The Hair Follicle and Immune Privilege

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This essay reviews the available evidence that the proximal hair follicle epithelium generates and maintains an area of relative immune privilege during a defined segment of the hair cycle (i.e., during anagen). This immune privilege is chiefly characterized by a very low level of expression of MHC class Ia antigens and by the local production of potent immunosuppressive agents, such as α-MSH and TGF-β1. We discuss the putative functions of immune privilege of the anagen hair bulb, favoring the view that immune privilege serves mainly to sequester anagen- and/or melanogenesis-associated autoantigens from immune recognition by autoreactive CD8+ T cells. On this basis, we develop how the “immune privilege collapse model” of alopecia areata pathogenesis was conceived. In our discussion of the clinical implications of immune privilege, we outline the currently available evidence in support of this still hypothetical scenario to explain the initiation, progression, and termination of alopecia areata lesions. We review the most recent evidence from our laboratory that α-MSH, IGF-1, and TGF-β1 can downregulate IFN-γ-induced ectopic MHC class I expression in human anagen hair bulbs in vitro. Finally, we suggest that hair follicle–derived α-MSH, IGF-1, and TGF-β1 form part of a constitutively active “IP restoration machinery” of the anagen hair bulb, which we propose to be recruited whenever the hair follicle suffers immune injury. Finally, we sketch some particularly promising avenues for future investigation into the far too long ignored hair follicle immune privilege. Keywords: MHC class I/Qa-2/α-MSH/TGF-β1/IGF-1/alopecia areata/autoimmunity, macrophage. JID Symposium Proceedings 8:188–194, 2003

It has been an object of long-standing fascination among immunologists and transplant surgeons that there are a few well-defined tissue compartments in the mammalian body that are immunologically “privileged.” These include the anterior chamber of the eye, the testis, the central nervous system behind the blood–brain barrier, and the hamster cheek pouch (Head and Billingham, 1985; Streilein, 1993; Brent, 1997; Janeway et al., 2001; Niederkorn, 2002). Originally, the term “immune privilege” (IP) was coined to illustrate that a given tissue environment that hosts an allotransplant awards the transplanted cells relative protection from rejection by the host immune system (Head and Billingham, 1985; Brent, 1997). With the realization that the establishment of IP in the fetomaternal placental unit is vital for avoiding fetal rejection (Mellor and Munn, 2000; Erlebacher, 2001) and that ocular IP is indispensable for normal eye function (Niederkorn, 2002; Niederkorn, this issue), this narrow definition was extended to tissue sites in which a number of mechanisms collaborate to suppress a cytotoxic immune attack on the cells and antigens harbored within these sites.

IP is established and maintained e.g. by: (Streilein, 1993; Brent, 1997; Mellor and Munn, 2000; Erlebacher, 2001; Fuzzi et al., 2002; Niederkorn, 2002):

- Downregulation or absence of classical MHC class I expression, which sequesters (auto)antigens in tissue sites and hinders their presentation to CD8+ T cells with a matching T cell receptor
- Local production of potent immunosuppressants such as TGF-β1, IL-10, and α-MSH
- Functional impairment of antigen-presenting cells
- Absence of lymphatics
- Establishment of extracellular matrix barriers to hinder immune cell trafficking
- Expression of nonclassical MHC class I molecules (such as the MHC class Ib molecules HLA-G in humans and Qa-2 in mice)
- Expression of Fas Ligand (FasL) in order to delete autoreactive, Fas-expressing T cells

With increasing progress in IP research, an ever more complex picture is evolving concerning the immunosuppressive mechanisms that are recruited to guarantee immunotolerance of antigens presented in or emanating from areas of IP. For example, a local downregulation of the level of tryptophan below a threshold required for normal T cell function contributes to the state of tolerance toward the fetal allograft, which is brought about by the tryptophan–catabolizing enzyme indoleamine 2,3-dioxygenase in the fetomaternal interface (Munn et al., 1998; Mellor and Munn, 2000). Inhibition of complement activation (Xu et al., 2000) and a placenta-specific, β2-microglobulin–dependent process linked to MHC class I antigen presentation (Hobbs et al., 2002) are additional newly recognized mechanisms that favor maternal immunotolerance of pregnancy; uterine natural killer cells may also be involved (Erlebacher, 2001).

