44]. Perifollicular macrophages are seen in the mouse dermis and the subcutis (panniculus) at post-natal day 1 (P1), with numbers increasing and peaking at around P20 [53]. In human scalp, CD68⁺/MHC class II⁺ macrophages are mostly distributed in the perifollicular basal lamina, while CD1a⁺/MHC class II⁺ Langerhans cells are located at the distal hair follicle epithelium; both display low numbers in the dermal papilla and the proximal hair follicle [8].

A role for Langerhans cells in the hair follicle during catagen has been proposed by D. J. Tobin. He suggests that LCs help remove melanin from hair follicles in healthy human scalp [74]. During catagen, pigmentation, which comprises the transport of melanin to the follicular dermal papilla, occurs simultaneously with the hair cycle [74]. Through microscopy analysis, Tobin shows that Langerhans cells, which were identified based on their distinctive cytoplasmic granules, endocytose and transport melanosomes from the regressing hair matrix to the dermal papilla during early catagen. This role elucidates a system whereby the hair follicle can dispose of excess pigment during catagen. Additionally, the LCs of the hair follicle can serve as a pool that replenishes the LCs of the human epidermis after ultraviolet B (UVB) exposure [15]. Two days after UVB exposure, which ablates the LCs in the epidermis while sparing the LCs in the hair follicle as seen through immunohistochemistry, LCs from the ORS proliferate and migrate to the epidermis [15]. Moreover, the LCs that repopulate the epidermis display a distinct phenotype from the

resident epidermal LCs, in that they are deficient in the induction of B7 protein but not CD40 costimulatory molecules [15].

In 1970, Parakkal [51] described a physiological role of macrophages in hair follicle regression during catagen. He found that macrophages cluster around the hair follicle and are in charge of phagocytosing and breaking down the collagen fibers in the basal lamina in mice and rats. Also, activated macrophages clusters can be observed, in normal uninflamed undamaged mouse skin, close to the distal hair follicle (HF) epithelium during telogen, anagen, and catagen suggesting that they have a physiological role in homeostatic hair follicle regeneration [12]. Intercellular adhesion molecule (ICAM)-1 is upregulated within clusters of macrophages restricted to mouse skin and has been found to be dependent on the hair cycle [46]. The ICAM-1 ligand, lymphocyte function associated antigen (LFA)-1, is also hair cycle-dependent and highly expressed in follicle-associated macrophages and LCs [46]. Moreover, ICAM-1 knockout mouse show premature hair follicle regression suggesting ICAM-1 has a role in catagen inhibition. Another factor implicated in catagen regulation is fibroblast growth factor (Fgf)-5, which is localizes in skin macrophages at the hair follicle [66], and its expression has been shown to be dependent on the hair cycle, inhibit hair growth in anagen, and induce the anagen-catagen transition when injected subcutaneously into mouse [67].

Osaka, *et al.*, found that intracutaneous transplantation of activated bone marrow-derived macrophages into the skin of wild type mouse is enough to cause hair growth proportional to the numbers of transplanted macrophages [50]. They also found that macrophages accelerate the proliferation of epidermal basal cells, hair follicle cells, and stem cells, suggesting that macrophages can promote entrance to anagen. Furthermore, Castellana, *et al.*, found that macrophages promote activation of hair follicle stem cells inducing the entrance to anagen [6]. They observed an apoptosis-induced decrease of F4/80⁺ CD11b⁺ Gr1⁺ macrophages at the end of telogen, and showed that genes regulating hair follicle stem cell (HFSC) activation are upregulated at this stage. Moreover, they showed that macrophage depletion with clodronate, which induces macrophage apoptosis, promotes premature entry into anagen, and is associated with the activation of HFSC through the B-catenin/Wnt signaling pathways. Furthermore, Amberg, *et al.*, found that inflammatory macrophages and dermal resident macrophages have different effects in the hair cycle. They showed that resident macrophages inhibit activation of hair follicle stem cells, preventing the entrance to anagen, while inflammatory infiltrating macrophages promote anagen [2].

