IMMUNE CELL REGULATION OF THE HAIR CYCLE

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ABSTRACT

The ability to manipulate the mammalian hair cycle will lead to novel therapies and strategies to combat all forms of alopecia. Thus, in addition to the epithelial-mesenchymal interactions in the hair follicle, niche and microenvironmental signals that accompany the phases of growth, regression and rest need to be scrutinized. Immune cells are well-described in skin homeostasis and wound healing, and have recently been shown to play an important role in the mammalian hair cycle. In this review, we will summarize our current knowledge of the role of immune cells in hair cycle control, and discuss their relevance to human hair cycling disorders. Increased attention to this aspect of the hair cycle will provide new avenues to manipulate hair regeneration in humans, and provide better insight into developing better *ex vivo* models of hair growth.

KEY WORDS: Macrophages; T cells, Mast cells, Anagen, Catagen, Telogen

INTRODUCTION

Alopecia, or loss of hair, is caused by the disrupted integrity of the hair follicle (HF), a *sine qua non* appendage of mammalian skin. The main role of the hair follicle is to produce the keratinaceous hair shaft, whose maximum length *in situ* is dictated by the length of the growth phase of the hair cycle, which varies across body sites and between species [1]. The hair cycle is a complex, orchestrated flux of stem cells arising from distinct populations of keratinocytes of the HF, with the slowest cycling population in the bulge region, responding to morphogenetic and inhibitory signals coming from neighbouring cells [2, 3]. Disorders of the hair follicle include classically immune-mediated alopecias like alopecia areata (AA) and cicatricial alopecias (eg. discoid lupus erythematosus, lichen planopilaris), or non-immune mediated alopecias like androgenetic alopecia and telogen effluvium. These various disorders occur when the co-ordination of the hair cycle and functions of the hair follicle stem cells (HFSCs) have become impaired.

At steady state, after the HF has completed morphogenesis (a process that begins *in utero*), hair follicles go through cyclic phases of growth (anagen), regression (catagen), and relative rest (telogen), whereby keratinocyte proliferation, differentiation and quiescence are precisely co-ordinated. This co-ordination is partially controlled by intrinsic properties of the HFSC, but is also reliant on the HF microenvironment, made up of surrounding cells and tissues that contribute to the HFSC niche [4]. These cell types include the dermal papilla (DP) [5, 6], arrector pili muscle [7], adipose tissue [8], blood vessels [9], and even lymphatics [10]. Historically, immune cells have been described to be closely associated with hair follicles [11], with characteristic compositions at each stage of the hair cycle [12], but their influence on the HFSC niche has only recently been uncovered.

In this review, we have assembled the current knowledge on immune cells in and around the HF. We detail the role of immune cells in various stages of HF cycling (in both mouse and humans), and discuss immune cells in context of common human diseases that result from interference of the normal hair cycle.

IMMUNE CELLS AND TISSUE HOMEOSTASIS

Specialized macrophages, known as microglia, in the central nervous system have long been known to be responsible for the maintenance of synaptic health and optimal function of neurons [13]. More recently, immune cells, in addition to their nascent functions of antigen surveillance and microbial defense, have started to emerge as significant contributors to tissue homeostasis in a variety of other tissues. For example, another subset of macrophages have adapted to facilitate propagation of membrane potential and contractility of cardiomyocytes in the heart [14]. In the bone marrow, immune cells such as macrophages, neutrophils and T regulatory (T reg) cells double up as components of the hematopoietic stem cell niche [15-18], as do specialized macrophages in the skeletal muscle [19].

In the interfollicular epidermis (IFE) of the skin, Langerhans cells and dendritic epidermal T cells (DETCs) are found in close approximation with basal keratinocyte stem cells [20], while DETCs have been shown to support the IFE niche via insulin-like growth factor (IGF)-1 [21], and wound healing via fibroblast growth factor (FGF)-7 [22].

Immune cells are recruited to the developing HF during embryogenesis, and have been described to change dynamically with the hair cycle [23]. The HF epithelium is also densely populated by DETCs in both mice and humans [24-26]. Likewise, dermal macrophages are found to be associated with almost every stage of the hair cycle [27, 28], and may play multiple roles in the maintenance of the HF.

SPONTANEOUS HAIR CYCLING

The hair cycle is an exquisitely orchestrated series of cellular interactions between the epidermal keratinocytes and mesenchymal “control centre” in the DP. Evolutionarily, the hair cycle functions to provide a protective coat of fur for mammals, which has the capacity to regenerate easily when plucked, to molt and shed periodically, or change colour in order to adapt to temperate climates with changing seasons. The spontaneous hair cycle is the rhythmic progression of anagen-catagen-telogen-anagen in an unperturbed animal, and can be disrupted, induced and stimulated experimentally.

In rodents and other small mammals, the spontaneous hair cycle is tightly synchronized across their entire coat for the first few months of life, and anagen can be observed spreading from the anterior to the posterior integument in waves. Inbred laboratory strains like the C57BL/6 mouse are an ideal model animal to observe hair cycling because the phases of anagen, catagen and telogen are predictable according to their age up to about 80-100 days after birth (post-natal day 80-100, or P80-P100), at which time they enter their third, asynchronous anagen. The co-ordination of the synchronous waves of anagen in these animal models is currently under investigation [29], and may involve juxtacrine signals from the dermal papilla, IFE, subdermal adipose tissue, and very likely also the immune cells (summarized in Table 1 and Figure 1).

Catagen

At the end of HF morphogenesis, the completed terminal HF enters the first catagen, around P19 in a C57BL/6 mouse [30]. This process involves apoptosis of the keratinocytes that form the outer root sheath (ORS) and matrix [31], and a loss in volume of the DP (caused by a loss of extracellular matrix and migration of cells out of the DP [32]). The HF begins to regress, and the bulb (consisting of the few remaining matrix keratinocytes and the DP) is drawn away from the subcutaneous adipose tissue towards the epidermis (Figure 1A).

The decision for a terminal HF to enter catagen is likely a combination of signals from the microenvironment, including bone morphogenetic protein (BMP) signaling from the underlying adipose tissue [33]. In human HFs

in culture, transforming growth factor (TGF)- β 1 and TGF β 2 induce catagen and are likely produced by the DP [34, 35]. Interferon (IFN)- γ , which is typically produced by lymphoid cells of the immune system, is also a potent inducer of catagen [36]. FGF-5 from perifollicular macrophages have also been implicated in promoting catagen [37], while FGF-5 mutations result in trichomegaly of the eyelashes in humans [30, 38], and the long-haired angora phenotype in several mammalian species, including mice [39] and dogs [40, 41]. This knowledge has allowed researchers to disrupt the FGF-5 gene in cashmere goats, resulting in gene-edited animals with prolonged anagen that result in longer coats for the manufacture of clothing [42].

Mast cells, which are the arbiters of allergic inflammation, also play a role HF regression [43]. Mast cell degranulation is associated with catagen HFs, and granule contents substance P and adrenocorticotrophic hormone (ACTH) induce catagen in mouse HFs *in vivo*. Mast-cell deficient mice also have delayed catagen entry, as do neurokinin-1 knock-out mice that cannot respond to substance P secretion [44]. Interestingly, the HF contains mast cell precursors in mice [45], which can differentiate into mature mast cells under appropriate conditions, and contribute to wound healing in the skin [46]. Mast cells are also associated with HFs in human skin [47], and substance P produced by mast cells may contribute to HF regression by upregulating nerve growth factor (NGF) and its receptor p75NTR on HF ORS keratinocytes [48].