Not all of these mechanisms may be present in each recognized IP site, and their composition and relative importance vary between sites. Also, IP likely enrolls additional mechanisms yet to be discovered, some of which are probably related to the highly effective immune evasion strategies exploited by viruses and malignant cells in order to escape immune elimination.
by CD8+ T cells (Paul, 1999; Janeway et al, 2001; Strauss & Strauss, 2002).

It is noteworthy that IP is a relative, not an absolute, state. For example, even though fetal cells expressing paternal antigens can leave the fetomaternal placental unit and do evoke a maternal response (Tafuri et al, 1995), and even though paternal DNA is found in the lesions of one of the classical dermatoses associated with pregnancy (PEPH), fetal rejection is usually not a consequence. This suggests that the critical issue is whether, and to what extent, a state of tolerance to the alloantigen-bearing cells is established and how well immunotolerance is maintained throughout the persistence of these antigens.

It is with this rapidly evolving research background—dominated by insights gained into ocular and placental IP—that the hair follicle enters the picture.

THE ANAGEN HAIR FOLLICLE AS A SITE OF IMMUNE PRIVILEGE

More than three decades ago, Billingham discovered that the anagen hair bulb—the unique factory for pigmented hair shafts that represents one of the hallmarks of mammalian species (Paus and Cotsarelis, 1999; Cotsarelis and Millar, 2001)—provides a special milieu that permits transplanted allogeneic cells to escape limitation by the host immune system. Billingham noted that, as expected, black ear skin epidermis transplanted onto skin beds of genetically incompatible white guinea pigs quickly lost its pigmentation as a sign that the foreign melanocytes had been rejected. Surprisingly, however, black hair shafts soon thereafter began to pierce the (now white) epidermis, indicating that at least some donor melanocytes have survived in the host hair bulbs and have contributed to the formation of fully pigmented hair shafts.

Yet, the immunological research community, transplantation surgeons, skin biologists, and most investigative dermatologists have largely ignored these early indications of the existence of hair follicle IP. In fact, standard immunology and dermatology textbooks, as well as the IP literature, typically do not even mention the hair follicle in the context of IP, even though our ignorance about the immune system of the hair follicle is vast number of potential IP sites, which are highly accessible to experimental analysis and manipulation.

It was not until the study of rat hair follicle MHC class I expression (Westgate et al, 1991) and subsequent systematic attempts to characterize what was until then the obscure immunobiology of the murine and human hair follicle (Paus et al, 1994a; Hofmann et al, 1996; Paus et al, 1998; Tokura et al, 1998; Paus et al, 1999; Christoph et al, 2000) that Billingham’s landmark observation was rescued. It was surprising that it took so long for the concept of the hair follicle as an IP site to resurface, because it had been reported 20 years earlier (Harrist et al, 1983) that the human anagen hair bulb displays one of the key features of IP: the absence or very-low-level of MHC class I expression (Head and Billingham, 1985; Streilein, 1993; Brent, 1997). This striking downregulation of MHC class I expression in the proximal epithelium of anagen hair bulbs was confirmed for human (Bröcker et al, 1987), rat (Westgate et al, 1991), and murine hair follicles (Paus et al, 1994a); it was then re-analyzed in greater detail in human anagen-VI scalp hair follicles (Christoph et al, 2000).

Additional features of hair follicle biology strongly support the concept that anagen hair bulbs in all mammalian species indeed enjoy a relative IP:

- Anagen hair bulbs show a greatly reduced number of antigen-presenting cells (CD1a+ cells or ultrastructurally identified Langerhans cells), which appear to be functionally impaired because they do not express MHC class II antigens (Christoph et al, 2000).
- Unlike the outer root sheath distal from the infundibulum of the sebaceous gland, the anagen hair bulb is almost devoid of intraepithelial T cells; in mice, no γδ-TCR + lymphocytes are found below the bulge region (Paus et al, 1994c). Human anagen-VI scalp hair follicles show CD4+ T cells extremely rarely, and a CD8+ lymphocyte is almost never caught trafficking through the proximal follicle epithelium (Christoph et al, 2000).
- Even the melanocytes of the pigmentary unit of human scalp hair follicles, which is located around and above the dermal papilla in anagen, show no or very low levels of MHC class I expression (Ito et al, submitted; Moseley et al, 1997).
- Like other immunoprivileged tissues (Head and Billingham, 1985; Streilein, 1993), the hair bulb is devoid of lymphatics and is ensheathed by a special extracellular matrix barrier which may combine to hinder immune cell trafficking (Westgate et al, 1991; Stenn and Paus, 2001).