The role of macrophages in cutaneous salt regulation

The kidneys are classically considered the primary regulators of sodium (Na⁺) and fluid homeostasis. The kidneys monitor Na⁺ levels and control blood pressure (BP) through the renin-angiotensin system [64]. Non-renal regulators of blood pressure include the cardiovascular, neural and endocrine systems [7]. Remarkably, during the past decade the skin has emerged as new regulator of blood pressure. Growing data point towards a skin-based control of blood pressure through local balance of sodium and water [69]. The idea of sodium storage in the skin and its induction by a high salt diet (HSD) was first investigated in the 1900's [25]. In 2000, Heer, *et al.*, re-explored this system, which does not cause water

accumulation in humans [21]. Humans subjects were fed high amounts of sodium chloride (NaCl), then fluid compartments, metabolic and sodium balances were measured. The authors found that plasma volume increased proportionally to NaCl intake, whereas total body water or extracellular volume did not increase, suggesting that water moves from the interstitial to the intravascular compartment without extracellular volume expansion. Another group suggested that the fact that retention of sodium does not cause extracellular volume expansion indicates that Na⁺ is accumulated in an osmotically inactive form, which bypasses renal control [70]. They performed similar experiments in salt-sensitive Dahl rats, that develop hypertension in response to high Na⁺ diet. As a result, these rats showed a decreased capacity to store osmotically inactive Na⁺, which made them prone to fluid retention and resulted in hypertension. Furthermore, they establish that the skin serves as a reservoir for the osmotically inactive sodium, which increases after a high salt diet [71], and they suggest that polymerization of negatively-charged glycosaminoglycans (GAGs) could facilitate its storage in the skin [73].

The mononuclear phagocyte system (MPS), which includes macrophages and dendritic cells, has also been implicated in the regulation of skin salt homeostasis and blood pressure [35]. In experiments where mice were fed a high sodium diet, ear histology displayed hyperplastic lymph capillaries with increased interstitial infiltration of vascular endothelial growth factor C positive mononuclear phagocytes and increased levels of tonicity-responsive enhancer binding protein (TonEBP) (Figure II). Infiltration of MPS cells into mouse skin following a high salt diet, points to a role in regulating cutaneous salt homeostasis. Thus, in order to assess whether the response to the defects caused by a HSD was dependent on mononuclear phagocytes, mice were treated with clodronate, which depletes MPS cells, and fed a HSD. After depletion, hyperplastic lymph capillaries were absent, and the increase in the levels of VEGF-C positive MPS cells and expression of TonEBP was abolished. TonEBP, also known as nuclear factor of activated T cells (NFAT)-5, is a transcription factor in charge of maintaining cellular homeostasis in response to hypotonic or hypertonic stress [20]. The fact that mononuclear phagocyte depletion prevented the upregulation of TonEBP suggests that macrophages positively regulate the response to osmotic stress. Additionally, the increase in sodium was followed by an elevation in GAG charge density and proteoglycan levels, which supports the theory that GAGs facilitate Na⁺ storage in the skin. Moreover, a second study also shows that clodronate depletion of MPS cells results in the loss of the homeostatic response in the skin of rats after consumption of a high salt diet [34]. Similarly, they demonstrate that MPS cell depletion results in a decrease in TonEBP and VEGF-C expression and causes salt-sensitive hypertension in rats [34]. They propose that macrophages regulate cutaneous interstitial volume and promote blood pressure homeostasis through the following mechanism: macrophages express TonEBP to balance osmotic stress caused by excess sodium in the skin, resulting in the production of VEGF-C, which promotes the generation of lymph capillaries and the expression of endothelial nitric oxide synthase (eNOS) buffering the development of salt sensitive hypertension [34, 72]. This mechanism is further supported by the conditional deletion of TonEBP in mice MPS cells, which were generated in LysM^{Cre} TonEBP^{fl/fl} mice [75]. The conditional knockout, along with a HSD, caused a decrease in VEGF-C mRNA and protein levels, accompanied by a reduction in skin lymph capillary density. Also, mutant mice showed impaired clearance of

skin chloride and suffered from salt-sensitive hypertension [75]. This mechanism was also shown to play a role in salt homeostasis and blood pressure in healthy humans and patients suffering from chronic kidney disease [63]. In these subjects, VEGF-C levels were shown to be regulated by sodium consumption further validating the proposed mechanism.

It is well known that chemotaxis is essential to recruit macrophages during inflammation [5]. Muller, *et al.*, explored the concept of macrophage chemotaxis in relation to osmotic stress [45]. Notably, they found that murine bone marrow derived macrophages and peritoneal macrophages, travel towards sodium chloride (NaCl) but not urea or mannitol gradients, indicating that NaCl induced hypertonic stress, as opposed to general osmolality, is a chemotactic stimulus that recruits macrophages *in vitro*. In addition, they transfected RAW264.7 macrophages with mouse Tag-TonEBP and used kanamycin resistance to select for clones overexpressing TonEBP, to show that TonEBP is dispensable during salt dependent macrophage chemotaxis. These results are consistent with the data obtained during the conditional deletion of TonEBP in MPS cells, which did not affect macrophage recruitment and infiltration to the skin [75]. Additionally, the authors find that only bone marrow derived (BMD) macrophages and peritoneal macrophages, but not dendritic cells, are attracted to the excess NaCl, suggesting that sodium-induced chemotaxis is specific to macrophages and excludes the rest of the cells in the mononuclear phagocyte system. It is important to note that although in this review we mostly show that the NFAT5/TonEBP-induced response is anti-inflammatory (Figure II), other groups have shown that NFAT5 is necessary to generate a pro-inflammatory response in the setting of infection or inflammation independently of osmotic stress [5, 27]