Cellular debris (apoptotic keratinocytes and redundant extracellular matrix, ECM) from the regressing HF are cleared by macrophages [49], and possibly neighbouring keratinocytes, which appear to take on phagocytic abilities [50]. As the HF regresses, the DP reduces in size and migrates upwards, leaving a “fibrous streamer” of degenerating elastic fibres [51]. Macrophages are involved in the clearance of this streamer, as histiocytes and multinucleated giant cells are observed in association with it [52]. The control of DP size during the hair cycle is currently poorly understood, and may be a combination of fluctuations in cell number and ECM components [32, 53]. Whether immune cells are involved in this process is still unknown.

Telogen

At the end of catagen, the HF enters the telogen phase, whereby the DP has reduced in size, and is located subjacent to the base of the so-called “permanent portion” of the HF (Figure 1B). During this phase of “rest”, the bulge and ORS keratinocytes are mitotically quiescent, and the hair shaft (“club hair”) remains anchored in the HF. This apparent “quiescence” of the HF is the result of active inhibitory macro- and microenvironment signals from cell types comprising the HF niche, including the Krt6+ inner bulge [54], arrector pili muscle [7], subdermal adipocytes [33], and HF-associated immune cells [55]. In the period immediately following catagen, the HF is in a state of refractory telogen, in which the threshold for anagen re-entry is higher, due in part to the increased concentrations of BMPs secreted by neighbouring adipocytes [33]. By mid-telogen, these inhibitory signals are reduced, and anagen is more readily induced by experimental means such as plucking, depilation and topical application of drugs or chemicals.

Immune cell numbers wax and wane according to the phase of the hair cycle, but both T regulatory (T reg) cells and macrophages are most abundant during mid-telogen [56-58], consistent with their roles in tissue homeostasis and remodeling. Systemic administration of clodronate liposomes during mid-telogen, which ablates phagocytic cells (including macrophages, but also other cell types), has been shown to induce early anagen re-entry in mice. Single-cell RNA sequencing of murine macrophages during telogen identified a rare subset of TREM2⁺ macrophages (coined “trichophages”) that produce Oncostatin M (OSM) [55], a potent inhibitor of HFSC proliferation and differentiation [59]. OSM maintains HFSC quiescence via the JAK-STAT5 pathway, and anagen can be induced by topical Janus kinase (JAK)-inhibitors in wild-type C57BL/6 mice in telogen [60]. More specific methods of inhibiting macrophages in the dermis during telogen (ie. with genetic, immunological and pharmacological means) also initiates the telogen-to-anagen transition in C57BL/6 mice [55].

Anagen

The beginning of the growth phase (“anagen”) starts with proliferation in the DP, which produces stimulatory signals such as Wnt10b [61], FGF-2, FGF-7 [62] and BMP antagonists [33] to induce proliferation of the matrix keratinocytes in the hair germ (HG). The proliferation of the HG sends a signal (like Sonic Hedgehog (Shh) [63]) to the HF bulge to activate the quiescent, long-lived HFSCs that stimulates proliferation of cells that will populate the ORS of the new anagen HF [64] (Figure 1C).

The telogen-to-anagen transition is a clinically relevant phase of the hair cycle, as understanding its control might allow us to replicate it in patients with various forms of recalcitrant alopecia. In mouse skin, the density of perifollicular macrophages decreases at the end of telogen [55, 56], suggesting a process that is coupled to anagen re-entry, perhaps via macrophage apoptosis and release of Wnt ligands [56].

The anagen HF interacts with the immune system in a way that promotes immune privilege, and prevents autoimmune attack of the shifting antigens in the growing HF. MHC class I expression anagen keratinocytes are upregulated [65], and a relative immunosuppression is observed in the murine skin [66].

As anagen progresses, the DP enlarges in size, and the HF begins to extend into the adipose tissue. The adipose layer also thickens during anagen, in a manner that appears to be synchronised with the HF cycle [67]. Adipocytes provide a supportive niche for the growing anagen HF by producing a myriad adipokines like leptin [68] and adiponectin [69]; and growth factors, including macrophage colony-stimulating factor (M-CSF) [70]. While macrophage-stimulating protein (MSP) has a direct effect on the anagen HF [71], its influence on the immune milieu itself during anagen has not been reported.

Human HFs cultured *ex vivo* were shown to be maintained in anagen with insulin, IGF-1 and IGF-2, and withdrawal of these factors led to catagen entry [72]. Whether DETCs are the source of IGF-1 in humans, as in the mouse during induced anagen [24], is currently unknown. The involvement of immune cells during

spontaneous anagen remains largely unexplored, as most studies on this phase of the hair cycle employ on the induced anagen model in small laboratory mammals.

Exogen/Kenogen

There are two sub-phases of the cycle that are independent of the cycling phases (anagen-catagen-telogen) that captures the physical shedding of the club hair from the follicle ("exogen") [73], and a short period whereby a telogen HF is without a hair shaft ("kenogen") [74].

The term exogen refers to the physical shedding of the club fibre (Figure 1D). To ensure a constant coat of fur in small mammals, the club hair fibre and growing fibre can reside in the same HF. Shedding of the club hair is an active proteolytic process [75], and whether immune cells are involved is unknown. Some intriguing data in birds suggests that there may be a link with immune cells and skin appendage retention; the relative force required to remove feathers (and escape predators) is larger in bird species with a stronger anti-parasitic T cells mediated immune response [76].

Kenogen is not usually observed on the coats of mammals, and is more of a phenomenon restricted to human scalp follicles. Kenogen is common when the club hair is shed without a new shaft being produced, the empty follicle is in kenogen. This state may be related to the miniaturized, refractory HFs in androgenetic alopecia (AGA) [77]. Interestingly, in humans, HFs in AGA are associated with deposits of immunoglobulins along the basement membrane [78], and a lymphocytic immune infiltrate [79], the significance and composition of which remains uncharacterized.

INDUCED HAIR CYCLING

Experimental models of the hair cycle in laboratory animals are conceptually distinct from the spontaneous hair cycle. These techniques are convenient for synchronizing the hair cycle over the entire animal in adulthood so that observations are easily recorded and studied (Figure 2). Plucking telogen hairs in a C57BL/6 mouse is known to induce a reliable anagen, with a characteristic time-course that can be perturbed and easily examined in the laboratory [80]. Similar effects are seen with chemical depilation, or within and around accidental or experimental wounds, all of which are associated with a significant inflammatory infiltrate [81]. Wounding-induced anagen progression is abnormal in mice lacking DETCs, suggesting shared mechanisms in tissue allostasis [82]. Evolutionarily, this allows the mammal to regenerate its coat of fur quickly and efficiently after environmental or predatorial insults. While many of the signals that coordinate induced anagen are similar to those in spontaneous anagen, there may be subtle differences that must be considered when interpreting these studies.

Early clues that spontaneous and induced hair cycling are distinct processes come from observations made by Sano et al, in which epidermal-specific STAT3 knock-out mice had delayed spontaneous anagen entry, but normal induced anagen entry with plucking or phorbol 12-myristate 13-acetate (PMA) application [83]. The threshold for telogen hairs to enter anagen in small mammals and rodents after injury is hence quite low, and compounds that stimulate anagen experimentally may not be relevant to the spontaneous hair cycle, or to the hair cycle in humans.

Plucking telogen hairs in mice appear to induce anagen of the surrounding HFs via a diffusible signal, and is contingent on the density and number of plucked HFs. Chen et al showed that there was threshold density of plucked HFs that stimulated anagen in their unplucked neighbours, and this signal was mediated by the release of chemokine (C-C motif) ligand 2 (CCL2) and subsequent recruitment of inflammatory macrophages that stimulated anagen by secreting tumour-necrosis factor (TNF)- α [58].

