
Alopecia universalis associated with impaired interleukin-4 production and low serum IgE level

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A 17-year-old female presented with alopecia universalis, which appeared following widespread eczematous dermatitis. An immunohistochemical study demonstrated that CD8⁺ T cells infiltrated into hair follicles with satellite cell necrosis of keratinocytes. The precedence of the autosensitization dermatitis suggested that T cells concerned with the eczematous reaction also attacked hair follicles, or alternatively, that those T cells sequentially activated another population of cytotoxic T cells. We found that she had a continuously low level of serum IgE (<1 units/ml), and this abnormality urged us to investigate interleukin (IL)-4 production of T cells. Although the patient had a normal number of CD4⁺ T cells, the production of IL-4, but not IL-10, was profoundly impaired as assessed by a reverse transcriptase polymerase chain reaction analysis, suggesting the possible relationship between the baldness and IL-4 reduction. The autoimmune hair loss might occur via activation of cytotoxic T cells and Th₁ cells as a result of a relief from IL-4 control. (J Am Acad Dermatol 2007;57:S22-5.)

Accumulating evidence has suggested that alopecia areata is a T-cell mediated, tissue-restricted autoimmune disease of the hair follicle.¹ A wide range of clinical presentations can occur, from the loss of a single patch of hair to complete loss of hair on the scalp (alopecia totalis) or over the entire body (alopecia universalis). The hair follicle is an immunologically privileged organ that is protected from the attack by cytotoxic T lymphocytes (CTLs) by decreasing major histocompatibility complex (MHC) class I expression.^{2,3} In some pathologic conditions, however, attenuated down-regulation of MHC class I expression or the activation of CTLs leads to the attack on hair follicles and resultant hair loss. Here, we report a patient with alopecia universalis who exhibited such an aberrant immunological state. Our case is characterized by the precedence of a widespread eczematous eruption before the occurrence of alopecia, and the attenuation of interleukin (IL)-4 production that we noticed with an extremely

low level of IgE. The possible relationship between hair loss and IL-4 was discussed.

CASE REPORT

A 17-year-old Japanese female was referred to our department in March, 1995 because of a 1-month history of an exudative, itchy, eczematous eruption on the earlobes, external meatus, and palpebral regions with facial edema. On admission, her eruption spread to all four extremities, forming itchy red papules. A biopsy specimen from the lesion demonstrated an upper dermal infiltrate of lymphocytes, some of which invaded the epidermis with epidermal spongiosis and vesicles. A tentative diagnosis of contact dermatitis and subsequent autosensitization dermatitis was made, although results of patch test to all the five topical drugs and cosmetics that she had used were negative. On blood examination, she had a normal leukocyte count but eosinophilia (17%; 1,343/ μ l). Other blood cell counts and routine chemistry were all normal, except for an extremely low level of IgE (<1 units/ml; normal, 30-250 units/ml). She was treated with oral prednisolone (15 mg/day for 2 weeks) and topical steroid ointments, followed by oral ketotifen (2 mg/day for 2 weeks).

The patient's eruption completely improved following the aforementioned therapies, but in the interim the hair on her scalp began to fall out. Over the next year, she lost all of the hair on her scalp, nose, axillae, and pubic area, and was diagnosed as having alopecia universalis (Fig 1, A). A biopsy

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Funding sources: None.

Conflicts of interest: None declared.

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0190-9622/\$32.00

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doi:10.1016/j.jaad.2006.12.032

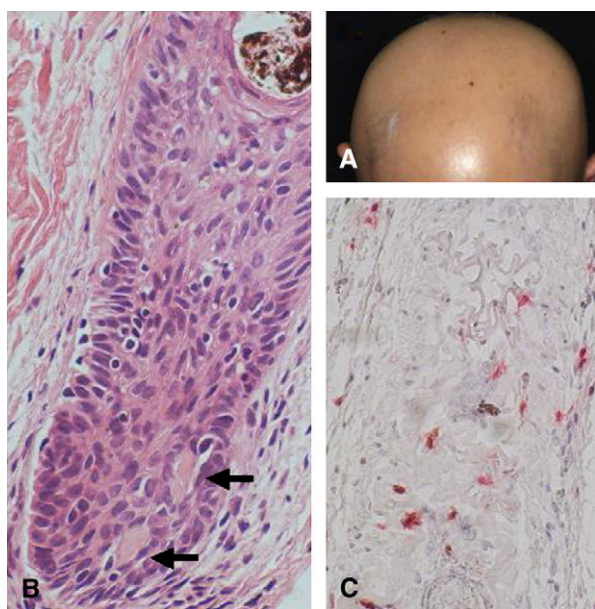


Fig 1. Clinical appearance and histologic pictures. **A**, Complete baldness of the scalp in March, 1996. **B**, Histologic picture showing a hair follicle into which lymphocytes infiltrate with satellite necrosis of keratinocytes (arrows). **C**, Immunohistochemical staining for CD8, showing infiltration of CD8⁺ T cells into a destroyed hair follicle. (**B**, Hematoxylin–eosin stain; **C**, avidin-biotin stain; **B** and **C**, original magnification: $\times 100$.)