In the fetomaternal placental unit, expression of the nonclassical MHC class I (MHC class Ib) molecules HLA-E and HLA-G are thought to play an important role in establishing fetal immunotolerance (Fuzzi et al, 2002). When we discovered that mammalian skin also expresses functionally related MHC class Ib molecules (e.g., Qa-2) and that, by immunohistology, this Qa-2 expression in murine skin appears to be restricted to the hair follicle epithelium (Paus et al, 1994a), we had to ask whether these molecules play a role in hair follicle IP similar to that played by HLA-E and HLA-G in maternal immunotolerance of the fetus. Qa-2 expression is found in the peri-infundibular region of the murine outer root sheath, however, which leads to the question of whether these MHC class Ib molecules are involved in the regulation of the intriguing, nonspecific anti-infection defenses of the hair follicle immune system (HIS) rather than in follicular IP (Paus et al, 1994a; Paus et al, 1999b).

Our own studies of normal human scalp hair follicle immunology have so far failed to produce any evidence of a role for FasL or natural killer cells in the establishment or collapse of hair follicle IP (Christoph et al, 2000).

Systemic studies of the depilation-induced murine hair cycle revealed that the relative MHC class I negativity correlates well with a downregulation of β2-microglobulin. This is seen only during anagen and is largely confined to compartments of the hair follicle epithelium that are continuously generated from hair follicle stem cells (e.g., hair matrix and inner root sheath) during each anagen phase, to be deleted during each apoptosis-driven hair follicle regression (catagen) (Paus et al, 1994a; Paus et al, 1998; Rückert et al, 1998). Thus, on the basis of currently available evidence, follicular IP is probably restricted to anagen that is, to the hair cycle phase during which follicular melanogenesis is switched on and pigmented hair shafts are actively generated—and does not extend to either the catagen or the telogen phase. Also, follicular IP appears to be primarily restricted to the hair follicle epithelium, as the follicular connective tissue sheath and dermal papilla are clearly MHC class I-positive in murine and human anagen-VI hair follicles (Paus et al, 1994a; Paus et al, 1998; Rückert et al, 1998; Christoph et al, 2000; Ito et al, submitted).1

1Ito T, Ito N, Bettermann A, et al: gIFN-induced ectopic MHC class I expression in the human anagen hair follicle is down-regulated by IGF-1, TGFβ1, and alpha-MSH. Implications for hair follicle immune privilege and alopecia areata. submitted.
WHAT IS THE FUNCTION OF HAIR FOLLICLE IMMUNE PRIVILEGE?

Why does a defined region of the newly constructed proximal hair follicle epithelium undergo the effort of actively downregulating MHC class Ia during each new anagen development? Studies of the rat hair cycle (Westgate et al., 1991) have encouraged the hypothesis that this IP is instrumental in maintaining anagen and that its collapse somehow contributes to the induction of hair follicle involution, in which perifollicular macrophages were fate to play a crucial role. While this hypothesis has not yet been formally disproven, it is, in our opinion, unconvincing.

Changes in the secretory activities of perifollicular macrophages may indeed be involved in regulating the anagen–catagen transformation of the murine hair follicle (perhaps by switching from the synthesis of FGF5, a functional antagonist of the potent catagen inducer FGF5, to production of FGF5) (Suzuki et al., 1998; Ito et al., 2003). This secretory switch does not seem to be in any way related to intraepithelial changes in MHC class I expression. However, if perifollicular MHC class Ia molecules (which, after all, are expressed on every nucleated cell outside of the IP area) is not a recognized trigger for macrophage activation. Also, macrophages do not even possess the machinery that would allow them to specifically recognize and respond to MHC class I-presented peptides (these professional phagocytes are, typically, fairly weak antigen presenters and engage in phagocytosis as well as in the processing and presentation of antigenic peptides via MHC class II (Paul, 1999; Janeway et al., 2001).