Depilatory creams such as Nair or Veet are commonly used to loosen HFs and induce a chemical depilation. The active ingredients include salts of thioglycolic or thiolactic acid, which disrupt disulfide bonds in the keratinaceous hair shafts, allowing the hairs to be “gently” removed. This process also synchronizes the hair cycle in mice, and begins a phase of induced anagen, which is not observed after close shaving. Using this method, T regulatory (T reg) cells have been postulated to be recruited to HFs after depilation in order to stimulate HFSC proliferation and differentiation via Jagged1-Notch signaling [57]. However, exposure to harsh chemicals also results in a degree of inflammation in the interfollicular epidermis [84], and might induce cytokine/chemokine release characteristic of a wound response. In fact, models of induced skin inflammation with topical imiquimod also promotes the telogen-to-anagen transition in mice [85]. In the epidermis, there is an increase in DETCs following depilation [24], in a manner reminiscent of the wound healing process [22].

Application of topical compounds onto mouse telogen skin has also been used to induce anagen entry. In addition to compounds such as Shh and Wnt agonists which are known to kick start HFSC activation and proliferation, topical ciclosporin has been used to induce the hair cycle in mice. Intriguingly, ciclosporin is used in humans as a steroid-sparing immunosuppressant for organ transplant, and inflammatory diseases, with the known side effect of hypertrichosis. Both topical ciclosporin application and depilation induced anagen in mice that was accompanied by mast cell degranulation at the point of anagen entry [86, 87]. Mast cell stimulators and inhibitors were also able to induce and inhibit anagen entry in mice, respectively, suggesting that they may also play a role in induced anagen entry.

Anagen has also been induced in murine skin with various techniques including fractional carbon dioxide laser [88], microneedling (with and without application of other chemicals) [89], and mechanical stretch [90]. Chemokines, in particular CCL2, released by tissue damage might recruit anti-inflammatory, or “M2-like” macrophages, which are associated with tissue regeneration and homeostasis. Chu et al show that these

“alternatively activated” macrophages might induce HF anagen by producing growth factors (IGF-1 and hepatocyte growth factor, HGF) [90].

Hair follicle neogenesis has been observed within larger wounds of rodents and some small mammals, and represents a solution to regenerate a continuous coat of fur in a scar, which normal remains hairless in humans. This process (also known as wound-induced HF neogenesis, WIHN) occurs in large wounds [91], and recapitulates the process of HF morphogenesis [92]. WIHN is distinct from the anagen re-entry of the HFs in the wound periphery, although both appear to be dependent on TNF-induced AKT/ β -catenin signaling via macrophages [93]. Within the murine scar, macrophages with an “M2-like” phenotype producing FGF-2 and IGF-1 are necessary for WIHN [94], as are $\gamma\delta$ -T cells in the murine dermis that are recruited and secrete FGF-9 to activate dermal fibroblasts to start the HF morphogenesis cascade [95]. Human skin is almost devoid of $\gamma\delta$ -T cells, which is consistent with the non-existent HF regeneration in human scars.

HAIR CYCLING IN HUMAN DISEASE

Unlike mammals with a coat of fur which predominantly contains HFs in telogen, 90% of human scalp hair is in the growing anagen stage, reflected in the need for frequent haircuts. The anagen phase of human HFs last between 2-9 years [96], making studying the human hair cycle extremely challenging. Most of the knowledge about the human hair cycle is inferred from observations and experiments carried out in mammalian (mostly murine) fur. This has obvious limitations, not least as human HFs grow in a mosaic pattern, and cycle independently of neighbouring follicles, making microenvironmental regulation of the cycle extremely difficult to assess.

Recently, careful study of microdissected follicular units from hair transplant surgery has allowed us to validate the existence of these pathways (in particular Wnt signaling) in human HFs at the telogen-to-anagen transition [97]. Similar techniques show dynamic macrophage polarization associated with different stages of the hair cycle, with a possible Wnt-active macrophage subset during the beginning of anagen [98]. However, this method is still largely descriptive and correlative. Human HFs xenotransplanted onto immunodeficient mice is a model that might allow observation of human HF cycling [99], but this system is also artificial and does not take into account the extra-follicular macroenvironmental signals, particular from immune cells.

Disorders of hair cycling in humans can be disfiguring and cause significant psychological and social sequelae. Most conventional treatments for hair loss disorders are unsatisfactory and usually do not address the hair cycle proper, largely due to our lack of knowledge of the human hair cycle.

Alopecia areata (AA)

Pigmented, anagen HFs are the main target of this autoimmune-mediated alopecia [100]. Although the target antigen remains elusive, it is postulated to be upregulated during the anagen phase and may be melanocyte-associated [101], or part of the growing outer root sheath (ORS) [102]. The hair cycle, and its constant flux of self-antigens makes it susceptible to autoimmune attack, if not for the relative immune privilege of the HF [103]. Immune privilege is mediated in part by downregulation of major histocompatibility complex (MHC) and Natural killer group 2 member D (NKG2D)-ligands on the HF keratinocytes [104, 105] (Figure 3A). Aberrant upregulation of NKG2D-ligands ULBP3/ULBP6 on keratinocytes leads to recruitment and activation of NKG2D+ CD8+ cytotoxic T cells that destroy the HF matrix and induce a premature catagen, leading to non-scarring alopecia. The HF enters telogen, and eventually re-enters the hair cycle and begins the next anagen phase. However, if the autoimmune process is still present, the growing anagen HF is attacked as soon as it begins to produce the anagen-associated autoantigen, and is arrested in mid-anagen, and forced into a premature catagen yet again. Repeated attacks and truncation of the hair cycle ultimately leads to a miniaturized, exhausted and dystrophic, empty HF in a state specific to AA known as nanogen [106]. Discovery of the role of cytotoxic NKG2D+ T cells in this process has led to the use of JAK inhibitors for the treatment of AA, as IFN- γ and IL-15 (crucial cytokines for NKG2D+ T cell survival) signal via the JAK-STAT pathway [107, 108]. Recently, another subset of regulatory *invariant* NKT (iNKT) cells are postulated to suppress NKG2D+ T cell activity to promote regrowth and remission in AA [109]. Impairment in T regulatory (T reg) cells have also been described in both lesional AA [110] as well as in the circulatory system, a possible permissive factor for the pathogenesis of AA.

Pre-clinical experiments with JAK inhibitors also showed that JAK inhibition in normal C57BL/6 mice (without AA) was also able to induce a telogen-to-anagen transition without overt inflammation [60]. Transcriptomic analysis also showed dynamic JAK-STAT signaling across the spontaneous hair cycle in C57BL/6 mice, suggesting JAK inhibitor-induced anagen might more closely resemble spontaneous anagen. Further, JAK-STAT5 signaling in HFSC is associated with stem cell quiescence, and is downstream of prolactin (PRL) during pregnancy [111], or OSM (from perifollicular trichophages) during normal telogen [55]. Thus, future treatments for AA using JAK inhibitors might address both the immune-mediated attack on the HF, and the hair cycle itself, in order to stimulate regrowth in patients [112].

The role of mast cells in the hair cycle (facilitating catagen and anagen in the mouse) is confounded by their increased presence around human HFs in lesional AA [113]. Mast cells in this context appear to upregulate pro-inflammatory markers, make contacts with CD8+ cytotoxic cells, and may play a role in immune privilege collapse and antigen presentation. As AA episodes have been associated with stressful life events, mast cells (via substance P) may play a significant role in the collapse of immune privilege [48].

Androgenetic alopecia (AGA)

AGA, also known as male- or female-pattern baldness, is the most common form of alopecia, and affects up to 40-60% the general adult population [114-116]. AGA is largely viewed as a cosmetic issue, but has enough effect on self-image and psychological well-being to drive significant health-seeking behavior and spending among patients. FDA-approved therapies for AGA (topical minoxidil, oral finasteride and some LLLT devices) have inconsistent efficacy, and potentially unacceptable side effects, prompting patients to spend up to US\$3.5 billion a year on unproven baldness treatments [117].

