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# E-selectin and Interleukin-2 Receptor α-chain Expression in Alopecia Universalis

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**A study of the histopathological abnormalities in a case of alopecia universalis was accompanied by immunohistochemical analysis of the expression of intercellular adhesion molecule-1 (ICAM-1) and E-selectin (formerly known as endothelial leukocyte adhesion molecule-1) within the skin. ICAM-1 expression on follicular epithelium co-localized with intraepithelial mononuclear cells (MNC) positive for the interleukin-2 receptor α-chain (IL-2R) or HLA-DR. Aberrant expression of E-selectin was observed on dermal endothelium. Although restricted to one case, these new observations concerning the expression of E-selectin and IL-2R in alopecia universalis are consistent with the view that extravascular trafficking of MNC into follicular epithelium may play a key role in the pathogenesis of alopecia universalis and that use of agents that interfere with this process may be an effective therapeutic strategy. *Key words: Adhesion molecules; T-cells.***

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Alopecia universalis (AU) is an idiopathic disorder, characterized by the loss of all body hair. An important component of the pathology of AU is a reduction in the capillary network of the hair bulbs that is required for normal hair growth (1). The association of AU with a variety of autoimmune disorders (2–5), as well as abnormal in vitro humoral and cell-mediated immune responses, suggests an immunologic etiology for the disease. Moreover, AU has been linked to the presence of HLA-DR5, which may determine disease severity in affected individuals (6).

Direct immunofluorescent examination of affected scalps has failed to demonstrate the presence of anti-follicular antibodies. However, ectopic expression of HLA-DR by epithelial cells in the presumptive cortex and root sheaths of hair follicles in active lesions has been reported (7). There is also evidence that follicular epithelial cells in alopecia areata express the adhesion molecule intercellular adhesion molecule-1 (ICAM-1) (8) which may be important in T-cell trafficking in the skin. In addition, a role for T-lymphocytes has been implicated by the observation that cyclosporin A (CsA) administration stimulates hair growth in AU (9, 10) and in AU skin grafts transplanted onto athymic nude mice (11). In this study, we examined the expression both of lymphocyte activation antigens and of the adhesion molecules ICAM-1 and E-selectin (formerly known as endothelial leukocyte adhesion molecule-1) in a case of AU.

## MATERIAL AND METHOD

### *Patient*

The patient is a 32-year-old female with a history of AU diagnosed 14 years ago. She was born with severe eczema that required hospitalization and extensive use of steroid-containing creams. As a child, her medical history was remarkable for infectious mononucleosis and a fever of undetermined origin. She first noted her hair falling out in clumps in December 1978, and within 2 months she was totally bald. Sparse regrowth of scalp hair was followed by the onset of hair loss from her arms, eyelashes and eyebrows. The fine black hair that returned was quite different from the original hair that was dark brown and thick. One year later the new hair began falling out in clumps and she consulted a dermatologist who recommended no therapy as regrowth of the hair was not anticipated. Very shortly, all of her hair was gone. A year and a half after the initial episode of hair loss a diagnosis of AU was made. Local steroid therapy with creams and injections was followed by growth of some white hair. After 8 months, all medications were discontinued and a naturopathic powder containing poison ivy resin was tried. This produced an extended body rash, followed by some regrowth of hair on the eyebrows and of eye lashes. She stopped the resin treatment at 3 months because of the severity of the rash and all of her hair again fell out. While she was pregnant in 1985 and 1986, hair started to regrow but within days of delivery, total alopecia returned.