In addition, compared to telogen, the number of activated MHC class II-positive inter- and perifollicular macrophages rises dramatically long before murine anagen development has been completed, only to drop sharply well before anagen VI is terminated. And there is no catagen-associated increase in the number of CD4+ or CD8+ perifollicular T cells (Paus et al., 1998). Furthermore, intraepithelial macrophages can be detected only very rarely in the regressing murine hair follicle epithelium, and this exclusively during the very latest stages of catagen development (Paus et al., 1998; Paus et al., 1999). Finally, because both murine and human hair follicles are shielded by an MHC class I-positive connective tissue sheath throughout all hair cycle stages (Christoph et al., 1999a; Paus et al., 1999b), it is interesting to note that, during hair follicle morphogenesis in mice, in contrast to the epidermis, almost the entire hair follicle epithelium is MHC class I-negative by immunohistology and why only during anagen.

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Most recently, Norris proposed that the lack of MHC class I expression in the proximal hair follicle may be “intended to protect the hair follicle from induction of auto-immunity during regression of the catagen follicle” (Norris, in press; this issue). He proposes that the phagocytosis of apoptotic cell fragments during catagen, upon peptide processing and presentation by MHC class I-positive neighboring hair follicle keratinocytes, entails the risk of inducing an immune response against self antigens, thus necessitating a downregulation of MHC class I. IP is a hallmark of anagen, not of catagen; however, so, contrary to what this hypothesis would predict, it is precisely during each normal catagen phase (at least in mice) that MHC class I antigens in the regressing follicle epithelium become strongly upregulated (Paus et al., 1994a; Paus et al., 1998), and this usually without any self-destructive immunological consequences. Taken together, these counter-arguments render the “autoimmunity protection” hypothesis unsatisfactorily as an explanation for the establishment of follicular IP.

The characteristic inflammatory cell attack on lesional hair follicles in AA almost exclusively targets anagen hair bulbs in the

from immune-mediated injury (as proposed by Niederkorn this issue), because the available immunohistological evidence suggests that these follicle regions are MHC class I-positive throughout the hair cycle. The bulge region and its neighboring outer root sheath sections above and below clearly do express HLA-A, HLA-B, HLA-C, and B2-microglobulin in human anagen scalp hair follicles, and the bulk of MHC class I-negative follicle keratinocytes is located far proximal to the bulge (Christoph et al., 2000; Ito et al., submitted). In fact, it may be one of the inbuilt “Achilles’ heels” of the hair follicle not to properly shield its crucial stem cell regions from immune-mediated injury: Even a few activated macrophages or cytotoxic T cells suffice to eliminate the entire organ by destroying the hair follicle’s regenerative capacity by an attack on its bulge region stem cells (Hermes and Paus, 1998; Eichmüller et al., 1998; Paus et al., 1999b; Paus and Cotsarelis, 1999; Cotsarelis and Millar, 2001).

In mice, the peri- and infrainfundibular outer root sheath constitutively expresses ICAM-1 and can thus attract LFA-1 expressing immunocytes (e.g., macrophages) (Mühler-Röver et al., 2001). Not surprisingly, this and the bulge region are the preferred sites for the occurrence of dense perifollicular inflammatory cell infiltrates under stress (Arck et al., 2001; Arck et al., 2003) or even in physiological circumstances: Activated macrophages can “immunosurgically” remove individual (malignant?) hair follicles by “programmed organ deletion” (Eichmüller et al., 1998; Paus et al., 1999). It is conceivable that an individual’s risk of (auto)immune attack on the bulge region, for example, in the course of lupus erythematosus, morphea, graft-versus-host disease, or lichen planopilaris, as a result of which it degenerates beyond repair, is related to the relative expression level of MHC class Ia and Ib molecules as well as to the constitutive or induced ICAM-1 expression level in this region. Yet this fails to explain for what purpose IP may be needed in the much more proximally located hair bulb epithelium and why only during anagen.

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The process of active pigment production (i.e., anagen-III/VI hair follicles).

Recovering hair follicles in AA patients typically generate white hair shafts.