The pathognomonic features in AGA is the miniaturization of HFs, with reduction in the size of DP during each hair cycle, leading to thinner hair shafts [118] (Figure 3B). The number of follicles in a follicular unit also decreases, and hair density declines in an androgen-dependent manner. This leads to the characteristic bitemporal recession and thinning over the vertex in men [119], and diffuse thinning with accentuation of the central parting in women [120]. Interestingly, occipital scalp HFs are spared from this process in men, thus they are ideal donor follicular units in hair transplant surgery. Adipocytes, which appear to support anagen in mice, are also under investigation for the treatment of AGA in the form of stem cells derived from the stromal-vascular fraction enriched through centrifugation [121], or their conditioned media derived from *ex vivo* cell culture [70].

Histologically, perifollicular infundibular inflammation has been described in AGA [78, 79], but the nature of this infiltration remains uncharacterized. Whether immune cells like macrophages, mast cells or T regs contribute to the microenvironment of HFs, and participate in the miniaturization process, remains an open question. Further, the basic nature of miniaturized HFs, how they relate to the normal hair cycle, and whether the miniaturization process can be reversed, is poorly understood.

The discovery of WIHN has prompted investigation into novel therapeutic modalities for AGA that aim to recreate the wound microenvironment, and assess whether inducing tissue injury might rejuvenate miniaturized HFs [122]. Platelet-rich plasma, harvested autologously from the patient's venous blood, has been shown to have some effect on increasing hair density and preventing hair shaft miniaturization [123, 124], likely via the release of cytokines and growth factors from activated platelets [125]. These factors are commonly found in healing wounds and include platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), HGF and IGF-1, and an array of pro- and anti-inflammatory cytokines [126]. Other techniques like microneedling [127] and fractional non-ablative laser treatment have also been attempted alone, or in combination with drugs or growth factors [128, 129], in order to boost hair regrowth in AGA patients, with modest results [130]. Low-level laser therapy (LLLT) using visible red light laser-emitting LED devices may hold promise in stimulating hair growth in AGA [131], and the mechanism, which remains under investigation, may involve modulation of the local immune system and vasculature to promote a wound healing-like milieu [132].

Telogen effluvium (TE)

Episodes of physical or emotional stress have been linked to the disruption of the hair cycle in humans [133]. This is exemplified by the normally self-limiting condition of TE, commonly experienced around 3-6 months post-partum [134], whereby the HFs that entered telogen *en masse* during the period of stress undergo shedding or “teloptosis” at the same time a few weeks or months later [135]. Other stressful events that may precipitate TE include crash or fad diets, surgeries, or even starting a new medication for unrelated medical issues [136]. The sudden hair loss may lead to significant despair, and requires detailed history-taking, examination and investigations to exclude other forms of acute and diffuse non-scarring alopecias [137]. Scalp biopsies, if performed at all, may show more than 25% of HFs in telogen [136]. Treatment is usually supportive after confirmation of the diagnosis, and relief accompanies the emergence of the next cycle of anagen hairs.

The actual mechanism of hair cycle disruption in human HFs is currently unknown. Stressful situations, including pregnancy, may be associated with high levels of serum mediators such as cortisol and prolactin, which have a suppressive effect on immune cells or HFSCs themselves [138] (Figure 3C), leading to the premature termination of anagen. Increased mast cells are found in their degranulated state near catagen HFs in TE [139], and substance P produced by mast cells may play a role in the induction of stress-associated keratinocyte apoptosis [140]. This apoptosis in humans may be mediated by TNF- α , which are upregulated in the mast cells themselves upon exposure to substance P. The roles of extra-medullary PRL, mast cells, substance P and TNF signaling in stress-associated hair cycling defects such as TE and AA have been proposed [141], but their precise mechanism is yet to be elucidated.

Metabolic stress may be another mechanism, as manipulating glycolytic and oxidative substrates in HFSCs seem to impact the hair cycle in mice [142, 143]. Unfortunately, the diagnosis of acute TE is normally made several months after the precipitating event, and the molecular and cellular fluxes at this time are frequently missed. A small subset of patients progress to chronic TE, whereby hair shedding persists beyond 6 months, and represents a more lasting shift in the hair cycle which can last for years [144, 145]. Studying the local immune and metabolic changes in this group of patients may provide insight into the mechanism of hair cycle control in humans.

CONCLUSION AND FUTURE DIRECTIONS

Current strategies to therapeutically manipulate the hair cycle are focused on the activation of HFSC or DP cells and their precursors. Engineered skin constructs and organoids currently also only include these epithelial and mesenchymal components. So far, none of these models have been shown to be able to cycle spontaneously. Expanding our knowledge into the supportive cells of the HF niche, in particular the immune cells, is essential for the recreation of a functional HF *ex vivo*.

The role of the auxillary cells in HF cycling have gradually been uncovered in the past decade. Not only do immune cells play an important role in HF homeostasis, we have described in this review how perturbations in follicle cycling can be initiated by alterations in the immune environment. Macrophage subsets appear to have pleiotropic and context-dependent roles at various stages of the spontaneous and induced hair cycle in mice, as do T reg cells and mast cells. It is important to understand the function of these cells in human hair cycling in order to recapitulate the HF niche in future tissue engineering efforts.

Currently, most of our understanding of the immune infiltrate during the human hair cycle is descriptive, and confined to mostly anagen HFs [12], which is the predominant phase found in human scalps. *Ex vivo* analysis of human HFs are limited to anagen and catagen follicles [98], and telogen hairs are difficult to dissect and maintain in culture. Functional studies done in mice and small mammals have been extrapolated to human HF biology in order to develop new treatments, but actual mechanistic studies in humans are lacking. Several new technologies and advances are now available for researchers to probe the human hair cycle.

Single-cell transcriptomics to identify immune cell subsets in human skin will allow identification of rare populations from limited tissue (ie. 4mm punch biopsies). These populations may be easily overlooked in immunohistochemical or immunofluorescence studies, or bulk RNA sequencing. Sampling hair-bearing skin from other body sites using this technology will allow us to uncover the distinct immunophenotype associated with different hair cycle stages, potentially identifying new therapeutic targets to combat hair loss. Discovering how the immune macroenvironment can epigenetically regulate transitions through the follicle cycle will open up exciting new avenues for research.

Similar to single-gene hair disorders described in the last century, the new era of biologic and immunotherapy in dermatology and other diseases will provide valuable insight into the biological pathways relevant in human hair cycling. Clinical vigilance for side effects affecting the hair cycle in patients receiving these treatments will give us clues to signaling pathways underlying the human hair cycle, and the cells involved can be confirmed by cross-referencing with single-cell transcriptomic or proteomic data. Just like prostaglandin analogues were serendipitously found to induce eyelash trichomegaly in glaucoma patients [146], recent reports suggest that EGFR inhibitors also cause eyelash trichomegaly and Hh blockade with vismodegib results in moustache trichomegaly [147]. Tocilizumab, an IL-6 monoclonal antibody, is also postulated to affect the human hair cycle [148]. A systematic, standardized way of tracking and analyzing the human hair cycle is required to monitor these effects.

While we have gleaned much useful information in mammalian hair cycling from our murine counterparts, it's time to start systematically and thoroughly studying the mechanisms that underpin the human hair cycle. Only with this concerted effort will novel treatments and platforms be developed for all forms of hair loss in our patients.