### *Immunohistochemistry*

While the patient was attending an outpatient clinic at the University of Pittsburgh Medical Center in 1992, an elliptical biopsy of scalp skin was taken under local anaesthesia (lidocaine). The tissue was mounted in OCT mounting medium (Miles Inc, Elkhart, IN, USA), snap-frozen in arcton/liquid nitrogen and stored at −70°C until sectioned. Normal skin from healthy adult volunteers was examined also for purposes of comparison. Formalin-fixed and paraffin-embedded tissue was used for light microscopy. Vertical, 5-micron sections were stained with hematoxylin and eosin. Immunohistochemistry was accomplished using two-micron thick cryostat sections as described previously (12). Sections were fixed in acetone for 5 min, air dried, then incubated for 1 h at room temperature with predetermined optimal dilutions of appropriate primary mouse IgG anti-human monoclonal antibodies directed against CD4 or CD8 (Becton Dickinson, San Jose, CA), CD25 (IL-2R α chain) (Dako Corp., Carpinteria, CA), HLA-DR (Becton Dickinson), ICAM-1 (Gen Trak Inc., Plymouth Meeting, PA) or E-selectin (Biosource Int., Camarillo, CA). The sections were incubated for 30 min with biotin-conjugated horse anti-mouse IgG, diluted 1:200 in 4% v/v normal human serum in Dulbecco's phosphate-buffered saline (PBS) and then treated for 30 min with avidin biotin complex peroxidase diluted in PBS. The color reaction was developed for 6 min, using a peroxidase chromogen kit (AEC; Biomedia Corp., Foster City, CA) according to the manufacturer's directions. Negative controls included the omission of primary antibody and the use of irrelevant antibody subclass control reagents. Sections were counterstained lightly with hematoxylin. Positively stained cells were examined at ×400 magnification in a minimum of five sequential grid fields.

## RESULTS AND DISCUSSION

The most prominent histological abnormalities, compared with normal skin, were changes in the structure of the hair follicles, with loss of openings, and thickening of the walls of small

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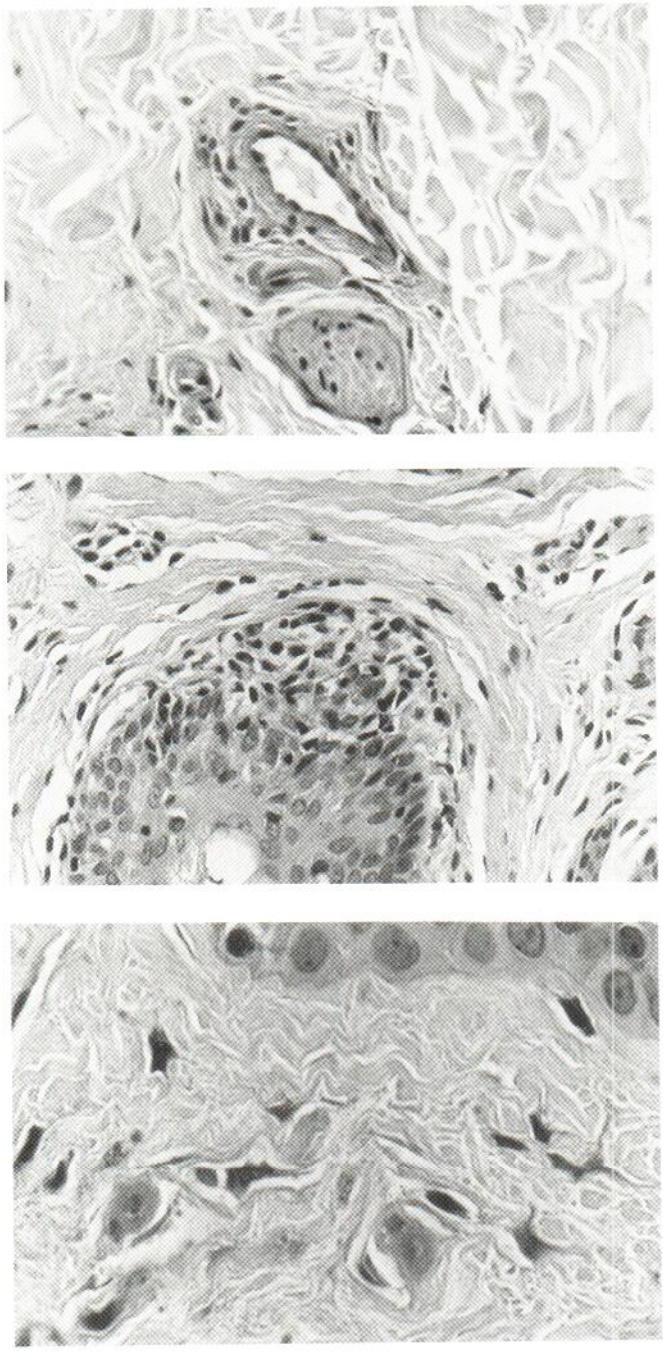