specimen from her scalp disclosed lymphocytic infiltration into hair follicles (Fig 1, B). Immunohistochemically, the infiltrating cells were mostly CD8⁺/CD3⁺ T cells with a smaller number of CD4⁺ T cells (Fig 1, C). Repeated examinations of her peripheral blood between 1995 and 1997 showed that her eosinophil counts fluctuated from 234/ μ l to 1,652/ μ l (normal, <400/ μ l), but it was enigmatic that the level of IgE was under detection level (<1 units/ml) at any of five times that she was examined.

The patient's alopecia was treated with topical steroid cream or lotion, but no therapeutic response was observed during the next 6 months. For her own personal reasons, she was lost to follow-up. According to her mother, however, her hair began to regrow 2 years later, and she completely recovered from alopecia by the time she reached the age of 20. Currently, at the age of 28 years, she has no recurrence and is in good health.

Cytokine expression in PBMCs

Because IgE production is controlled by IL-4,⁴ we investigated the production and expression of IL-4 by the patient's T cells. A flow cytometric analysis of her peripheral blood mononuclear cells (PBMCs) in March, 1996 contained the following populations:

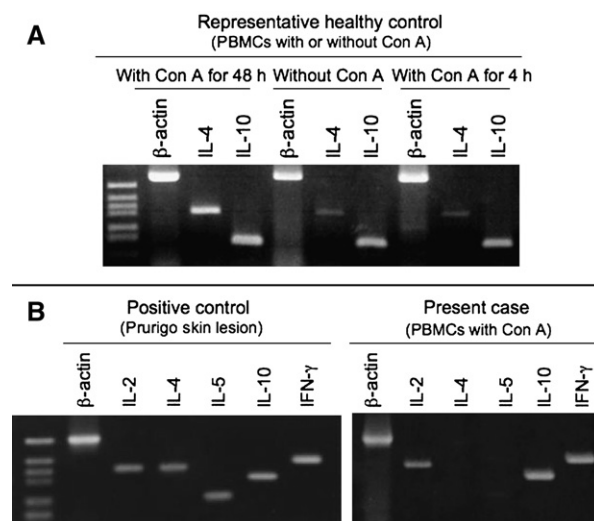


Fig 2. Cytokine mRNA expression pattern of the patient's lymphocytes. **A**, Normal individual's PBMCs, which were freshly isolated (without Con A) or cultured with 5 μ g/ml Con A for 4 or 48 hours, were subjected to RT-PCR. **B**, The patient's PBMCs were cultured for 48 hours and the expression of various cytokines were tested. As a positive control, a biopsy specimen from a skin lesion of prurigo subacuta was used because of its expression of all the cytokines tested.⁵ Total RNA was prepared from these samples, and a RT-PCR analysis was performed as described previously.^{5,6} The sizes of predicted base pairs were 661 for β -actin, 458 for IL-2, 456 for IL-4, 291 for IL-5, 352 for IL-10, and 501 for IFN γ .

CD3, 63%; CD4, 40%; CD8, 26%; CD7, 82%; CD20, 10%; CD56, 29%; and HLA-DR, 14%; indicating normal T cell (normal CD4/CD8 ratio) and B cell percentages and a slightly high natural killer cell percentage. When cultured in 96-well plates for 72 hours and tested in ³H-thymidine incorporation, the patient's PBMCs proliferated well in response to concanavalin A (Con A) at 5 μ g/ml (112,319 $\pm$ 2,761 cpm *vs* 1,841 $\pm$ 167 cpm of non-addition control). To see the production of IL-2 and IL-4, PBMCs were cultured (2×10^6 cells/well of 1.2 ml medium) in duplicate with Con A at 5 μ g/ml for 72 hours, and the concentration of IL-4 and IL-2 in the supernatants was measured by enzyme-linked immunosorbent assay. Although IL-2 was produced at a concentration of 22.6 units/ml, IL-4 was under detection level (<31 pg/ml). Because this undetectable level of IL-4 was the same as the level in normal healthy subjects, we investigated mRNA expression of IL-4 along with other cytokines in the following study.