FIGURE LEGENDS

Figure 1. Schematic of the spontaneous mammalian hair cycle, with current knowledge in mouse and human hair cycle depicted in embedded tables. A) The previous hair cycle, or HF morphogenesis, ends with catagen whereby the HF regresses. Keratinocyte apoptosis, and shrinking of the DP, are accompanied by mast cell and macrophage infiltration of the HF in mice. Macrophages secrete FGF-5, which has been shown to induce catagen in many mammalian species. FGF-5 mutations in humans result in delayed catagen and prolonged anagen, leading to trichomegaly of the eyelashes. B) The telogen HF is mitotically quiescent, but immune cells like macrophages and T reg cells have been described to be in proximity with these HFs in mice. Quiescence of HFSCs during telogen is controlled by many factors secreted by HF niche cells, which have been shown to include macrophages. There is currently no data on the immune cell infiltrate associated with human telogen HFs. Grey dotted box denotes the “permanent portion” of the HF which is present at every stage of the hair cycle, as opposed to the “cycling portion” that grows and regresses with each cycle. C) Anagen entry is marked by proliferation in the HFSCs in the matrix and bulge. Macrophage apoptosis is postulated to be involved in this process in both mice and humans. The involvement of immune cells in the spontaneous hair cycle in both mice and humans is currently poorly studied as most experiments utilize induced anagen models. D) Physical shedding of the club hair fibre is known as exogen. HF – hair follicle; HFSC – hair follicle stem cell; ORS – outer root sheath.

Figure 2. Methods of inducing anagen experimentally in laboratory mammals (usually mice). Mice in the telogen phase may be subjected to plucking, chemical depilation or wounding. The immune cells that have been shown to be recruited after some, but not all, of these procedures include mast cells [44], macrophages [58, 90], T reg cells [57], DETCs [24] and $\gamma\delta$ T cells [82], depending on the extent of injury. Growth factors and cytokines produced by immune cells play a role in stimulating anagen re-entry. Unlike spontaneous anagen, this new anagen phase is not associated with a club hair fibre. These mechanisms have not been shown to be present in human hair-bearing skin.

Figure 3. Human diseases of the hair cycle and potential immune cell involvement. A. Alopecia areata is characterized by loss of immune privilege of anagen HFs. Upregulation of “danger signals” such as MHC Class I and ULBP3/ULBP6 on ORS and matrix keratinocytes attracts NKG2D+ cytotoxic T cells that form the characteristic “swarm of bees” around the bulb region of the HF. B. Androgenetic alopecia (AGA) results from progressive miniaturization of terminal HFs, with a poorly characterized immune infiltrate around the infundibulum. The hair shaft becomes finer, and the DP size is reduced. Further work is required to elucidate the role of immune cells in AGA. C. Telogen effluvium is a biological response to physiological or emotional stress that is also poorly characterized and has no effective and proven treatments. The premature and usually synchronized telogen leads to shedding of club hairs *en masse*. Immune involvement in this condition is also pending interrogation.

TABLE 1. IMMUNE CELLS IMPLICATED IN MAMMALIAN HAIR CYCLE

	MURINE HAIR CYCLE	HUMAN HAIR CYCLE
CATAGEN	<p>Mast cells accumulate and degranulate during catagen [43].</p> <p>Macrophages may induce catagen by producing FGF-5 [37], and may clear debris from regressing HF [49].</p>	<p>Macrophages undergo apoptosis and shift to an “M2-like” phenotype [98].</p> <p>FGF-5 mutations in humans result in delayed catagen (prolong anagen) – cellular source yet to be identified [38]</p>
TELOGEN	<p>Macrophages numbers increase during mid-telogen [56], and a subset of TREM2+ macrophages maintain HFSC quiescence by producing OSM [55].</p> <p>Ablation of macrophages using clodronate liposomes [56], or genetic/ pharmacologic methods [55] leads to anagen.</p>	No data at the time of publication.
EARLY ANAGEN	<p>Numbers of macrophages decrease, possibly by apoptosis and release of Wnt ligands that stimulate anagen [56].</p> <p>T regs cluster around HFSC during the telogen-anagen transition [57].</p>	Downregulation of MHC Class I molecules on keratinocytes [12].
MID-LATE ANAGEN	<p>Adipocyte niche produces macrophage-stimulating protein that maintains anagen [71].</p>	<p>High density of perifollicular macrophages and mast cells.</p> <p>MHC Class I downregulation and low numbers of NK cells [12].</p>

REFERENCES

1. Stenn, K.S. and R. Paus, *Controls of hair follicle cycling*. Physiol Rev, 2001. **81**(1): p. 449-494.
2. Rompolas, P. and V. Greco, *Stem cell dynamics in the hair follicle niche*. Semin Cell Dev Biol, 2014. **25-26**: p. 34-42.
3. Jaks, V., M. Kasper, and R. Toftgard, *The hair follicle-a stem cell zoo*. Exp Cell Res, 2010. **316**(8): p. 1422-8.
4. Hsu, Y.C., L. Li, and E. Fuchs, *Emerging interactions between skin stem cells and their niches*. Nat Med, 2014. **20**(8): p. 847-56.
5. Jahoda, C.A. and A.J. Reynolds, *Dermal-epidermal interactions--follicle-derived cell populations in the study of hair-growth mechanisms*. J Invest Dermatol, 1993. **101**(1 Suppl): p. 33S-38S.
6. Matsuzaki, T. and K. Yoshizato, *Role of hair papilla cells on induction and regeneration processes of hair follicles*. Wound Repair Regen, 1998. **6**(6): p. 524-30.
7. Fujiwara, H., et al., *The basement membrane of hair follicle stem cells is a muscle cell niche*. Cell, 2011. **144**(4): p. 577-89.
8. Festa, E., et al., *Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling*. Cell, 2011. **146**(5): p. 761-71.
9. Li, K.N., et al., *Skin vasculature and hair follicle cross-talking associated with stem cell activation and tissue homeostasis*. Elife, 2019. **8**.
10. Yoon, S.Y., et al., *An important role of cutaneous lymphatic vessels in coordinating and promoting anagen hair follicle growth*. PLoS One, 2019. **14**(7): p. e0220341.
11. Paus, R., T. Christoph, and S. Muller-Rover, *Immunology of the hair follicle: a short journey into terra incognita*. J Invest Dermatol Symp Proc, 1999. **4**(3): p. 226-34.
12. Christoph, T., et al., *The human hair follicle immune system: cellular composition and immune privilege*. Br J Dermatol, 2000. **142**(5): p. 862-73.
13. Perry, V.H. and S. Gordon, *Macrophages and microglia in the nervous system*. Trends Neurosci, 1988. **11**(6): p. 273-7.
14. Hulsmans, M., et al., *Macrophages Facilitate Electrical Conduction in the Heart*. Cell, 2017. **169**(3): p. 510-522 e20.
15. Hirao, A., F. Arai, and T. Suda, *Regulation of cell cycle in hematopoietic stem cells by the niche*. Cell Cycle, 2004. **3**(12): p. 1481-3.
16. Winkler, I.G., et al., *Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs*. Blood, 2010. **116**(23): p. 4815-28.
17. Edinger, M., et al., *CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation*. Nat Med, 2003. **9**(9): p. 1144-50.