*Fig. 1. (a)* Mononuclear cell infiltration and thickening of the wall of a small vein in the upper dermis (×1,200). *(b)* Mononuclear cell infiltration and localized disruption of a hair follicle; both connective tissue and corresponding epithelium are affected (×1,200). *(c)* Prominent and numerous cells with dendritic morphology in the upper dermis. A mononuclear cell within the epidermis is also evident (×3,000).

vessels, accompanied by moderate inflammation. All hair follicles demonstrated an absence or abnormalities of hair tissue (both cortex and medulla). All hair follicles and most dermal papillae showed a decrease in the amount or absence of vessels, especially capillary loops. Similar observations compared with normal scalp have been reported previously (13, 14). The remaining vessels (mostly veins and capillaries) demonstrated thickening of the wall and mononuclear cell infiltration (Fig. 1a). Connective tissue surrounding some vessels had degenerated. The mononuclear cell infiltrate of the epidermis and follicles was comprised characteristically of scattered lymphocytes (Fig. 1b), which did not show affiliation with any particular cell type. Some cells at the level of the dermal papillae exhibited distinct dendritic cell morphology (Fig. 1c).

$CD4^+$ T (helper) cells which outnumbered $CD8^+$ (T cytotoxic/suppressor) cells by about 5:1 were present within the epithelium of the hair follicles, as well as in the perifollicular matrix (Fig. 2a). There were also abundant follicular and perifollicular $HLA\text{-}DR^+$ cells (Fig. 1b); a minority of these cells were $IL\text{-}2R^+$ (Fig. 1c). E-selectin staining (Fig. 2d) was evident on dermal endothelial cells, but not in follicles (Fig. 2e), whilst strong expression of ICAM-1 (Fig. 2f) was observed both on follicular epithelium and also on endothelial cells. In psoriatic skin, studied for comparison, there were abundant $CD4^+$ (predominantly) and $CD8^+$-cells in the dermis and epidermis; $IL\text{-}2R^+$ MNC were also evident, particularly in the epidermis. Strong HLA-DR staining was seen on skin-infiltrating MNC and on vascular endothelium, which stained intensely for ICAM-1 and E-selectin. Patchy ICAM-1 staining of basal keratinocytes was also noted. A few, very isolated MNC were detected in normal skin; adhesion molecule staining was absent.

The histopathological changes in this patient with long-standing AU reflect the duration of the disease and correlate well with previous observations (15–17), although the decrease in density of capillary loops and the appearance of cells with dendritic morphology have not usually been described. The peribulbar infiltration of $CD4^+$ T cells and of $HLA\text{-}DR^+$ and $IL\text{-}2R^+$ cells was associated with aberrant follicular epithelial cell staining for ICAM-1 and endothelial cell E-selectin expression. The co-localization of follicular epithelium ICAM-1 expression with intraepithelial T-cells in AU is in agreement with a previous report concerning alopecia areata (9). Upregulation of adhesion molecule expression is found invariably in inflammatory responses. Our novel finding of $IL\text{-}2R^+$ intraepithelial cells in association with ICAM-1 and E-selectin expression extends previous observations and adds credence to the view that ICAM-1 and HLA-DR on epithelial cells may be induced secondarily by cytokines derived from skin-infiltrating, activated T-cells. The therapeutic implication of these findings is that agents such as CsA and FK 506, which directly inhibit T-cell activation (and consequently cytokine production and adhesion molecule expression) (18) may prove useful in disease management, especially if available as an effective topical formulation (11, 19, 20) for human use.