PBMCs from the patient and healthy subjects were cultured with Con A at 5 μ g/ml for 48 hours and subjected to a reverse transcriptase polymerase chain reaction analysis, as described previously.^{5,6} As a positive control, we chose a skin lesion of

subacute prurigo, because the specimen expressed mRNA for all the cytokines tested.⁵ As represented by Fig 2, A, freshly isolated PBMCs (without Con A) from a normal control expressed mRNA for IL-4 as well as another Th₂ cytokine, IL-10. When cultured with Con A for 48 hours, the level of IL-4 expression was increased (with Con A for 48 hours). We examined the expression of IL-4 in 10 healthy controls and found that all the healthy subjects had positive IL-4 expression as reported previously.^{5,6} In the patient's PBMCs, however, while interferon- γ (IFN γ), IL-2, and IL-10 were positively expressed, no signal for IL-4 or IL-5 was detected (Fig 2, B). Although IL-5 message was also undetectable by this RT-PCR in PBMCs from normal individuals and even atopic patients, the absence of expression of IL-4 was definitely abnormal. Thus, the ability of T cells to produce IL-4 was impaired in this patient.

DISCUSSION

We diagnosed this patient as having alopecia universalis because of hair loss over the entire body, histologic and immunohistochemical infiltration of CD8⁺ T cells into the hair follicles, and the completely curable nature of the disease. The patient's alopecia was preceded by autosensitization dermatitis. Although the precise mechanism underlying these two sequential events remains unclear, T cells concerned with the eczematous reaction also might attack hair follicles. Alternatively, those T cells might sequentially activate another population of T cells cytotoxic to the hair follicle. In any case, the precedence of dermatitis implies that the patient's alopecia was caused by transiently-disordered T cell immunity.

Although our patient had a normal number of CD4⁺ T cells, their IL-4 production was impaired as assessed by RT-PCR analysis. The reduced production of IL-4 is strongly supported by the markedly low level of serum IgE. IL-4 is one of the cytokines produced by Th₂ cells. However, the patient had eosinophilia, which is induced by IL-5 overproduction,⁴ and her PBMCs expressed mRNA for IL-10 at a comparable level to a healthy subject. Thus, among Th₂ cytokines, IL-4 seems to be preferentially downmodulated, and this IL-4 decrease might be closely associated with the patient's baldness. The transcription factors GATA-3 and Stat6 play a central role in regulating Th₂ cell differentiation.⁷ It is possible that the signaling pathway involving these molecules was transiently-disordered in this patient.

Alopecia areata is regarded as a T-cell mediated, tissue-restricted autoimmune disease of the hair follicle. The predominant expression of Th₁ cytokines has been reported in this hair disorder,^{8,9}

although it is not necessarily consistent among studies.¹⁰ Because Th₁ cytokines stimulate CD8⁺ CTLs, Th₁ dominance is in accordance with immunological hair loss. Two possibilities may be put forward on the pathogenesis of this patient's alopecia. First, because IL-4 suppresses the function of Th₁ cells and CTLs,⁹ the decreased production of IL-4 may allow the CTL and its supporter Th₁ cells to attack hair follicles, as histologically and immunohistochemically seen in our case. In addition, the relative increase of IFN γ to IL-4 may augment the expression of MHC class I molecules, which are the target of CTLs.¹¹ Second, it is possible that IL-4 directly turns the hair cycle from telogen to anagen. Dermal papilla cells control the hair follicle growth through their elaboration of mitogenic factors and extracellular matrix components. With regard to cytokines capable of regulating hair growth, tumor necrosis factor- α , epidermal cell growth factor, IL-1 α , IL-1 β and transforming growth factor β 1 inhibit hair growth by downregulating dermal papilla cells,¹² while there has been no convincing report on hair growth-promoting cytokines. Only one study showed that IL-4 induces apoptosis in cultured human follicular keratinocytes, but not in dermal papilla cells.¹³

Taken together, these observations make it likely that in our patient the autoimmune hair loss occurred via activation of CTLs and Th₁ cells as a result of a relief from IL-4 control. Although we could not monitor chronological changes in the production/expression of T-cell cytokines throughout the clinical course, the spontaneous and complete recovery and the absence of recurrence provide a speculation that her T-cell immunological condition had been gradually normalized over the course of a couple of years. Our patient suggests that IL-4 plays an important role for hair growth in a certain condition.

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