18. Morrison, S.J. and D.T. Scadden, *The bone marrow niche for haematopoietic stem cells*. Nature, 2014. **505**(7483): p. 327-34.
19. Merly, F., et al., *Macrophages enhance muscle satellite cell proliferation and delay their differentiation*. Muscle Nerve, 1999. **22**(6): p. 724-32.
20. Parkinson, E.K., *Epidermal keratinocyte stem cells: their maintenance and regulation*. Semin Cell Biol, 1992. **3**(6): p. 435-44.
21. Sharp, L.L., et al., *Dendritic epidermal T cells regulate skin homeostasis through local production of insulin-like growth factor 1*. Nat Immunol, 2005. **6**(1): p. 73-9.
22. Jameson, J.M., et al., *Regulation of skin cell homeostasis by gamma delta T cells*. Front Biosci, 2004. **9**: p. 2640-51.
23. Paus, R., et al., *Generation and cyclic remodeling of the hair follicle immune system in mice*. J Invest Dermatol, 1998. **111**(1): p. 7-18.
24. Hashizume, H., Y. Tokura, and M. Takigawa, *Increased number of dendritic epidermal T cells associated with induced anagen phase of hair cycles*. J Dermatol Sci, 1994. **8**(2): p. 119-24.
25. Nagao, K., et al., *Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin*. Nat Immunol, 2012. **13**(8): p. 744-52.
26. Paus, R., et al., *Distribution and changing density of gamma-delta T cells in murine skin during the induced hair cycle*. Br J Dermatol, 1994. **130**(3): p. 281-9.
27. Eichmuller, S., 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**(3): p. 361-70.
28. Muneeb, F., J.A. Hardman, and R. Paus, *Hair growth control by innate immunocytes: Perifollicular macrophages revisited*. Exp Dermatol, 2019. **28**(4): p. 425-431.
29. Plikus, M.V. and C.M. Chuong, *Complex hair cycle domain patterns and regenerative hair waves in living rodents*. J Invest Dermatol, 2008. **128**(5): p. 1071-80.
30. Muller-Rover, S., et al., *A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages*. J Invest Dermatol, 2001. **117**(1): p. 3-15.
31. Lindner, G., et al., *Analysis of apoptosis during hair follicle regression (catagen)*. Am J Pathol, 1997. **151**(6): p. 1601-17.
32. Elliott, K., T.J. Stephenson, and A.G. Messenger, *Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: implications for the control of hair follicle size and androgen responses*. J Invest Dermatol, 1999. **113**(6): p. 873-7.
33. Plikus, M.V., et al., *Cyclic dermal BMP signalling regulates stem cell activation during hair regeneration*. Nature, 2008. **451**(7176): p. 340-4.

34. Foitzik, K., et al., *Control of murine hair follicle regression (catagen) by TGF-beta1 in vivo*. FASEB J, 2000. **14**(5): p. 752-60.
35. Foitzik, K., et al., *Towards dissecting the pathogenesis of retinoid-induced hair loss: all-trans retinoic acid induces premature hair follicle regression (catagen) by upregulation of transforming growth factor-beta2 in the dermal papilla*. J Invest Dermatol, 2005. **124**(6): p. 1119-26.
36. Ito, T., et al., *Interferon-gamma is a potent inducer of catagen-like changes in cultured human anagen hair follicles*. Br J Dermatol, 2005. **152**(4): p. 623-31.
37. Suzuki, S., 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**(6): p. 963-72.
38. Higgins, C.A., et al., *FGF5 is a crucial regulator of hair length in humans*. Proc Natl Acad Sci U S A, 2014. **111**(29): p. 10648-53.
39. Hebert, J.M., et al., *FGF5 as a regulator of the hair growth cycle: evidence from targeted and spontaneous mutations*. Cell, 1994. **78**(6): p. 1017-25.
40. Housley, D.J. and P.J. Venta, *The long and the short of it: evidence that FGF5 is a major determinant of canine 'hair'-itability*. Anim Genet, 2006. **37**(4): p. 309-15.
41. Dierks, C., et al., *Allelic heterogeneity of FGF5 mutations causes the long-hair phenotype in dogs*. Anim Genet, 2013. **44**(4): p. 425-31.
42. Li, G., et al., *Base pair editing in goat: nonsense codon introgression into FGF5 results in longer hair*. FEBS J, 2019.
43. Maurer, M., et al., *Activated skin mast cells are involved in murine hair follicle regression (catagen)*. Lab Invest, 1997. **77**(4): p. 319-32.
44. Arck, P.C., et al., *Mast cell deficient and neurokinin-1 receptor knockout mice are protected from stress-induced hair growth inhibition*. J Mol Med (Berl), 2005. **83**(5): p. 386-96.
45. Kumamoto, T., et al., *Hair follicles serve as local reservoirs of skin mast cell precursors*. Blood, 2003. **102**(5): p. 1654-60.
46. Komi, D.E.A., K. Khomtchouk, and P.L. Santa Maria, *A Review of the Contribution of Mast Cells in Wound Healing: Involved Molecular and Cellular Mechanisms*. Clin Rev Allergy Immunol, 2019.
47. Weber, A., J. Knop, and M. Maurer, *Pattern analysis of human cutaneous mast cell populations by total body surface mapping*. Br J Dermatol, 2003. **148**(2): p. 224-8.
48. Peters, E.M., et al., *Probing the effects of stress mediators on the human hair follicle: substance P holds central position*. Am J Pathol, 2007. **171**(6): p. 1872-86.
49. Parakkal, P.F., *Role of macrophages in collagen resorption during hair growth cycle*. J Ultrastruct Res, 1969. **29**(3): p. 210-7.

50. Mesa, K.R., et al., *Niche-induced cell death and epithelial phagocytosis regulate hair follicle stem cell pool*. Nature, 2015. **522**(7554): p. 94-7.
51. Horenstein, M.G. and J.S. Jacob, *Follicular streamers (stelaes) in scarring and non-scarring alopecia*. J Cutan Pathol, 2008. **35**(12): p. 1115-20.
52. McCoy, S., A. Evans, and C. James, *Histological study of hair follicles treated with a 3-msec pulsed ruby laser*. Lasers Surg Med, 1999. **24**(2): p. 142-50.
53. Chi, W., E. Wu, and B.A. Morgan, *Dermal papilla cell number specifies hair size, shape and cycling and its reduction causes follicular decline*. Development, 2013. **140**(8): p. 1676-83.
54. Hsu, Y.C., H.A. Pasolli, and E. Fuchs, *Dynamics between stem cells, niche, and progeny in the hair follicle*. Cell, 2011. **144**(1): p. 92-105.
55. Wang, E.C.E., et al., *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): p. 654-669 e6.
56. Castellana, D., R. Paus, and M. Perez-Moreno, *Macrophages contribute to the cyclic activation of adult hair follicle stem cells*. PLoS Biol, 2014. **12**(12): p. e1002002.
57. Ali, N., et al., *Regulatory T Cells in Skin Facilitate Epithelial Stem Cell Differentiation*. Cell, 2017. **169**(6): p. 1119-1129 e11.
58. Chen, C.C., et al., *Organ-level quorum sensing directs regeneration in hair stem cell populations*. Cell, 2015. **161**(2): p. 277-90.
59. Yu, M., et al., *Interleukin-6 cytokine family member oncostatin M is a hair-follicle-expressed factor with hair growth inhibitory properties*. Exp Dermatol, 2008. **17**(1): p. 12-9.
60. Harel, S., et al., *Pharmacologic inhibition of JAK-STAT signaling promotes hair growth*. Sci Adv, 2015. **1**(9): p. e1500973.
61. Li, Y.H., et al., *Wnt10b promotes growth of hair follicles via a canonical Wnt signalling pathway*. Clin Exp Dermatol, 2011. **36**(5): p. 534-40.
62. Hwang, K.A., et al., *Adenosine stimulates growth of dermal papilla and lengthens the anagen phase by increasing the cysteine level via fibroblast growth factors 2 and 7 in an organ culture of mouse vibrissae hair follicles*. Int J Mol Med, 2012. **29**(2): p. 195-201.
63. Oro, A.E. and K. Higgins, *Hair cycle regulation of Hedgehog signal reception*. Dev Biol, 2003. **255**(2): p. 238-48.
64. Greco, V., et al., *A two-step mechanism for stem cell activation during hair regeneration*. Cell Stem Cell, 2009. **4**(2): p. 155-69.
65. Ruckert, R., et al., *MHC class I expression in murine skin: developmentally controlled and strikingly restricted intraepithelial expression during hair follicle morphogenesis and cycling, and response to cytokine treatment in vivo*. J Invest Dermatol, 1998. **111**(1): p. 25-30.