Recent studies suggest that high salt concentration affects the activation state of macrophages [3, 78]. The change in expression profile of human monocyte derived macrophages was studied after treatment with excess salt *in vitro* [78]. It was found that high salt induces the expression of pro-inflammatory genes, including chemokine ligands (CCL2, CCL8, CXCL1, CXCL2), cytokines (IL β -1 and IL-8), and toll-like receptors (TLR3 and TLR4), and displayed dose-response relationship. In addition, high NaCl inhibited the expression of anti-inflammatory molecules such as CCL18, CCL22, triggering receptor expressed in myeloid cells (TREM)-2 and mannose receptor C type 1 (MRC1). In macrophages treated with IL-4 or lipopolysaccharide (LPS) in combination with high salt, IL-4-induced expression of anti-inflammatory genes was inhibited while LPS induced expression of pro-inflammatory genes was promoted. Binger, *et al.*, [3] showed that the activation of anti-inflammatory macrophages, expressing IL-4 and IL-13, was significantly decreased by physiological amounts of salt, and was independent of sodium responsive factors such as NFAT5 and serum/glucocorticoid regulated kinase (SGK)-1. Additionally, they found that under these conditions the protein kinase B (AKT) and mechanistic target of rapamycin (mTOR) pathway was hindered.

Recently, Jantsch, *et al.*, [25] showed that during a parasitic infection, high sodium concentrations promote the activation of pro-inflammatory macrophages and helps control the infection independently of diet. They used magnetic resonance imaging (MRI) to visualize ^{23}Na in the skin of patients infected with *Leishmania major*, and found it was accumulated. They also establish that sodium accumulation correlates with the activation of

RAW 264.7 and BMD macrophages *in vitro*, which is shown by enhanced LPS-induced stimulation of NOS2, NO release and TNF release, promoting the elimination of *Leishmania major*.

The role of macrophages the regulation of inflammation, autoimmunity, and cancer in the skin

The phagocytosis theory proposed by Metchnikoff suggests that macrophages are key in preserving the harmony and integrity of the organism [68]. Macrophages constantly monitor the skin microenvironment for signals that indicate cell stress, tissue injury or infection [47]. They display a high degree of plasticity, and are able to change their genetic profile to promote or suppress inflammation in order to maintain homeostasis. The class of inflammatory stimulus determines the type of pathway induced [38]. Skin inflammation has physiological roles which include infection defense, tissue repair response, stress adaptation, and restoration of homeostasis [39]. After skin injury or malfunction or imbalance in the skin, macrophages undergo an adaptive inflammatory response with the goal of restoring homeostasis balance [39]. During host defense, controlled inflammation is usually beneficial for an organism, and involves the recruitment of factors necessary to manage the infection such as neutrophils and plasma proteins [39]. As a result of pathogen detection, macrophages are activated, and rapidly produce cytokines, chemokines, inflammatory mediators such as prostaglandins, leukotrienes and platelet activating factor [24]. Also, blood monocytes are recruited and differentiate into additional macrophages [24]. The end result of an effective inflammatory response is the eradication of the pathogen, inflammation resolution and repair. In any situation, inflammation is a form of adaptation that responds to stressful conditions to restore homeostasis [38], and macrophages are essential during this response.