Patched into one unifying scenario for hair follicle IP functioning, these background data suggest that the hair follicle IP is generated and maintained during each anagen phase (and then disassembled during catagen and telogen) in order to sequester potentially deleterious, anagen- and/or melanogenesis-associated autoantigens from immune recognition by appropriately sensitized CD8\(^+\) T cells with cognate receptors, primarily via down-regulation of MHC class I and by the local production and secretion of potent immunosuppressants (Paus et al., 1994b; Paus et al., 1999; see Fig 1).

This hypothesis was largely derived from a theoretical analysis of previously proposed and, in our assessment, unsatisfactory speculations on the pathogenesis of AA. These speculations focused on the putative role of autoantibodies or MHC class II-presented autoantigens recognized by CD4\(^+\) T cells (Dawber, 1997). At the time our hypothesis was published, some authorities even questioned whether or not AA represents a genuine autoimmune disease at all—a debate that in the meantime has been settled in favor of AA's autoimmune pathogenesis (Kalish and Gilhar, Norris, this issue).

Recognizing that AA can serve as a uniquely instructive model to illustrate the consequences of normal follicular IP collapse, we have ever since advocated the scientific scrutiny of the normal anagen hair follicle and AA as two highly educational sides of the same coin. That is, the systematic analysis of normal hair follicle IP during anagen and its collapse during AA will, in our view, not only bring major, direly needed progress in the clinical management of AA, a frequent and psychologically devastating hair loss disorder, but will also provide fresh insights into the biology and controls of IP in general (Paus et al., 1994b; Paus et al., 1999b).

Figure 1. Hypothetical scenario of alopecia areata (AA) pathogenesis by collapse of the hair follicle immune privilege. (for explanation, see text; modified after Paus et al., 1994b).

THE “IMMUNE PRIVILEGE COLLAPSE MODEL” OF ALOPECIA AREATA PATHOGENESIS

Our hypothesis was the first to apply Billingham’s concept of hair follicle IP to the pathogenesis of AA. Over the years, this long-ignored and still hypothetical concept has been modified slightly and extended to reflect recent progress in AA research (see Fig 1); still, its basic tenets have found ever increasing experimental support. Most important, Gilhar, Kalish, and colleagues have demonstrated that AA lesions can be induced by the transfer of MHC class I-restricted CD8\(^+\) T cells alone, that anagen hair follicle antigens are needed to stimulate T cells for effective triggering of AA lesions after cell transfer, and that these antigens can be substituted for by hair follicle melanocyte antigens (Gilhar et al., 1998; Kalish and Gilhar, this issue).

The “immune privilege collapse model” develops the scenario of AA pathogenesis (Fig 1) described in the following paragraphs.

Triggered by infectious foci, bacterial superantigens, psychomotorial stressors, skin microtrauma, or other damage to the hair follicle, and possibly aided by as yet ill-defined predisposing immunogenetic factors, a peri- and/or intrafollicular rise in AA IFN-\(\gamma\) secretion ectopically upregulates MHC class Ia expression in the proximal hair follicle epithelium. Namely, this occurs in the normally MHC class I-negative hair matrix of anagen hair bulbs, thus seriously endangering maintenance of the hair follicle IP. Now follicular autoantigens can be ectopically presented in the normally MHC class I-negative epithelial hair bulb and are no longer sequestered. Once the hair follicle enters anagen and, at the latest, when its pigmentary unit engages in active melanogenesis (i.e., during anagen III/VI [Slominski and Paus, 1993]), as yet obscure anagen- and/or melanogenesis-associated autoantigens are exposed to the skin immune system. In the event that a given individual has pre-existing autoreactive CD8\(^+\) cells, which must receive appropriate costimulatory signals and help...
from CD4+ T cells (and, possibly, additional signals via CD4 as well), a cytotoxic T cell attack is launched on the hair matrix. This attack activates a vicious circle of secondary, follicle-damaging autoimmune phenomena, whose quality and magnitude largely determine the resulting degree of hair follicle damage (dystrophy) and thus the actual clinical manifestation, progression, and course of AA (see Fig 1).

