# Collapse of Immune Privilege in Alopecia Areata: Coincidental or Substantial?

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**Only indirect evidence supports the concept that a collapse of immune privilege (IP) in hair follicles leads to the development of alopecia areata (AA). In this issue, Kang *et al*. provide further evidence to support this theory, demonstrating downregulation of the expression of several genes important for the immunosuppressive environment in lesional and perilesional areas of AA.**

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## The immune privilege of critical organs

Investigators have long been curious about the ability of critical organs to survive for long periods of time, protected from inflammatory reactions and foreign invasion. Even in an immune-compromised environment, various critical organs and tissues are protected from immune-mediated inflammation that might otherwise affect the function of vital tissues and threaten organ survival. The Dutch ophthalmologist van Dooremaal (1873) described the phenomenon of immune privilege (IP) by demonstrating the prolonged survival of mouse skin grafts placed in the anterior chamber of a dog's eye. Later, Medawar (1948) studied the fate of allogenic skin grafts transplanted into the anterior chamber of the eye and brains of rabbits, discovering that, in contrast to allografts inserted into other body sites (including the skin itself), those transplanted into the eye and brain survived much longer. Thus, historically, IP sites are defined as areas in the body where foreign tissue grafts can survive for extended or indefinite periods of time, whereas similar grafts placed in conventional body sites are acutely rejected. IP is most probably crucial for the survival of three organs: the brain, the eye, and the pregnant uterus. In an evolutionary sense, these organs are vital for survival, and immune-mediated inflammation in any of these organs could have devastating consequences.

## Immune privilege of hair follicles

Over the past two decades, it has been suggested that normal hair follicles represent an IP site, indicating a possible evolutionary change in the role of hair over the ages. The Billingham mouse experiment (Billingham and Silvers, 1971) provided the first clue regarding the immune protection of hair follicles—in a model of skin grafts of black guinea pigs transplanted to the white skin of an incompatible recipient strain, the donor's melanocytes immigrated and survived for a long time within host hair follicles, in sharp contrast to their fate among epidermal melanocytes.

The characteristic features of the eye's IP might serve as an example of pure IP: lack of lymphatic drainage, immunosuppressive effects of the aqueous humor itself, low major histocompatibility complex (MHC) expression, low numbers of Langerhans cells or other antigen-presenting cells, and inhibition of natural killer cells. Similarly, normal hair follicles do not express MHC class I and class II molecules; there are only a few Langerhans cells around and within hair follicles, and they have functional impairment because they do not express MHC class II molecules, which normally play roles in antigen presentation. Furthermore, immunosuppressive cytokines are expressed prominently by the follicular epithelium. These cytokines are believed to maintain the IP of the hair follicles and to induce peripheral tolerance (Gilhar *et al*., 2007). Ito *et al*. (2008) demonstrated the strong expression of the potent NK-cell inhibitor MIF, which prevents the accumulation of NK cells around and within hair follicles. Recently, Petukhova *et al*. (2010) described a genome-wide association study in which they identified several genetic susceptibility loci for alopecia areata (AA), most of which were clustered in eight genomic regions. The spectrum of affected gene activities implies the involvement of both acquired and innate immunity in the disease. Significant associations include the *ULBP* genes, which encode activating ligands for the NK cell receptor, NKG2D. NKG2D/ULBP3 engagement might be responsible for the destructive effect observed in affected tissues. Normally, *ULBP3* is not present in hair follicles, but in Petukhova and colleagues' study ULBP3 proteins were abundant in the hair follicles affected by alopecia areata. Collectively, these findings support a role for IP in hair growth.

## Collapse of IP in AA: true or false?

AA is a noncontagious, incurable skin disease that appears mostly as bald patches of the scalp. The disease is characterized by a complexity of events, and multiple genes, hormones, growth factors, and inflammatory molecules have been found to play roles in its pathogenesis. Previously, we showed that it is possible to transfer AA to scalp explants on severe combined immunodeficiency mice by injecting autologous scalp-derived T cells from patients with AA (Gilhar *et al*., 1998). The scalp T cells were cultured with a follicular homogenate prior to injection into the explants. The histological and immunohistological profile of the explants demonstrated characteristic features of AA, including

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increased expression of MHC class I and II molecules by the follicular epithelium. Paus *et al*. (1994) hypothesized that the induction of MHC class I molecules on hair follicle epithelium leads to the collapse of IP because ectopic MHC class I molecule expression is the logical key prerequisite for autoantigens being exposed at all. These investigators speculated that the collapse of IP is the key to the induction of AA. Hair follicle IP is considered to have involvement at multiple levels in AA, including roles for cell receptors, cytokines, chemokines, and physical barriers. Thus, IP collapse would presumably require several steps rather than simply the induction of MHC class I and II molecule expression. Direct functional evidence for IP collapse could be provided by showing that "IP-intact" mouse hair follicles can harbor and immune-protect an alloantigen, whereas "IP-defective" hair follicles would reject such an antigen. However, to date, only indirect evidence supports the concept of IP collapse in the pathogenesis of AA. Therefore, the study by Kang *et al*. (2010, this issue) is of particular importance because the investigators therein report solid data demonstrating the downregulation of IP genes in uninvolved scalp areas of patients with AA.