In addition to inflammation, macrophages and LCs also have a role in cancer prevention in the skin. Studies have shown that sporadic deletion of macrophages and LCs results in the development of basal cell carcinomas [29]. Basal cell carcinoma (BCC) represents the most common cancer in humans, and it is caused by mutations in the tumor suppressor gene protein patched homolog 1 (PTCH1), which results in uncontrolled activation in the hedgehog pathway [43]. It is considered a keratinocyte carcinoma and resembles differentiated basal cells of the epidermis and its accessory structures [26]. Recently, König, *et al.*, showed that macrophages and Langerhans cells are necessary for limiting the growth of BCCs in mice [29]. They used a conditional knock out mouse, a BCC model with the *Ptch1* gene floxed and tamoxifen inducible Cre recombinase downstream of the ROSA26 promoter (*Ptch1^{flox/flox} ERT^{+/-}*), to show that BCC tumors are infiltrated with macrophages and LCs. Depletion of macrophages, dermal DCs and epidermal LCs with intraperitoneal injections of clodronate liposomes results in augmentation of BCC growth with a reduction in the number of stromal cells and decreased levels of fibroblast markers (vimentin and prolyl 4-hydroxylase) [29]. Effective depletion of macrophages was confirmed through Mac1 and F4/80 levels, while depletion of LCs and DCs was confirmed MHC class II in the epidermis and the dermis, respectively. Moreover, topical treatment with imiquimod, a drug that is FDA-approved to treat superficial BCC, results in increased infiltration of macrophages and T cells in mice [2]. In addition, tumor peptide-pulsed intra-dermal

Langerhans cell vaccines have been shown to be effective at eliciting immunity against melanoma antigens in Phase I clinical trials in patients with stage III or IV melanoma [58].

During homeostasis, LCs also promote tolerogenic responses to self-antigens in the skin [9]. To assess the role of LCs in immune tolerance in the skin, a group crossed systemic lupus erythematosus mice (MRL-lpr and MRL/MpJ-Fas^{+/+}) which develop a syndrome that resembles lupus in humans, with B6 mice, in which the LC-specific gene CD207 (Langerin) is attached to the diphtheria toxin (DT) receptor, and a single dose of DT temporarily abates Langerin+Langerhans cells [28]. Mouse serum showed skin self-antigens (Dsg-3), and cells isolated from these mice displayed an immune response when cultured with Dsg-3. The authors conclude that autoimmune-prone mice, LCs are capable of protecting mice from acquiring autoimmune dermatitis, and maintaining skin tolerance [28].

CONCLUSION

The present review summarizes the currently available evidence, which supports multiple roles of macrophages and Langerhans cells in maintaining cutaneous homeostasis. We review how macrophages are present during all three stages of wound repair, and that they are essential to obtain wound closure and full tissue regeneration. We explore mechanisms by which macrophages and Langerhans cells are involved in hair follicle regeneration. Macrophages digest excess ECM during hair follicle regression (catagen) and stimulate follicular stem cells to enter the active stage of hair growth (anagen), while Langerhans cells help eliminate excess hair follicle melanin during catagen. We summarize the evidence that supports a macrophage-based model during salt balance in the skin. Lastly, we illustrate how macrophages and LCs are involved in the regulation of inflammation and autoimmunity, and in the regression of skin cancer. The studies included in this review, which show different experimental approaches for the ablation of myeloid cells in the skin are limited. More specific genetic models are required to clearly define the role of macrophages in the skin compared to the role of the rest of the myeloid-derived cells. Understanding the array of functions of Langerhans cells and macrophages during skin homeostasis, spatially and temporally, is essential for the development of future therapies that can aid in the restoration of homeostasis after the origination of trauma, stress or pathologic conditions such as cancer or autoimmune diseases in the skin.

Acknowledgments

This work was supported by a grant from the Doris Duke Charitable Foundation (15-007374) to ORC.

References

1. Alonso L, Fuchs E. The hair cycle. *J Cell Sci.* 2006; 119:391–393. DOI: 10.1242/jcs02793 [PubMed: 16443746]
2. Amberg N, Holmann M, Stulnig G, et al. Effects of Imiquimod on Hair Follicle Stem Cells and Hair Cycle Progression. *J Invest Dermatol.* 2016; 136:2140–2149. DOI: 10.1016/j.jid.2016.06.613 [PubMed: 27377695]
3. Binger KJ, Gebhardt M, Heinig M, et al. High salt reduces the activation of IL-4- and IL-13-stimulated macrophages. *J Clin Invest.* 2015; 125:4223–4238. DOI: 10.1172/JCI80919 [PubMed: 26485286]