In contrast, the thought-provoking and innovative concept of AA pathogenesis most recently proposed by David Norris hypothesizes that AA is a consequence of insufficient hair follicle IP during catagen (Norris, in press; this issue). This theory suffers from a lack of evidence that catagen hair follicles are the primary target of an autoimmune attack in AA and that there is any IP established in catagen hair follicles in the first place. Indeed, current evidence supports only anagen-III/VI hair follicles as primary targets of immune injury in early AA lesions, and provides strong data that suggest the establishment of IP in the anagen hair bulb (see above).

**IMMUNE PRIVILEGE RESTORATION: LEADS FROM IN VITRO MANIPULATIONS OF MHC CLASS I EXPRESSION IN THE HUMAN ANAGEN HAIR FOLLICLE**

We previously showed that, compared to IL-1 and TNF-α, IFN-γ offers the most potent cytokine stimulus for ectopic MHC class I expression in murine pelage hair follicles in vivo (Rückert et al, 1998). We recently succeeded in developing a standardized and highly reproducible in vitro assay that recreates the key feature of IP collapse postulated above: the ectopic upregulation of HLA-A,B,C expression in the matrix of normal anagen hair follicles. Using this new in vitro assay and very sensitive immunostaining techniques, as well as in situ hybridization and RT-PCR, we could confirm that IFN-γ is indeed a very potent stimulator of ectopic MHC class I expression in microdissected, organ-cultured human scalp hair follicles from healthy donors in anagen VI (Ito et al, submitted).

The new in vitro model allows screening for candidate agents that effectively downregulate IFN-γ-induced, ectopic MHC class I expression in human anagen hair follicles as the most important prerequisite for IP restoration. In fact, three immunomodulators known to be locally produced in the anagen hair bulb—α-MSH, TGF-β1, and IGF-1 (Slominski et al, 1993; Botschkaev et al, 1999; Paus et al, 1999; Slominski et al, 2000)—are all capable of downregulating ectopic MHC class I expression, on both the protein and the mRNA level, when added to the culture medium after IFN-γ administration (Ito et al, submitted).

We are encouraged and intrigued by these findings because they provide a pragmatic and effective perspective on how IP restoration in AA can be achieved by administrations of natural immunomodulators that the hair follicle itself not only generates (Slominski et al, 1993; Botschkaev et al, 1999; Paus et al, 1999; Slominski et al, 2000) but also employs as key regulators of hair follicle cycling during the anagen-catagen switch (i.e., TGF-β and IGF-1 (Stenn and Paus, 2001; Cotsarelis and Millar, 2001)). It is tempting to speculate that these very same factors are also recruited by the anagen hair follicle to maintain and restore its IP (see Fig 1). In addition, the production/secretion of α-MSH, TGF-β1, and IGF-1 may potentiate each other in order to strongly downregulate ectopic MHC class Ia expression in the anagen hair bulb as a means of IP restoration. On the other hand, hair follicle-derived α-MSH, TGF-β1, and IL-10—well-recognized natural immunosuppressants (Luger et al, 1998; Paul, 1999; Janeway et al, 2000)—could efficiently dampen the secondary autoimmune phenomena that we envision as driving force behind the specific clinical characteristics and the progression of AA in any given patient. In this scenario, termination or progression of AA is determined largely by how efficiently the follicle’s own molecular tools for IP restoration and general immunosuppression operate in the face of an ongoing autoimmune attack (see Fig 1).

In the large group of AA patients with uncomplicated forms of this disease and frequent spontaneous remission, AA’s self-limited nature may be interpreted to reflect the presence of a very efficient “IP restoration machinery” of the hair follicle that could operate along the lines sketched in Fig 1. Instead, patients suffering from rapid disease progression and the recalcitrance of lesional AA skin to regrow hair even after the administration of potent immunosuppressants, such as glucocorticosteroids or cyclosporine A, may represent individuals with constitutively imperfect or insufficient IP restoration capacity.

Three simple yet clinically important messages can be distilled from this view of hair follicle IP and AA pathogenesis:

1. Rather than targeting the secondary autoimmune phenomena associated with AA, as most of our currently employed treatment regimens continue to do, the primary goal of effective AA management must be to restore the hair follicle’s lost or compromised IP—both for preventing the progression of AA lesions and for inducing hair regrowth.