66. Hofmann, U., et al., *The anagen hair cycle induces systemic immunosuppression of contact hypersensitivity in mice*. Cell Immunol, 1998. **184**(1): p. 65-73.
67. Chase, H.B., W. Montagna, and J.D. Malone, *Changes in the skin in relation to the hair growth cycle*. Anat Rec, 1953. **116**(1): p. 75-81.
68. Yang, C.C., et al., *Leptin of dermal adipose tissue is differentially expressed during the hair cycle and contributes to adipocyte-mediated growth inhibition of anagen-phase vibrissa hair*. Exp Dermatol, 2015. **24**(1): p. 57-60.
69. Won, C.H., et al., *Hair growth-promoting effects of adiponectin in vitro*. J Invest Dermatol, 2012. **132**(12): p. 2849-51.
70. Park, B.S., et al., *Hair growth stimulated by conditioned medium of adipose-derived stem cells is enhanced by hypoxia: evidence of increased growth factor secretion*. Biomed Res, 2010. **31**(1): p. 27-34.
71. McElwee, K.J., et al., *Macrophage-stimulating protein promotes hair growth ex vivo and induces anagen from telogen stage hair follicles in vivo*. J Invest Dermatol, 2004. **123**(1): p. 34-40.
72. Philpott, M.P., D.A. Sanders, and T. Kealey, *Effects of insulin and insulin-like growth factors on cultured human hair follicles: IGF-I at physiologic concentrations is an important regulator of hair follicle growth in vitro*. J Invest Dermatol, 1994. **102**(6): p. 857-61.
73. Milner, Y., et al., *Exogen, shedding phase of the hair growth cycle: characterization of a mouse model*. J Invest Dermatol, 2002. **119**(3): p. 639-44.
74. Rebora, A. and M. Guarrera, *Kenogen. A new phase of the hair cycle?* Dermatology, 2002. **205**(2): p. 108-10.
75. Higgins, C.A., G.E. Westgate, and C.A. Jahoda, *From telogen to exogen: mechanisms underlying formation and subsequent loss of the hair club fiber*. J Invest Dermatol, 2009. **129**(9): p. 2100-8.
76. Møller, A.P., J.T. Nielsen, and J. Erritzøe, *Losing the last feather: feather loss as an antipredator adaptation in birds*. Behavioral Ecology 2006. **17**(6): p. 1046-56.
77. Guarrera, M. and A. Rebora, *The Higher Number and Longer Duration of Kenogen Hairs Are the Main Cause of the Hair Rarefaction in Androgenetic Alopecia*. Skin Appendage Disord, 2019. **5**(3): p. 152-154.
78. Young, J.W., et al., *Cutaneous immunopathology of androgenetic alopecia*. J Am Osteopath Assoc, 1991. **91**(8): p. 765-71.
79. Magro, C.M., et al., *The role of inflammation and immunity in the pathogenesis of androgenetic alopecia*. J Drugs Dermatol, 2011. **10**(12): p. 1404-11.
80. Johnson, E. and F.J. Ebling, *The Effect of Plucking Hairs during Different Phases of the Follicular Cycle*. J Embryol Exp Morphol, 1964. **12**: p. 465-74.

81. Qin, W., U. Baran, and R. Wang, *Lymphatic response to depilation-induced inflammation in mouse ear assessed with label-free optical lymphangiography*. Lasers Surg Med, 2015. **47**(8): p. 669-76.
82. Kloepper, J.E., et al., *Loss of gammadelta T Cells Results in Hair Cycling Defects*. J Invest Dermatol, 2013. **133**(6): p. 1666-9.
83. Sano, S., et al., *Two distinct signaling pathways in hair cycle induction: Stat3-dependent and -independent pathways*. Proc Natl Acad Sci U S A, 2000. **97**(25): p. 13824-9.
84. Amberg, N., et al., *Effects of Depilation Methods on Imiquimod-Induced Skin Inflammation in Mice*. J Invest Dermatol, 2017. **137**(2): p. 528-531.
85. Amberg, N., et al., *Effects of Imiquimod on Hair Follicle Stem Cells and Hair Cycle Progression*. J Invest Dermatol, 2016. **136**(11): p. 2140-2149.
86. Paus, R., et al., *Mast cell involvement in murine hair growth*. Dev Biol, 1994. **163**(1): p. 230-40.
87. Botchkarev, V.A., et al., *Hair cycle-dependent changes in mast cell histochemistry in murine skin*. Arch Dermatol Res, 1995. **287**(7): p. 683-6.
88. Bae, J.M., et al., *Hair regrowth through wound healing process after ablative fractional laser treatment in a murine model*. Lasers Surg Med, 2015. **47**(5): p. 433-40.
89. Jeong, K.H., et al., *Effect of mycophenolic acid on proliferation of dermal papilla cells and induction of anagen hair follicles*. Clin Exp Dermatol, 2015. **40**(8): p. 894-902.
90. Chu, S.Y., et al., *Mechanical stretch induces hair regeneration through the alternative activation of macrophages*. Nat Commun, 2019. **10**(1): p. 1524.
91. Ito, M., et al., *Wnt-dependent de novo hair follicle regeneration in adult mouse skin after wounding*. Nature, 2007. **447**(7142): p. 316-20.
92. Wang, X., et al., *Principles and mechanisms of regeneration in the mouse model for wound-induced hair follicle neogenesis*. Regeneration (Oxf), 2015. **2**(4): p. 169-181.
93. Wang, X., et al., *Macrophages induce AKT/beta-catenin-dependent Lgr5(+) stem cell activation and hair follicle regeneration through TNF*. Nat Commun, 2017. **8**: p. 14091.
94. Kasuya, A., T. Ito, and Y. Tokura, *M2 macrophages promote wound-induced hair neogenesis*. J Dermatol Sci, 2018. **91**(3): p. 250-255.
95. Gay, D., et al., *Fgf9 from dermal gammadelta T cells induces hair follicle neogenesis after wounding*. Nat Med, 2013. **19**(7): p. 916-23.
96. Saitoh, M., M. Uzuka, and M. Sakamoto, *Human hair cycle*. J Invest Dermatol, 1970. **54**(1): p. 65-81.
97. Hawkshaw, N.J., et al., *Deciphering the molecular morphology of the human hair cycle: Wnt signalling during the telogen-anagen transformation*. Br J Dermatol, 2019.
98. Hardman, J.A., et al., *Human perifollicular macrophages undergo apoptosis, express Wnt ligands and switch their polarisation during catagen*. J Invest Dermatol, 2019.

99. Oh, J.W., et al., *A Guide to Studying Human Hair Follicle Cycling In Vivo*. J Invest Dermatol, 2016. **136**(1): p. 34-44.
100. Messenger, A.G., D.N. Slater, and S.S. Bleehen, *Alopecia areata: alterations in the hair growth cycle and correlation with the follicular pathology*. Br J Dermatol, 1986. **114**(3): p. 337-47.
101. Paus, R., A. Slominski, and B.M. Czarnecki, *Is alopecia areata an autoimmune-response against melanogenesis-related proteins, exposed by abnormal MHC class I expression in the anagen hair bulb?* Yale J Biol Med, 1993. **66**(6): p. 541-54.
102. Wang, E.H.C., et al., *Identification of Autoantigen Epitopes in Alopecia Areata*. J Invest Dermatol, 2016. **136**(8): p. 1617-1626.
103. Paus, R., S. Bulfone-Paus, and M. Bertolini, *Hair Follicle Immune Privilege Revisited: The Key to Alopecia Areata Management*. J Invest Dermatol Symp Proc, 2018. **19**(1): p. S12-S17.
104. Petukhova, L., et al., *Genome-wide association study in alopecia areata implicates both innate and adaptive immunity*. Nature, 2010. **466**(7302): p. 113-7.
105. Ito, T., et al., *Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack*. J Invest Dermatol, 2008. **128**(5): p. 1196-206.
106. Peckham, S.J., S.B. Sloan, and D.M. Elston, *Histologic features of alopecia areata other than peribulbar lymphocytic infiltrates*. J Am Acad Dermatol, 2011. **65**(3): p. 615-620.
107. Xing, L., et al., *Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition*. Nat Med, 2014. **20**(9): p. 1043-9.
108. Gilhar, A., A. Keren, and R. Paus, *A new humanized mouse model for alopecia areata*. J Invest Dermatol Symp Proc, 2013. **16**(1): p. S37-8.
109. Ghraieb, A., et al., *iNKT cells ameliorate human autoimmunity: Lessons from alopecia areata*. J Autoimmun, 2018. **91**: p. 61-72.
110. Speiser, J.J., et al., *Regulatory T-cells in alopecia areata*. J Cutan Pathol, 2019. **46**(9): p. 653-658.
111. Goldstein, J., et al., *Calcineurin/Nfatc1 signaling links skin stem cell quiescence to hormonal signaling during pregnancy and lactation*. Genes Dev, 2014. **28**(9): p. 983-94.
112. Wang, E.C.E., Z. Dai, and A.M. Christiano, *Novel therapies for alopecia areata: The era of rational drug development*. J Allergy Clin Immunol, 2018. **141**(2): p. 499-504.
113. Bertolini, M., et al., *Abnormal interactions between perifollicular mast cells and CD8+ T-cells may contribute to the pathogenesis of alopecia areata*. PLoS One, 2014. **9**(5): p. e94260.
114. Tang, P.H., et al., *A community study of male androgenetic alopecia in Bishan, Singapore*. Singapore Med J, 2000. **41**(5): p. 202-5.
115. Paik, J.H., et al., *The prevalence and types of androgenetic alopecia in Korean men and women*. Br J Dermatol, 2001. **145**(1): p. 95-9.