4. Blanpain C, Fuchs E. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat Rev Mol Cell Biol.* 2009; 10:207–217. DOI: 10.1038/nrm2636 [PubMed: 19209183]
5. Cantaert T, Baeten D, Tak PP, et al. Type I IFN and TNF α cross-regulation in immune-mediated inflammatory disease: basic concepts and clinical relevance. *Arthritis Res Ther.* 2010; 12:219.doi: 10.1186/ar3150 [PubMed: 21062511]
6. Castellana D, Paus R, Perez-Moreno M. Macrophages contribute to the cyclic activation of adult hair follicle stem cells. *PLoS Biol.* 2014; 12:e1002002.doi: 10.1371/journal.pbio.1002002 [PubMed: 25536657]
7. Chopra S, Baby C, Jacob JJ. Neuro-endocrine regulation of blood pressure. *Indian J Endocrinol Metab.* 2011; 15(Suppl 4):S281–288. DOI: 10.4103/2230-8210.86860 [PubMed: 22145130]
8. Christoph T, Muller-Rover S, Audring H, et al. The human hair follicle immune system: cellular composition and immune privilege. *Br J Dermatol.* 2000; 142:862–873. [PubMed: 10809841]
9. Clausen BE, Kel JM. Langerhans cells: critical regulators of skin immunity? *Immunol Cell Biol.* 2010; 88:351–360. DOI: 10.1038/icb.2010.40 [PubMed: 20351747]
10. Dovi JV, He LK, Dipietro LA. Accelerated wound closure in neutrophil-depleted mice. *J Leukoc Biol.* 2003; 73:448–455. [PubMed: 12660219]
11. Driskell RR, Clavel C, Rendl M, et al. Hair follicle dermal papilla cells at a glance. *J Cell Sci.* 2011; 124:1179–1182. DOI: 10.1242/jcs.082446 [PubMed: 21444748]
12. Eichmuller S, Van Der Veen C, Moll I, et al. Clusters of perifollicular macrophages in normal murine skin: physiological degeneration of selected hair follicles by programmed organ deletion. *J Histochem Cytochem.* 1998; 46:361–370. [PubMed: 9487118]
13. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol.* 2007; 127:514–525. DOI: 10.1038/sj.jid.5700701 [PubMed: 17299434]
14. Fuchs E. Scratching the surface of skin development. *Nature.* 2007; 445:834–842. DOI: 10.1038/nature05659 [PubMed: 17314969]
15. Gilliam AC, Kremer IB, Yoshida Y, et al. The human hair follicle: a reservoir of CD40+ B7-deficient Langerhans cells that repopulate epidermis after UVB exposure. *J Invest Dermatol.* 1998; 110:422–427. DOI: 10.1046/j.1523-1747.1998.00162.x [PubMed: 9540986]
16. Ginhoux F, Guilliams M. Tissue-Resident Macrophage Ontogeny and Homeostasis. *Immunity.* 2016; 44:439–449. DOI: 10.1016/j.immuni.2016.02.024 [PubMed: 26982352]
17. Golpon HA, Fadok VA, Taraseviciene-Stewart L, et al. Life after corpse engulfment: phagocytosis of apoptotic cells leads to VEGF secretion and cell growth. *FASEB J.* 2004; 18:1716–1718. DOI: 10.1096/fj.04-1853fje [PubMed: 15345697]
18. Goren I, Allmann N, Yogev N, et al. A transgenic mouse model of inducible macrophage depletion: effects of diphtheria toxin-driven lysozyme M-specific cell lineage ablation on wound inflammatory, angiogenic, and contractive processes. *Am J Pathol.* 2009; 175:132–147. DOI: 10.2353/ajpath.2009.081002 [PubMed: 19528348]
19. Guilliams M, Ginhoux F, Jakubzick C, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol.* 2014; 14:571–578. DOI: 10.1038/nri3712 [PubMed: 25033907]
20. Halterman JA, Kwon HM, Wamhoff BR. Tonicity-independent regulation of the osmosensitive transcription factor TonEBP (NFAT5). *Am J Physiol Cell Physiol.* 2012; 302:C1-8.doi: 10.1152/ajpcell.00327.2011 [PubMed: 21998140]
21. Heer M, Baisch F, Kropp J, et al. High dietary sodium chloride consumption may not induce body fluid retention in humans. *Am J Physiol Renal Physiol.* 2000; 278:F585–595. [PubMed: 10751219]
22. Hettinger J, Richards DM, Hansson J, et al. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol.* 2013; 14:821–830. DOI: 10.1038/ni.2638 [PubMed: 23812096]
23. Ishida Y, Gao JL, Murphy PM. Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. *J Immunol.* 2008; 180:569–579. [PubMed: 18097059]
24. Janeway C. Immunobiology : the immune system in health and disease. Garland Science, New York. 2005