2. IP restoration therapy does not require any prior knowledge of the relevant key autoantigens or the specific autoreactive T cells, and it can resort to the administration of well-known nonspecific immunomodulators that chiefly downregulate ectopic MHC class I expression in the anagen hair bulb.

3. The therapeutic use of powerful natural immunosuppressants such as α-MSH and TGF-β1 along with the anagen-promoting and catagen-suppressing (as well as MHC class I-suppressing) cytokine IGF-1, all of which are locally generated in the hair follicle itself and may be part of the follicle’s own “IP restoration machinery”; promises to tilt the balance in favor of successful IP restoration. These agents also promise to carry with them minimal risks of toxicity, which may be further reduced by their topical application (e.g., via hair follicle-targeted liposome preparations).

**OPEN QUESTIONS AND FUTURE PROSPECTS**

We suspect that many additional mechanisms recruited during the generation and maintenance of the hair follicle IP are yet to be discovered and that we have seen only the “tip of the iceberg.” Important new leads in this respect arise from recent progress in reproductive immunology, which indicates that local tryptophan catabolism is important as a means of impairing T cell function in the fetomaternal placental unit (Munn et al, 1998; Mellor and Munn, 2000). Additionally, a constitutive inhibition of complement activation may support the maintenance of IP here (Xu et al, 2000); a specific β2-microglobulin-dependent process linked to MHC class I antigen presentation controlled by a TATA-binding protein is also critical for maternal immunotolerance of pregnancy (Hobbs et al, 2002).

Therefore, we are currently applying these leads to the study of hair follicle IP, and we are actively investigating which of the mechanisms recognized to underlie ocular IP (Streilein, 1993; Niederkorn, 2002, this issue) are relevant to the hair follicle IP as well. This work promises instructive new insights into the characteristics and controls of hair follicle IP in our search for a hair follicle equivalent of the “anterior chamber–associated immuneDeviation” (ACAIMD) of the normal eye, with its antigen-specific downregulation of TH1 immune responses. We also hope to understand how the molecular composition of the aqueous humor of the anterior eye chamber (with its DTH-suppressive factors like TGF-β, α-MSH, VIP, and CGRP) (Streilein et al, 1993; Niederkorn, 2002) compares to the cytokine and neuropeptide signaling milieu in which the anagen hair bulb
“bathes.” Already, the presence of these factors has been identified in the hair bulb or in the perifollicular neural networks (Slominski et al., 1993; Paus et al., 1997; Paus et al., 1998; Slominski et al., 2000; Peters et al., 2001), and synchronized anagen development in mice is recognized to produce a profound inhibition of TH1 immune responses (contact hypersensitivity) (Hofmann et al., 1996; Tokura et al., 1997; Hofmann et al., 1998).

While pursuing this approach—that is, wherever possible, draw to instructive analogies between follicular, fetomaternal, and ocular IP—a few open, key questions deserve more careful consideration.

For example, is it not surprising that during anagen the MHC class I-negative hair follicle epithelium does not regularly fall victim to the attack of NK cells programmed to recognize and eliminate MHC class I-negative cells (Karre, 1997; Janeway and ocular IP/C246a few open, key questions deserve more careful consideration.

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Given that nonclassical MHC class I molecules such as Qa-2 are capable of deactivating NK cells (Vilches and Parham, 2002), does the MHC class I-negative follicle epithelium express any such MHC class Ib antigens? That we did not find Qa-2 expression in this region (Paus et al., 1994a) certainly does not rule out that other NK inhibitory cell surface or secreted molecules are expressed in the anagen hair bulb.

Is there actually a small subpopulation of patients with AA in whom the collapse of the hair follicle IP is initiated not by ectopic MHC class I expression (as we have proposed for the majority of AA patients (Paus et al., 1994a); see Fig 1) but rather by the failure to suppress NK cell activities directed against MHC class I-negative hair follicle cells during anagen?