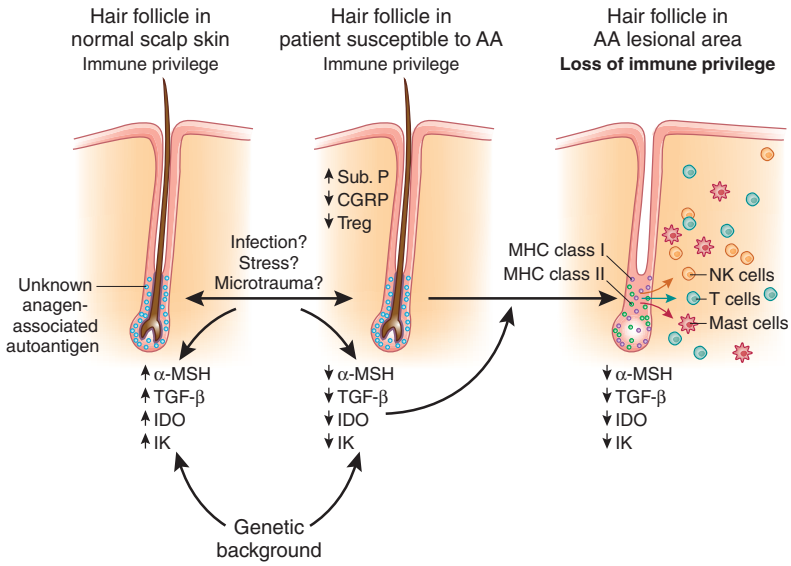


**Figure 1. Pathogenic model of alopecia areata.** The normal hair follicle represents a site of immune privilege (IP). The guardians of IP include immunosuppressive cytokines such as α-melanocyte stimulating hormone (α-MSH), transforming growth factor-β (TGF-β), IK, indoleamine 2,3-dioxygenase (IDO), and IL-10. Patients with a specific genetic background are susceptible to developing alopecia areata (AA), most probably by downregulation of this immunosuppressive environment. It has been suggested that events such as stress, infection, or microtrauma might lead to downregulation of immunosuppressive cytokines. This downregulation enables the accumulation of natural killer (NK) cells around hair follicles. Furthermore, stress or other trauma may also alter the production of neuropeptides, including substance P (SP) and calcitonin gene–related peptide (CGRP). SP may upregulate the production of nerve growth factor, which in turn induces accumulation of mast cells around hair follicles. SP causes degranulation of mast cells, leading to a release of large amounts of TNF-α, which is known to inhibit hair growth. Furthermore, SP induces accumulation of $CD8^+$ cells and induce these cells to produce large amounts of IFN-γ. IFN-γ, produced by the activated $CD8^+$ cells and the NK cells, induces expression of major histocompatibility complex (MHC) class I molecules in the lower part of the follicular epithelium, resulting in presentation of follicular autoantigens to the $CD8^+$ cells and loss of IP. IFN-γ also may induce MHC class II molecule expression by the follicular epithelium, leading to a second wave of $CD4^+$ cells that may bolster $CD8^+$ activity via released cytokines. Treg, regulatory T cell.

**Lesional versus perilesional areas in AA**

A crucial question is whether the collapse of hair follicle IP in AA is a secondary event of the "storm"-like events that precipitate in AA. Kang *et al*. (2010) provide data regarding IP gene expression in both lesional and unaffected perilesional areas. Comparing gene and protein expression in involved and uninvolved areas might indicate patients' susceptibility to hair loss. Indeed, several genes were upregulated in both areas, including *IL-1Ra* and *CD80*. Previously, it was suggested that *IL-1Ra* is associated with AA and involved in its pathogenesis. CD80 molecules are expressed by dendritic cells and are needed to activate T cells. The current study showed that FasL, a molecule known to induce apoptosis of activated T cells and neutrophils, was decreased significantly in lesional but not in unaffected perilesional areas. It is well recognized that FasL is crucial in promoting eye, brain, and fetal IP. The potent immunosuppressive cytokines, α-melanocyte-stimulating hormone (α-MSH) and transforming growth factor-β (TGF-β), which are assumed to maintain hair follicle IP and are known to be decreased in the involved areas of AA, were downregulated in both the involved and uninvolved areas.