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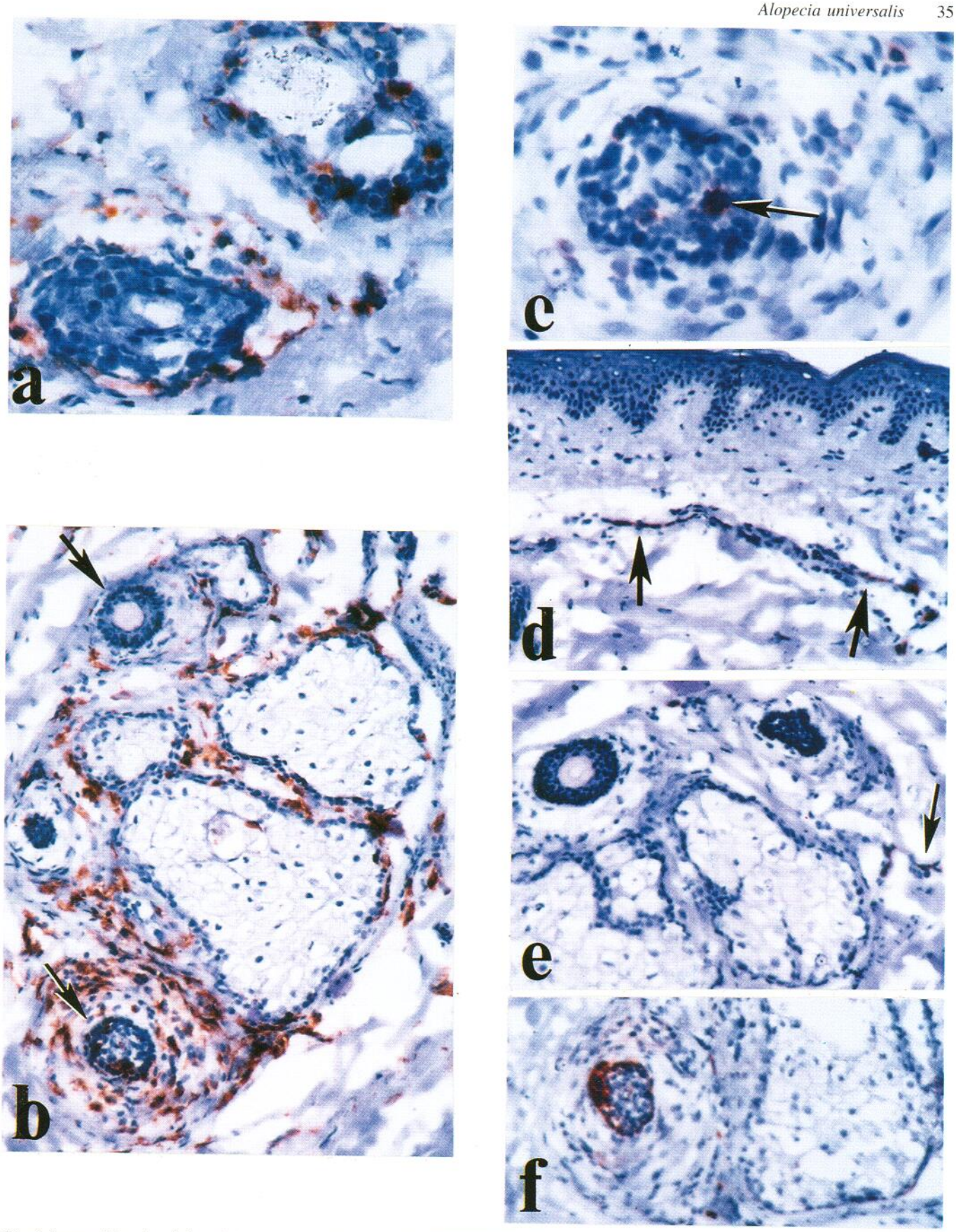


*Fig. 2.* Immunohistochemical analysis of lesional skin: *(a)* $CD4^+$ mononuclear cells within hair follicles and in the surrounding dermal matrix; *(b)* follicular and perifollicular HLA-DR staining, showing positive cells within a disorganized hair follicle *(lower arrow)* and also on capillary endothelium; *(c)* $IL\text{-}2R^+$ cells *(arrow)* within a hair follicle; *(d)* and *(e)* E-selectin staining endothelial cells *(arrows)* in adjacent fields; *(f)* ICAM-1 staining of cells within an inflamed hair follicle. *(a, c)* ×1,200; *(b, d–f)* ×500.

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