Hair Follicle Immune Privilege Revisited: The Key to Alopecia Areata Management

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The collapse of the immune privilege (IP) of the anagen hair bulb is now accepted as a key element in AA pathogenesis, and hair bulb IP restoration lies at the core of AA therapy. Here, we briefly review the essentials of hair bulb IP and recent progress in understanding its complexity. We discuss open questions and why the systematic dissection of hair bulb IP and its pharmacological manipulation (including the clinical testing of FK506 and α-melanocyte-stimulating hormone analogs) promise to extend the range of future therapeutic options in AA and other IP collapse-related autoimmune diseases.

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HAIR FOLLICLE IMMUNE PRIVILEGE: INCEPTION AND BASICS

Rupert Billingham recognized in the skin of guinea pigs that transplanted heterologous epidermal melanocytes can escape immune elimination when they migrate to the anagen hair bulb epithelium of genetically incompatible host hair follicles (HFs) (Billingham and Silvers, 1971). This striking finding was largely ignored until Gill Westgate recalled attention to the concept of HF immune privilege (IP) (Westgate et al., 1991). This encouraged the proposal that hair bulb IP collapse constitutes a crucial element in HF pathobiology, namely in alopecia areata (AA) (Paus et al., 1993) and triggered a series of studies to better define HF immunology (e.g., Christoph et al., 2000; Hoffman et al., 1996; Paus et al., 1994a, 1994b, 1994c, 1998, 2005; Wang et al., 2014).

The central role of IP collapse in AA pathobiology has now become widely accepted in the field (Gilhar et al., 1998; Gilhar et al., 2012, 2016; Kang et al., 2010; McElwee et al., 2013; Pratt et al., 2017; Wang et al., 2014), and formal functional proof that anagen HFs do exhibit a relative IP has been provided in mouse models (Alli et al., 2012; Gao et al., 2017; Giangreco et al., 2012).

Since its first inception by Peter Medawar (1948) the term *IP* has been extended to reflect a dynamic immunoinhibitory state established by complex tissue-specific mechanisms that suppress inflammation and promote immune tolerance (Engelhardt et al., 2017). Although the few tissues that enjoy IP differ in their IP state and characteristics, the most crucial common mechanisms shared by them are (i) low or absent expression of major histocompatibility complex (MHC) class Ia/β2 microglobulin expression, thus rendering self-peptide presentation ineffective or impossible, and (ii) the creation of an immunoinhibitory milieu by the generation of secreted immunosuppressants (Engelhardt et al., 2017; Joyce and Fearon, 2015; Kinori et al., 2011; Nasr et al., 2005; Noso et al., 2015; Paus et al., 2005; Taylor, 2016). Also, IP tissues can induce a state of tolerance against antigens that manage to escape immune sequestration (Taylor, 2016) and down-regulate T-cell activation and proliferation (Eleftheriadis et al., 2016; Ma et al., 2017; Tan and Bharath, 2009).

All of these mechanisms are established in the hair bulge IP and hair bulb IP of murine and human HFs (Christoph et al., 2000; Harrist et al., 1983; Meyer et al., 2008; Paus et al., 2005), with the bulb IP being the one that is relevant to AA pathogenesis. Although the functions of HF IP remain speculative, current evidence suggests that the main function of the anagen hair bulb IP may be to sequester immunogenic, melanogenesis-associated, and/or other HF antigens produced in the anagen hair bulb from immune recognition (Paus et al., 1993, 2005). With increasing insights into the immunological features of the HF over the past decade, the understanding of HF IP has attained new layers of complexity (Bertolini et al., 2016; Breitkopf et al., 2013; Ito and Tokura, 2014; Kang et al., 2010; Kinori et al., 2012; Paus et al., 2005; Wang et al., 2014). These, and potential mechanisms for how hair bulb IP is actively maintained, is threatened, collapses, and may best be repaired during AA management are discussed below.

AA AS A STEREOTYPIC HAIR FOLLICLE RESPONSE PATTERN

AA typically begins with focal hair loss in uninflamed skin, which in some individuals progresses to universal hair loss. Histologically, AA lesions show a dense inflammatory infiltrate around the hair bulb of melanogenically active anagen HFs (Miteva et al., 2012). This autoaggressive infiltrate causes both premature termination of anagen, forcing the HF into catagen (Oh et al., 2016), and major HF dystrophy (Gilhar et al., 2012). Therefore, AA represents both an HF cycling and a hair growth disorder.

The preference of AA for fully pigmented anagen VI HFs has been used to explain the sparing of white HFs and has invited the hypothesis that melanogenesis-associated autoantigens are the chief targets in AA (Paus et al., 1993). In AA, these autoantigens are most likely to be presented by MHC class Ia$^+$ cells in the anagen hair bulb epithelium

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Abbreviations: AA, alopecia areata; HF, hair follicle; IP, immune privilege; MHC, major histocompatibility complex; NK, natural killer

(Bertolini et al., 2014, 2016, 2017; Christoph et al., 2000; Ito et al., 2004; Kinori et al., 2012).

It has long been recognized that AA reflects a wide spectrum of hair loss phenotypes (Gilhar et al., 2012; Ikeda, 1965; Tosti et al., 2006). Moreover, AA clinical subtypes show some shared gene associations, fitting the model of AA as a polygenic disease with different presentations arising from a combination of genetic and environmental factors (Betz et al., 2015; Fischer et al., 2016; Petukhova et al., 2010). However, a phenocopy of AA lesions can be experimentally induced in healthy human scalp skin xenotransplants onto SCID mice under conditions where an antigen-specific autoimmune attack on the anagen HF and a genetic predisposition to AA are quite unlikely (Gilhar et al., 2013, 2016).