25. Jantsch J, Schatz V, Friedrich D, et al. Cutaneous Na⁺ storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. *Cell Metab.* 2015; 21:493–501. DOI: 10.1016/j.cmet.2015.02.003 [PubMed: 25738463]
26. Kasper M, Jaks V, Hohl D, et al. Basal cell carcinoma - molecular biology and potential new therapies. *J Clin Invest.* 2012; 122:455–463. DOI: 10.1172/JCI58779 [PubMed: 22293184]
27. Kim NH, Choi S, Han EJ, et al. The xanthine oxidase-NFAT5 pathway regulates macrophage activation and TLR-induced inflammatory arthritis. *Eur J Immunol.* 2014; 44:2721–2736. DOI: 10.1002/eji.201343669 [PubMed: 25044064]
28. King JK, Philips RL, Eriksson AU, et al. Langerhans Cells Maintain Local Tissue Tolerance in a Model of Systemic Autoimmune Disease. *J Immunol.* 2015; 195:464–476. DOI: 10.4049/jimmunol.1402735 [PubMed: 26071559]
29. Konig S, Nitzki F, Uhmann A, et al. Depletion of cutaneous macrophages and dendritic cells promotes growth of basal cell carcinoma in mice. *PLoS One.* 2014; 9:e93555.doi: 10.1371/journal.pone.0093555 [PubMed: 24691432]
30. Lee J, Tumber T. Hairy tale of signaling in hair follicle development and cycling. *Semin Cell Dev Biol.* 2012; 23:906–916. DOI: 10.1016/j.semcdb.2012.08.003 [PubMed: 22939761]
31. Leibovich SJ, Ross R. The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am J Pathol.* 1975; 78:71–100. [PubMed: 1109560]
32. Lucas T, Waisman A, Ranjan R, et al. Differential roles of macrophages in diverse phases of skin repair. *J Immunol.* 2010; 184:3964–3977. DOI: 10.4049/jimmunol.0903356 [PubMed: 20176743]
33. Ma Y, De Castro Bras LE, Toba H, et al. Myofibroblasts and the extracellular matrix network in post-myocardial infarction cardiac remodeling. *Pflugers Arch.* 2014; 466:1113–1127. DOI: 10.1007/s00424-014-1463-9 [PubMed: 24519465]
34. Machnik A, Dahlmann A, Kopp C, et al. Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. *Hypertension.* 2010; 55:755–761. DOI: 10.1161/HYPERTENSIONAHA.109.143339 [PubMed: 20142563]
35. Machnik A, Neuhofer W, Jantsch J, et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med.* 2009; 15:545–552. DOI: 10.1038/nm.1960 [PubMed: 19412173]
36. Madsen DH, Leonard D, Masedunskas A, et al. M2-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway. *J Cell Biol.* 2013; 202:951–966. DOI: 10.1083/jcb.201301081 [PubMed: 24019537]
37. Martin P. Wound healing--aiming for perfect skin regeneration. *Science.* 1997; 276:75–81. [PubMed: 9082989]
38. Medzhitov R. Inflammation 2010: new adventures of an old flame. *Cell.* 2010; 140:771–776. DOI: 10.1016/j.cell.2010.03.006 [PubMed: 20303867]
39. Medzhitov R. Origin and physiological roles of inflammation. *Nature.* 2008; 454:428–435. DOI: 10.1038/nature07201 [PubMed: 18650913]
40. Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat Rev Immunol.* 2008; 8:935–947. DOI: 10.1038/nri2455 [PubMed: 19029989]
41. Minutti CM, Knipper JA, Allen JE, et al. Tissue-specific contribution of macrophages to wound healing. *Semin Cell Dev Biol.* 2016; doi: 10.1016/j.semcdb.2016.08.006
42. Mirza R, Dipietro LA, Koh TJ. Selective and specific macrophage ablation is detrimental to wound healing in mice. *Am J Pathol.* 2009; 175:2454–2462. DOI: 10.2353/ajpath.2009.090248 [PubMed: 19850888]
43. Mohan SV, Chang AL. Advanced Basal Cell Carcinoma: Epidemiology and Therapeutic Innovations. *Curr Dermatol Rep.* 2014; 3:40–45. DOI: 10.1007/s13671-014-0069-y [PubMed: 24587976]
44. Moresi JM, Horn TD. Distribution of Langerhans cells in human hair follicle. *J Cutan Pathol.* 1997; 24:636–640. [PubMed: 9449491]
45. Muller S, Quast T, Schroder A, et al. Salt-dependent chemotaxis of macrophages. *PLoS One.* 2013; 8:e73439.doi: 10.1371/journal.pone.0073439 [PubMed: 24066047]

