

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Clues from Alopecia Areata on the Role of Neuropeptides in the Initiation of Autoimmunity

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A fascinating question regarding the pathogenesis of alopecia areata is the potential linkage with the brain. Siebenharr *et al.* demonstrate that substance P fibers are increased in early lesions, and that substance P treatment induces catagen follicles along with activated CD8⁺ T cells. Potentially, neuropeptides serve as the initial insult resulting in loss of tolerance and autoimmune disease.

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In this issue of the *Journal of Investigative Dermatology*, Siebenharr *et al.* (2007) present data strongly suggesting a role for substance P in alopecia areata. This is of great interest because it provides a potential link between brain and body with respect to the initiation of autoimmune disease. There is considerable evidence supporting an autoimmune pathogenesis for alopecia areata (Gilhar and Kalish, 2006). Alopecia areata has a statistical association with autoimmune thyroiditis and HLA DQB1*03. Histologically, the condition is marked by an infiltrate of lymphocytes around hair follicles, associated with prema-

ture catagen, and conversion of terminal hairs to miniature anagen hairs. The hair follicle demonstrates evidence of immune activation including expression of major histocompatibility complex (MHC) class I and class II molecules as well as ICAM-1 on follicular epithelium, where these molecules are usually poorly expressed or absent. The T-cell infiltrate is characterized by perifollicular CD4⁺ cells and intrafollicular CD8⁺ cells as well as a T-helper 1 cytokine profile. Immunologic intervention such as treatment with allergic contact sensitizers can induce hair regrowth, supporting a role of immunology in the pathogenesis.

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Evidence for a role for T cells in the pathogenesis of alopecia areata is provided by the ability to transfer hair loss to human scalp grafts on severe combined immunodeficient (SCID) mice by injection of activated T cells derived from alopecia areata lesions (Gilhar *et al.*, 1998). The T cells must first be activated with hair follicle autoantigen to induce hair loss. Cooperation between CD4⁺ and CD8⁺ T cells is required for maximal hair loss. Melanocyte-derived peptides are capable of serving as autoantigens for inducing alopecia areata in this transfer system.

Murine alopecia areata is a well-characterized system that shares many histologic and immunologic features with the human condition (McElwee *et al.*, 1998). Female C3H/HeJ mice develop spontaneous alopecia areata with age at a rate of approximately 20% by 1 year. The immunohistologic features are similar to human disease, with perifollicular CD4⁺ cells, intrafollicular CD8⁺ cells, and expression of MHC class I and class II and ICAM-1 on follicular epithelium. It is possible to transfer hair loss to young C3H/HeJ mice by grafting involved skin. It is presumed that passenger lymphocytes in the skin mediate the transfer.

Alopecia areata provides an excellent model to study the initiation of autoimmunity. The proximal ("lower") hair follicle epithelium is deficient in expression of MHC class I and class II as well as numbers of dendritic antigen-presenting cells, rendering the hair follicle an immune-privileged site (Ito *et al.*, 2004). Additional mediators of immune privilege in the hair follicle include immunosuppressive cytokines (for example, transforming growth factor- α and α -melanocyte-stimulating hormone). As is noted above, with active disease, immune privilege is lost and the hair follicle epithelium expresses MHC class I and class II and ICAM-1 along dendritic antigen-presenting cells. Paus *et al.* (1993) have hypothesized that this loss of immune privilege is key to the induction of alopecia areata. Their proposal was tested by induction of hair follicle MHC class I and class II expression in C3H/HeJ mice by injection of IFN- γ . The mice developed alopecia areata at a significantly higher rate and at a younger age than their control

mice injected with saline (Gilhar *et al.*, 2005). Only genetically susceptible C3H/HeJ mice could be induced to lose hair by this treatment. Clinically relevant factors that may induce initial loss of immune privilege remain to be determined. It is unlikely that IFN- γ plays this initial role, because the primary source for this cytokine is activated T cells, and it would be difficult to postulate T-cell activation prior to loss of immune privilege. Neuropeptides and neurogenic

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inflammation have been proposed as a potential initial insult capable of triggering autoimmunity in a genetically susceptible host.

Many patients report that their initial episode of alopecia areata was induced by a specific stressful event. Although this finding has not been confirmed by population-based epidemiologic studies, experienced clinicians hear this history repeatedly. This intriguing story has fueled attempts to link autoimmunity with the brain. Immunomodulatory neuropeptides associated with alopecia areata include substance P and calcitonin gene-related peptide (CGRP) (Rossi *et al.*, 1997; Meyronet *et al.*, 2003; Botchkarev *et al.*, 2006). CGRP released from cutaneous nerves can induce mast-cell degranulation, with release of immunosuppressive tumor necrosis factor- α and IL-10. CGRP-containing neurons come in close proximity to Langerhans cells, and Langerhans-cell antigen-presenting function is inhibited by CGRP treatment. In addition, CGRP interacts with keratinocyte factors to promote melanization. Involved scalp has reduced cutaneous levels of CGRP, and serum levels of CGRP are depressed to about half of control values in patients with active alopecia areata. CGRP is also a potent inducer of vasodilatation of skin vasculature, which may be of significance to the hair cycle. The literature on substance P had been contradictory until clarified by the study of Siebenharr *et al.* (2007).

The key to understanding the role of substance P in alopecia areata is the demonstration by Siebenharr *et al.* (2007) that substance P-containing nerve fibers are greatly elevated in early lesions, and reduced in late lesions. Advanced lesions also expressed increased levels of the substance P-degrading enzyme neutral endopeptidase. Injection of substance P-treated agarose beads induced catagen hair follicles as well as mast-cell degranulation. Substance P treatment resulted in a modest accumulation of CD8⁺ T cells along with an impressive increase in granzyme expression by these CD8⁺ cells. Granzyme B is both a marker of CD8⁺-cell activation and an important effector molecule of possible relevance to alopecia areata pathogenesis. Neurokinin-1 receptors were expressed on CD8⁺ cells and monocytes, but not on mast cells. CD8⁺ cells infiltrating the follicular epithelium were found to express both neurokinin-1 and granzyme B.

The significance of this study is that it demonstrates that:

- (1) Substance P-containing neurons are increased in early lesions of alopecia areata.
- (2) Substance P treatment induces catagen hair follicles.
- (3) Substance P treatment results in recruitment of intrafollicular CD8⁺ cells expressing the effector molecule granzyme B as well as neurokinin-1 receptors.

The data suggest that substance P acts through immune activation of CD8⁺ cells. Intrafollicular granzyme B-positive CD8⁺ cells are a feature of alopecia areata. However, it is necessary to determine whether substance P induces catagen in immunodeficient or CD8 knockout mice. The authors reference data that substance P induces catagen in mouse strains not susceptible to alopecia areata. This raises questions regarding the nature of the genetic susceptibility to alopecia areata. Perhaps the limiting factor is the ability to develop a self-perpetuating CD8⁺ immune response after initial insult with substance P.

It is proposed that autoimmunity is induced by a loss of self-tolerance. In the case of immune-privileged sites, such as the hair follicle, loss of tolerance may

result from loss of immune privilege. Possible causes of such loss of immune privilege include infections, and trauma (for example, in the eye). However, data are now accumulating that suggest that neuropeptides may initiate autoimmunity. The study by Siebenharr *et al.* (2007) suggests that substance P may play such a role in initiating alopecia areata. It is probable that the ability of the autoimmune process to establish itself and propagate after the initial insult is governed by genetic factors.

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