Therefore, we have argued that AA should be considered as a stereotypic *response pattern* that any anagen HFs will show, irrespective of the genetic predisposition and the preexistence of autoreactive $CD8^+$ T cells, provided that HF IP collapses and that excessive IFN-γ signaling causes cytotoxic HF damage (Paus, in: McElwee et al., 2013). According to this concept, only a certain percentage of AA patients reflects the presence of an (auto)antigen-specific and CD8/NKG2D-dependent autoimmune disease, which is best termed *autoimmune AA* (AAA). Instead, other AA patients—perhaps those with the greatest likelihood of spontaneous remission—may mainly exhibit the hair loss-triggering consequences of nonspecific, IFN-γ–induced HF IP collapse, dystrophy, and premature catagen (Ito et al., 2005); therefore, AA patients may require personalized medicine management strategies (Paus, in: McElwee et al., 2013).

HF IP COLLAPSE AS THE CENTRAL PREREQUISITE OF THE AA HAIR LOSS PHENOTYPE

Regardless of whether or not AA is only a stereotypic HF response pattern, AA cannot develop without the following (Gilhar et al., 2012):

- the occurrence of a perifollicular inflammation around the anagen hair bulb;
- the induction of HF dystrophy, which leads to hair shaft breakage or shedding and/or production of a dysfunctional hair shaft; and, most important,
- hair bulb IP collapse.

The bulb IP exists only in anagen, because most of the MHC class Ia/β2 microglobulin-negative HF keratinocytes are deleted via apoptosis during catagen (Paus et al., 1994a, 2005). Therefore, once an inflammation-damaged anagen HF (Ito et al., 2004, 2005; Peters et al., 2007; Zarbo et al., 2017) has entered into catagen and has resided in telogen (Oh et al., 2016), it has the chance to completely reconstruct its bulb IP with entry into the next anagen phase—unless the newly developing anagen HF is attacked again.

Circumstantial evidence suggests that spontaneous restoration of HF IP is promoted by the intrafollicular production of endogenous IP guardians by outer root sheath keratinocytes (Breitkopf et al., 2013; Ito et al., 2004; Paus et al., 2005) and the release of immunoinhibitory neuropeptides from perifollicular sensory nerve fibers (Bertolini et al., 2016; Kinori et al., 2012). Therefore, the interindividual differences in susceptibility to AA, in course of the hair loss phenotype, in response to AA therapy, and in likelihood of spontaneous remission (Gilhar et al., 2012) may very well reflect constitutive, genetically determined differences in how easily the hair bulb IP collapses and how effectively HFs manage to repair their IP.

HF IP RESTORATION AS THE CORNERSTONE OF SUCCESSFUL AA MANAGEMENT

Thus, if one manages to protect and restore HF IP more effectively, AA progression can be stopped, and spontaneous hair regrowth is predicted to occur, because the HF damage associated with AA remains fully reversible.

However, the current standard therapy for AA (Kassira et al., 2017) may do little to restore HF IP. Although unequivocal placebo-controlled evidence that JAK/STAT inhibitors are effective in AA therapy is not yet available, one main reason why these therapeutics promote hair regrowth in many, though not all, AA patients (Alves de Medeiros et al., 2016; Jabbari et al., 2015; Kennedy Crispin et al., 2016; Liu et al., 2017; Mackay-Wiggan et al., 2016) may be because they block IFN-γ–mediated signaling (Xing et al., 2014) and thus the promotion of HF IP collapse, besides affecting HF cycling (Harel et al., 2015).

The relapse of hair loss often seen after AA patients have discontinued therapy with these promising new therapeutics and the development of refractoriness to this therapy in some patients over time (Kennedy Crispin et al., 2016; Mackay-Wiggan et al., 2016) underscore the ultimate goal of any successful AA therapy, that is, to help the HF restore its IP—the most effective safeguarding mechanism against AA relapse. Moreover, long-term therapy with potent JAK/STAT inhibitors, which block T- and natural killer (NK)-cell functions (Xing et al., 2014), must be expected to severely compromise intracutaneous tumor immunosurveillance and the defense against viral infection—concerns that are not circumvented by topical drug application (Kostovic et al., 2017). Therefore, caution is still advised regarding the long-term administration of JAK/STAT inhibitors to AA patients.

From a drug safety and IP restoration efficiency perspective, well-documented "IP guardians" such as synthetic α-MSH analogs (melanotan, alfamelanotide) need to be tested clinically in AA. These not only potently down-regulate MHC class Ia/β2 microglobulin expression in human anagen HFs (Ito et al., 2004), but they may also stimulate production of other HF IP guardians, besides exerting direct immunoinhibitory effects themselves (Brzoska et al., 2008; Chang et al., 2008). Although this strategy has long been advocated (Gilhar et al., 2012; Ito et al., 2004) and despite the introduction of α-MSH analogs into clinical dermatology, industry has not yet advanced these agents for clinical testing in AA.

Although glucocorticoids suppress T-cell functions, MHC class II expression, and many proinflammatory signaling pathways, they are much less effective in down-regulating MHC class Ia/β2 microglobulin expression (Truckenmiller et al., 2005) and stimulating the production of HF IP guardians, possibly explaining why glucocorticoids often disappoint in AA management.

Instead, FK506 (tacrolimus), ideally in a topical preparation that facilitates penetration and drug accumulation