Another critically important issue for both hair follicle IP and AA research is to firmly establish to what extent individuals, as well as hair follicles in distinct locations of the integument, differ in their constitutive level of MHC class Ia and Ib expression in the anagen hair bulb. What interindividual and interlocation differences can be identified in the constitutive expression levels of MHC class Ia-downregulating factors such as a-MSH, TGF-β1, and IGF-1, as well as the expression of the cognate receptors? Also, an individual’s sensitivity to IFN-γ-induced ectopic MHC class I expression in the anagen hair bulb may be genetically determined and may play a significant role in determining whether such ectopic MHC class I expression in the follicle, and with it the collapse of immune sequestration of anagen-associated autoregulants, occurs in response to various IFN-γ-upregulating stimuli (see Fig 1).

To clarify these issues is clinically very important because deviations in all of these parameters may well conspire to predispose selected individuals and hair follicle subpopulations to the development of AA while awarding relative protection to others. Such differences may help to explain why certain hair follicle populations are attacked much more frequently in AA than others and why a positive family history for AA predisposes to the development of the disease (Dawber, 1997). Certainly, we need to elucidate how any such expression differences are related to the increasingly recognized immunogenetic parameters that determine an individual’s relative risk of developing AA, particularly in families with a high prevalence of AA.

Prophylactic measures can be envisioned that prevent the onset of AA in high-risk individuals, and the spread of AA lesions in established disease, once we have learned more about the mechanisms of hair follicle IP. In particular we must investigate the role that local MHC class Ia-downregulatory signals generated in the anagen hair bulb itself play in follicular IP—and how patients with a constitutively flawed or insufficiently maintained hair follicle IP, or with a higher constitutive sensitivity to IFN-γ stimulation, can be treated to re-establish the full natural protection from immunologically mediated injury that healthy anagen follices enjoy.

Exciting work from the Botchkarev lab has implicated neurotrophins and the p75 neurotrophin receptor (p75NTR) in these immunoprotective mechanisms (Botchkarev, this issue). The previous discovery that neurotrophins, for which the hair follicle is both an important target and a source, are central elements of hair growth control during hair follicle development and cycling (Botchkarev et al., 2000; Botchkareva et al., 1999) has been extended to the study of murine perifollicular CD8+ T cells. Apparently, these are very sensitive to p75NTR stimulation, whose blockade or functional deletion results in a dramatic upregulation of CD8+ T cell numbers. This suggests that hair follicle-derived neurotrophins have important regulatory effects on perifollicular CD8+ T cells. Naturally, it remains to be clarified whether follicle-derived neurotrophins and p75NTR stimulation of CD8+ T cells are good or bad for maintaining the hair follicle IP—strictly depending on whether the intriguing observations made by Botchkarev and colleagues relate to cytotoxic or suppressor CD8+ T lymphocyte subpopulations, and on whether it is really the pathogenic, autoreactive CD8+ cytotoxic T cells that can be deleted by p75NTR stimulation (rather than beneficial suppressor T cells).

Given the central role that autoreactive CD8+ T cells play in our (still hypothetical) scenario of AA pathogenesis (without these lymphocytes, the proposed collapse of the hair follicle IP during AA would remain without deleterious consequences; see Fig 1), the neurotrophin track is a particularly appealing research avenue toward improved AA management because it does not require prior identification of the putative T cell-dependent autoantigen(s) that the majority of AA researchers now suspect to underlie AA pathogenesis (Kalish and Gilhar, this issue). In this sense, the newly recognized p75NTR link plays nicely into what is perhaps the most attractive feature of the “immune privilege collapse model” of AA pathogenesis: Long before the elusive (anagen-, melanocyte- and/or melanogenesis-associated?) autoantigen(s) attacked in AA has finally been identified, and long before specific T cell tolerance toward these antigens has been successfully engineered, our model promises that we can already treat AA very effectively, simply by restoring immune privilege to the follicle using well-defined and fairly easily applied agents, such as the first reasonable candidates now identified to downmodulate ectopic MHC class Ia expression in organ-cultured human scalp hair follicles.

Even in the unlikely event that the new focus in AA research on the still insufficiently explored IP of the normal anagen hair follicle that we strongly advocate here should ultimately fail to generate the benefits for the prevention and treatment of AA that we predict, this focus is guaranteed to produce a rich immunological harvest in terms of improving our general understanding of IP, antigen sequestration, immune tolerance, and MHC class I biology—in a uniquely accessible and easily manipulated, abundantly available research model that is only waiting to be rediscovered by professional immunologists.

REFERENCES


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