Furthermore, two striking immunosuppressive agents that have not been detected previously in AA were found to be decreased significantly, mostly in the perilesional areas: the lymphocyte proliferation inhibitor indoleamine 2,3-dioxygenase (IDO) and Red/IK. The role of IDO in the immune system remains a subject of active investigation, including its role in the eye. Recent studies have shown that enhanced IDO activity plays an important role in inducing immunological tolerance in many Th1-mediated autoimmune diseases, such as experimental autoimmune encephalomyelitis (Hou *et al*., 2009). The second immunosuppressive component that was significantly downregulated in the lesional and nonlesional areas of AA is the IK cytokine, which has been isolated as a factor that inhibits IFN-γ-induced expression of MHC class II antigens. Aberrant expression of MHC class II antigens has reportedly been recognized in the target organs of autoimmune diseases and has been associated with disease activity (Muraoka *et al*., 2006). Kang *et al*. (2010) provide novel evidence of two potent immunosuppressive components in normal scalp skin, supporting the role of IP in normal hair follicles. Even more importantly, the significant downregulation of immunosuppressive

## Clinical Implications

- Normal hair follicles are immune-protected sites that lack expression of cell surface molecules ordinarily associated with effective immunity.
- Recent studies indicate that alopecia areata is associated with a loss of this immune protection, leading to an effector T-cell response that is directed against the hair follicle itself.
- Current strategies in the development of new therapies include the use of immunologically active molecules that return the hair follicle to its state of immune protection.

components such as IK and IDO not only in lesional but also in perilesional, unaffected areas may indicate a collapse of the immunosuppressive environment prior to the massive cellular infiltration of perifollicular regions in lesional skin. This line of thought may support the notion that the collapse of IP plays an important role and is probably not a secondary event in the pathogenesis of AA.

### A scenario of events leading to alopecia areata

A crucial and so far unresolved question is "What are the initial events leading to the development of AA?" Several studies have incriminated IFN-γ in the pathogenesis of the disease (Gilhar *et al.*, 2007). However, it is unlikely that IFN-γ plays an initial role in the pathogenesis of AA because the primary source of IFN-γ is activated T cells (and it would be difficult to postulate activation of T cells prior to the loss of IP). It has been proposed that neuropeptides, such as the calcitonin gene–related protein (CGRP) and substance P (SP), might have initiating capability (Gilhar *et al.*, 2007). CGRP has been reported to have an immunosuppressive effect; a lack of CGRP results in vasoconstriction and hyperresponsiveness (including autoimmunity), both of which may play roles in the pathogenesis of AA. SP-containing nerves are present in greater numbers in early AA lesions. SP treatment results in a moderate accumulation of $CD8^+$ cells (Gilhar *et al.*, 2007). Thus, AA is suggested to develop as follows: downregulation of IK and IDO along with other immunosuppressive agents, combined with alterations in neuropeptide production following infection-associated stimuli, stress-associated neurogenic inflammation, or other microtrauma, facilitates the accumulation of activated immune cells and the production of IFN-γ (Figure 1).

It appears that IFN-γ is unique in inducing MHC class I molecules in the lower part of the follicular epithelium (Gilhar *et al.*, 2007). This induction may result in the presentation of follicular autoantigens and loss of peripheral tolerance. IFN-γ also induces the expression of MHC class II molecules by the follicular epithelium. MHC class II molecule expression by the affected hair follicles may result in a second wave of activated CD4 cells. The significant downregulation of IK enables IFN-γ to induce MHC class II molecule expression by the follicular epithelium. Thus, the study by Kang *et al.* (2010) may provide additional and important indirect evidence supporting the role of IFN-γ in AA.

### How close are we to finding novel treatments for AA?

The study by Kang and colleagues (2010), among others, could lead to the development of specific treatments for AA, which to date are unavailable. It has been suggested that the pathophysiology of AA involves multiple effectors and that interrupting only one pathway is insufficient for affecting the course of the disease. This is consistent with recent studies in which single-targeted psoriasis treatments did not demonstrate efficacy in AA. The results of the study by Kang *et al.* strengthen the concept that the reestablishment of IP would serve as a therapeutic manipulation to reverse the disease and prevent its progression. Cytokines with the potential to maintain human hair follicle IP, such as TGF-β, α-MSH, and IL-10, have been proposed for use in a novel therapeutic approach. A potent IK analog demonstrated its ability to ameliorate the progression of lupus nephritis (Muraoka *et al.*, 2006), whereas further studies into the exact nature of IDO in pathogenic mechanisms of AA may lead to the development of novel therapies for AA (Hou *et al.*, 2009). Our hope is that more studies such as those by Kang *et al.* will lead to a better understanding of the pathogenesis of AA and to a promise of useful therapeutic agents.

### CONFLICT OF INTEREST

The author states no conflict of interest.

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