116. Pathomvanich, D., et al., *A random study of Asian male androgenetic alopecia in Bangkok, Thailand*. Dermatol Surg, 2002. **28**(9): p. 804-7.
117. https://www.americanhairloss.org/hair_loss_treatment/.
118. Pantelireis, N. and C.A. Higgins, *A bald statement - Current approaches to manipulate miniaturisation focus only on promoting hair growth*. Exp Dermatol, 2018. **27**(9): p. 959-965.
119. Sinclair, R., *Male pattern androgenetic alopecia*. BMJ, 1998. **317**(7162): p. 865-9.
120. Ludwig, E., *Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex*. Br J Dermatol, 1977. **97**(3): p. 247-54.
121. Perez-Meza, D., et al., *Hair follicle growth by stromal vascular fraction-enhanced adipose transplantation in baldness*. Stem Cells Cloning, 2017. **10**: p. 1-10.
122. Chueh, S.C., et al., *Therapeutic strategy for hair regeneration: hair cycle activation, niche environment modulation, wound-induced follicle neogenesis, and stem cell engineering*. Expert Opin Biol Ther, 2013. **13**(3): p. 377-91.
123. Gentile, P., et al., *The Effect of Platelet-Rich Plasma in Hair Regrowth: A Randomized Placebo-Controlled Trial*. Stem Cells Transl Med, 2015. **4**(11): p. 1317-23.
124. Gupta, A.K., et al., *Platelet-Rich Plasma as a Treatment for Androgenetic Alopecia*. Dermatol Surg, 2019.
125. Li, Z.J., et al., *Autologous platelet-rich plasma: a potential therapeutic tool for promoting hair growth*. Dermatol Surg, 2012. **38**(7 Pt 1): p. 1040-6.
126. Eppley, B.L., J.E. Woodell, and J. Higgins, *Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing*. Plast Reconstr Surg, 2004. **114**(6): p. 1502-8.
127. Dhurat, R., et al., *A randomized evaluator blinded study of effect of microneedling in androgenetic alopecia: a pilot study*. Int J Trichology, 2013. **5**(1): p. 6-11.
128. Huang, Y., F. Zhuo, and L. Li, *Enhancing hair growth in male androgenetic alopecia by a combination of fractional CO2 laser therapy and hair growth factors*. Lasers Med Sci, 2017. **32**(8): p. 1711-1718.
129. Bertin, A.C.J., A. Vilarinho, and A.L.A. Junqueira, *Fractional non-ablative laser-assisted drug delivery leads to improvement in male and female pattern hair loss*. J Cosmet Laser Ther, 2018. **20**(7-8): p. 391-394.
130. Dabek, R.J., W.G. Austen, Jr., and B. Bojovic, *Laser-assisted Hair Regrowth: Fractional Laser Modalities for the Treatment of Androgenic Alopecia*. Plast Reconstr Surg Glob Open, 2019. **7**(4): p. e2157.
131. Liu, K.H., et al., *Comparative effectiveness of low-level laser therapy for adult androgenic alopecia: a system review and meta-analysis of randomized controlled trials*. Lasers Med Sci, 2019. **34**(6): p. 1063-1069.

132. Hawkins, D., N. Houreld, and H. Abrahamse, *Low level laser therapy (LLL T) as an effective therapeutic modality for delayed wound healing*. Ann N Y Acad Sci, 2005. **1056**: p. 486-93.
133. Rebora, A., *Telogen effluvium: an etiopathogenetic theory*. Int J Dermatol, 1993. **32**(5): p. 339-40.
134. Rebora, A., M. Guarrera, and F. Drago, *Postpartum telogen effluvium*. J Eur Acad Dermatol Venereol, 2016. **30**(3): p. 518.
135. Pierard-Franchimont, C. and G.E. Pierard, *Teloptyosis, a turning point in hair shedding biorhythms*. Dermatology, 2001. **203**(2): p. 115-7.
136. Harrison, S. and R. Sinclair, *Telogen effluvium*. Clin Exp Dermatol, 2002. **27**(5): p. 389-5.
137. Harrison, S. and W. Bergfeld, *Diffuse hair loss: its triggers and management*. Cleve Clin J Med, 2009. **76**(6): p. 361-7.
138. Arck, P.C., et al., *Neuroimmunology of stress: skin takes center stage*. J Invest Dermatol, 2006. **126**(8): p. 1697-704.
139. Grace, S.A., et al., *Presence of Mast Cells and Mast Cell Degranulation in Scalp Biopsies of Telogen Effluvium*. Int J Trichology, 2017. **9**(1): p. 25-29.
140. Arck, P.C., et al., *Indications for a 'brain-hair follicle axis (BHA)': inhibition of keratinocyte proliferation and up-regulation of keratinocyte apoptosis in telogen hair follicles by stress and substance P*. FASEB J, 2001. **15**(13): p. 2536-8.
141. Langan, E.A., et al., *Tumour necrosis factor alpha, interferon gamma and substance P are novel modulators of extrapituitary prolactin expression in human skin*. PLoS One, 2013. **8**(4): p. e60819.
142. Flores, A., et al., *Lactate dehydrogenase activity drives hair follicle stem cell activation*. Nat Cell Biol, 2017. **19**(9): p. 1017-1026.
143. Miranda, M., et al., *Topical Inhibition of the Electron Transport Chain Can Stimulate the Hair Cycle*. J Invest Dermatol, 2018. **138**(4): p. 968-972.
144. Whiting, D.A., *Chronic telogen effluvium*. Dermatol Clin, 1996. **14**(4): p. 723-31.
145. Garcia-Hernandez, M.J. and F.M. Camacho, *Chronic telogen effluvium: incidence, clinical and biochemical features, and treatment*. Arch Dermatol, 1999. **135**(9): p. 1123-4.
146. Yoshino, T., et al., *Eyelid and eyelash changes due to prostaglandin analog therapy in unilateral treatment cases*. Jpn J Ophthalmol, 2013. **57**(2): p. 172-8.
147. Riera-Monroig, J., et al., *Hedgehog-like moustache trichomegaly during treatment with vismodegib*. J Eur Acad Dermatol Venereol, 2019.
148. Vidon, C., et al., *Hair-cycle changes in two patients taking tocilizumab*. Joint Bone Spine, 2014. **81**(1): p. 100-1.

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