46. Muller-Rover S, Bulfone-Paus S, Handjiski B, et al. Intercellular adhesion molecule-1 and hair follicle regression. *J Histochem Cytochem*. 2000; 48:557–568. [PubMed: 10727297]
47. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol*. 2011; 11:723–737. DOI: 10.1038/nri3073 [PubMed: 21997792]
48. Nestle FO, Di Meglio P, Qin JZ, et al. Skin immune sentinels in health and disease. *Nat Rev Immunol*. 2009; 9:679–691. DOI: 10.1038/nri2622 [PubMed: 19763149]
49. Ojeh N, Pastar I, Tomic-Canic M, et al. Stem Cells in Skin Regeneration, Wound Healing, and Their Clinical Applications. *Int J Mol Sci*. 2015; 16:25476–25501. DOI: 10.3390/ijms161025476 [PubMed: 26512657]
50. Osaka N, Takahashi T, Murakami S, et al. ASK1-dependent recruitment and activation of macrophages induce hair growth in skin wounds. *J Cell Biol*. 2007; 176:903–909. DOI: 10.1083/jcb.200611015 [PubMed: 17389227]
51. Parakkal PF. Morphogenesis of the hair follicle during catagen. *Z Zellforsch Mikrosk Anat*. 1970; 107:174–186. [PubMed: 5447672]
52. Paus R. Principles of hair cycle control. *J Dermatol*. 1998; 25:793–802. [PubMed: 9990771]
53. Paus R, Van Der Veen C, Eichmuller S, et al. Generation and cyclic remodeling of the hair follicle immune system in mice. *J Invest Dermatol*. 1998; 111:7–18. DOI: 10.1046/j.1523-1747.1998.00243.x [PubMed: 9665380]
54. Peters T, Sindrilaru A, Hinz B, et al. Wound-healing defect of CD18(–/–) mice due to a decrease in TGF-beta1 and myofibroblast differentiation. *EMBO J*. 2005; 24:3400–3410. DOI: 10.1038/sj.emboj.7600809 [PubMed: 16148944]
55. Reinke JM, Sorg H. Wound repair and regeneration. *Eur Surg Res*. 2012; 49:35–43. DOI: 10.1159/000339613 [PubMed: 22797712]
56. Rodero MP, Licata F, Poupel L, et al. In vivo imaging reveals a pioneer wave of monocyte recruitment into mouse skin wounds. *PLoS One*. 2014; 9:e108212.doi: 10.1371/journal.pone.0108212 [PubMed: 25272047]
57. Rohani MG, McMahan RS, Razumova MV, et al. MMP-10 Regulates Collagenolytic Activity of Alternatively Activated Resident Macrophages. *J Invest Dermatol*. 2015; 135:2377–2384. DOI: 10.1038/jid.2015.167 [PubMed: 25927164]
58. Romano E, Rossi M, Ratzinger G, et al. Peptide-loaded Langerhans cells, despite increased IL15 secretion and T-cell activation in vitro, elicit antitumor T-cell responses comparable to peptide-loaded monocyte-derived dendritic cells in vivo. *Clin Cancer Res*. 2011; 17:1984–1997. DOI: 10.1158/1078-0432.CCR-10-3421 [PubMed: 21355077]
59. Rompolas P, Deschene ER, Zito G, et al. Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration. *Nature*. 2012; 487:496–499. DOI: 10.1038/nature11218 [PubMed: 22763436]
60. Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen*. 2009; 17:153–162. DOI: 10.1111/j.1524-475X.2009.00466.x [PubMed: 19320882]
61. Sheng J, Ruedl C, Karjalainen K. Most Tissue-Resident Macrophages Except Microglia Are Derived from Fetal Hematopoietic Stem Cells. *Immunity*. 2015; 43:382–393. DOI: 10.1016/j.immuni.2015.07.016 [PubMed: 26287683]
62. Sindrilaru A, Peters T, Schymeinsky J, et al. Wound healing defect of Vav3–/– mice due to impaired {beta}2-integrin-dependent macrophage phagocytosis of apoptotic neutrophils. *Blood*. 2009; 113:5266–5276. DOI: 10.1182/blood-2008-07-166702 [PubMed: 19147786]
63. Slagman MC, Kwakernaak AJ, Yazdani S, et al. Vascular endothelial growth factor C levels are modulated by dietary salt intake in proteinuric chronic kidney disease patients and in healthy subjects. *Nephrol Dial Transplant*. 2012; 27:978–982. DOI: 10.1093/ndt/gfr402 [PubMed: 21778278]
64. Sparks MA, Crowley SD, Gurley SB, et al. Classical Renin-Angiotensin system in kidney physiology. *Compr Physiol*. 2014; 4:1201–1228. DOI: 10.1002/cphy.c130040 [PubMed: 24944035]
65. Stenn KS, Paus R. Controls of hair follicle cycling. *Physiol Rev*. 2001; 81:449–494. [PubMed: 11152763]

66. Suzuki S, Kato T, Takimoto H, et al. Localization of rat FGF-5 protein in skin macrophage-like cells and FGF-5S protein in hair follicle: possible involvement of two Fgf-5 gene products in hair growth cycle regulation. *J Invest Dermatol.* 1998; 111:963–972. DOI: 10.1046/j.1523-1747.1998.00427.x [PubMed: 9856803]
67. Suzuki S, Ota Y, Ozawa K, et al. Dual-mode regulation of hair growth cycle by two Fgf-5 gene products. *J Invest Dermatol.* 2000; 114:456–463. DOI: 10.1046/j.1523-1747.2000.00912.x [PubMed: 10692103]
68. Tauber AI. Metchnikoff and the phagocytosis theory. *Nat Rev Mol Cell Biol.* 2003; 4:897–901. DOI: 10.1038/nrm1244 [PubMed: 14625539]
69. Titze J. Water-free sodium accumulation. *Semin Dial.* 2009; 22:253–255. DOI: 10.1111/j.1525-139X.2009.00569.x [PubMed: 19573004]
70. Titze J, Krause H, Hecht H, et al. Reduced osmotically inactive Na storage capacity and hypertension in the Dahl model. *Am J Physiol Renal Physiol.* 2002; 283:F134–141. DOI: 10.1152/ajprenal.00323.2001 [PubMed: 12060595]
71. Titze J, Lang R, Ilies C, et al. Osmotically inactive skin Na⁺ storage in rats. *Am J Physiol Renal Physiol.* 2003; 285:F1108–1117. DOI: 10.1152/ajprenal.00200.2003 [PubMed: 12888617]
72. Titze J, Machnik A. Sodium sensing in the interstitium and relationship to hypertension. *Curr Opin Nephrol Hypertens.* 2010; 19:385–392. DOI: 10.1097/MNH.0b013e32833aeb3b [PubMed: 20571401]
73. Titze J, Shakibaei M, Schafflhuber M, et al. Glycosaminoglycan polymerization may enable osmotically inactive Na⁺ storage in the skin. *Am J Physiol Heart Circ Physiol.* 2004; 287:H203–208. DOI: 10.1152/ajpheart.01237.2003 [PubMed: 14975935]
74. Tobin DJ. A possible role for Langerhans cells in the removal of melanin from early catagen hair follicles. *Br J Dermatol.* 1998; 138:795–798. [PubMed: 9666824]
75. Wiig H, Schroder A, Neuhofer W, et al. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. *J Clin Invest.* 2013; 123:2803–2815. DOI: 10.1172/JCI60113 [PubMed: 23722907]
76. Willenborg S, Eming SA. Macrophages - sensors and effectors coordinating skin damage and repair. *J Dtsch Dermatol Ges.* 2014; 12:214–221. 214–223. DOI: 10.1111/ddg.12290 [PubMed: 24580874]
77. Willenborg S, Lucas T, Van Loo G, et al. CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair. *Blood.* 2012; 120:613–625. DOI: 10.1182/blood-2012-01-403386 [PubMed: 22577176]
78. Zhang WC, Zheng XJ, Du LJ, et al. High salt primes a specific activation state of macrophages, M(Na). *Cell Res.* 2015; 25:893–910. DOI: 10.1038/cr.2015.87 [PubMed: 26206316]
79. Zhu Z, Ding J, Ma Z, et al. Systemic depletion of macrophages in the subacute phase of wound healing reduces hypertrophic scar formation. *Wound Repair Regen.* 2016; 24:644–656. DOI: 10.1111/wrr.12442 [PubMed: 27169512]

Figure I. Role of macrophages in skin homeostasis

Macrophages maintain and restore homeostasis of the skin through functions including wound repair, cancer defense, salt balance and hair regeneration.

Figure II. Mechanism of macrophage regulation of cutaneous salt balance and blood pressure

High concentration of sodium in the skin attracts macrophages that express the transcription factor TonEBP in response to osmotic stress. This results in the expression of VEGF-C, which promotes the development of lymph capillaries (yellow) and the production of nitric oxide synthase (eNOS), which induces blood vessel (red and blue) dilation decreasing blood pressure. Increased levels of NaCl induce macrophage expression of pro-inflammatory genes (IL-1, IL-8, TLR3, TLR4, CXCL1/2), and inhibit the expression of anti-inflammatory genes (CCL18, CCL22, TREM-2, MRC1).