HLA-DR Expression by Hair Follicle Keratinocytes in Alopecia Areata: Evidence That it Is Secondary to the Lymphoid Infiltration*

Emilio L. Khoury, M.D., Vera H. Price, M.D., and John S. Greenspan, B.D.S., Ph.D.
Departments of Stomatology (ELK, JSG) and Dermatology (VHP), University of California at San Francisco, and Kaiser Permanente Medical Center (VHP), San Francisco, California, U.S.A.

There is evidence suggesting that alopecia areata (AA) may have an autoimmune pathogenesis, and it was recently reported that keratinocytes in the bulb of some hair follicles affected by this condition express class II HLA (HLA-DR) antigens, which are not present on the same cells in normal tissue. Since it has been proposed that an analogous ectopic HLA-DR expression by epithelial cells in other organs might be an early event leading to organ-specific autoimmunity, we have investigated the sequence in which perifollicular mononuclear cell (MNC) infiltration and ectopic HLA-DR expression on keratinocytes appear in recent-onset and longstanding cases of AA by immunostainings of affected and unaffected areas with monoclonal antibodies against leukocyte and HLA-DR antigens.

In recent-onset AA lesions, ectopic HLA-DR expression on hair follicle keratinocytes was found only occasionally (in 3 out of 247 follicles examined) and was restricted to biopsies from the affected areas. This prevalence was significantly lower than the prevalence of hair follicles showing perifollicular MNC infiltrates in the same biopsies, and was also significantly lower than the prevalence of hair follicles showing ectopic HLA-DR expression on keratinocytes in the affected areas of longstanding cases. These findings suggest that in AA lesions the perifollicular MNC infiltration precedes the ectopic HLA-DR expression on hair follicle keratinocytes, and therefore argue against the notion of a primary role for that ectopic HLA-DR expression on epithelial cells in triggering the putative autoimmune response in AA. J Invest Dermatol 90:193–200, 1988

Although the identity of the specific target(s) in the affected hair follicles has not yet been elucidated, there is considerable evidence suggesting that the pathogenesis of alopecia areata (AA) may include autoimmune mechanisms [1–3]. Messenger and associates [4] have recently reported the ectopic expression of HLA-DR antigenic determinants by epithelial cells in the presumptive cortex and root sheaths of hair follicles in active lesions of AA. This same ectopic or aberrant HLA-DR expression has also been found in other human epithelial cells that are the target of better-defined organ-specific autoimmune diseases, particularly those affecting the thyroid gland [5], leading to the hypothesis [6] that an aberrant HLA-DR expression by normally HLA-DR-negative cells might confer on them the ability to efficiently present their own specific surface antigens to sensitized, MHC-restricted T inducer cells, which then, by recognizing those determinants in conjunction with self class II MHC specificities, would initiate an autoreactive immune response. Epithelial cells can also express HLA-DR determinants, however, when they are the target of alloimmune responses, in transplant rejection [7–9] and graft-versus-host reactions [10–12], even though in those cases the epithelial cells are presumably devoid of any primary abnormality.

Furthermore, since the ectopic HLA-DR expression in autoimmune conditions has been found in tissues already having significant lymphoid infiltration [13], a basic question is whether the aberrant HLA-DR expression by epithelial cells represents the initial event triggering the autoimmune response or, conversely, whether it is the immune response, through the autoreactive lymphoid infiltrates and local release of lymphokines, that induces the target epithelial cells to express those class II MHC antigens [14,15]. Alopecia areata offers a unique model to explore these alternative possibilities, because of the obvious appearance of the clinical lesions, prompting the patient to an early consultation and diagnosis, and the accessibility of the affected tissues. Therefore, in order to address that question we have investigated the sequence in which the perifollicular mononuclear cell (MNC) infiltration and the ectopic HLA-DR expression by hair follicle keratinocytes occur in vivo, as detected by monoclonal antibodies (MoAbs) in indirect immunofluorescence (IFL) stainings of frozen biopsies from recent-onset and longstanding AA lesions.
MATERIALS AND METHODS

Patients Two groups of 5 patients each, with either recent-onset (1–5 months) or longstanding (1.5–12 years) patchy lesions of AA (Table I) were biopsied after giving informed consent. None of the patients had received any systemic or topical treatment in the 3 months before the biopsies were obtained.

Biopsies Two 4-mm punch biopsies, one from the edge of an active patch of AA and the other from an unaffected area (or less affected area in the case of patients with more confluent long-standing lesions), at least 5 cm away from the biopsied affected area, were obtained simultaneously from each patient. The biopsies were immediately snap-frozen in liquid nitrogen and sectioned transversely in their entirety with a cryostat (Slee III, Slee Medical Equipment, London, England). Consecutive 5-μm sections (350–400 from each biopsy) were placed, similarly oriented, on numbered microscope slides and stored at −70°C until stained. Every 30–40 sections, one was stained with haematoxylin-eosin (H & E), in order to assess histologic features regarding depth level in the biopsy, number and appearance of hair follicles, other structures in the tissue, and the presence of mononuclear cell (MNC) infiltrates. These histologic characteristics were assessed in all biopsies without prior knowledge of their lesional or nonlesional origin.

Monoclonal Antibodies (MoAbs) Three commercially available, purified MoAbs (from Becton-Dickinson Monoclonals, Inc., Mountain View, California) were employed throughout this study, at a concentration of 20 μg/ml: (1) HLe-1 (from clone 2D1), which recognizes a cell-surface antigenic determinant common to all human leukocytes [16]; (2) HLA-DR (from clone L243), directed against a nonpolymorphic determinant in HLA-DR molecules [17]; (3) Leu 10 (from clone SK10), which reacts with an epitope present in HLA-DQw1 and HLA-DQw3 molecules [18]. In some of the stainings, an additional mouse IgG MoAb (Leu 6, from Becton-Dickinson), which on normal skin reacts only with epidermal Langerhans cells [19], was used.

Indirect Immunofluorescence (IFL) Staining Unfixed cryostat sections (3 with each MoAb), consecutive to those showing maximal MNC infiltration when stained with H & E and having at least 10 hair follicles, were used for the IFL stainings. In those biopsies from the unaffected area of recent-onset cases in which no perifollicular MNC infiltrates could be detected in the initial screening, IFL stainings were performed on sections from the same level as that used in the biopsy from the affected area of that particular patient. The sections were incubated for 30 min at room temperature in a humidified chamber with the prediluted MoAbs. After 2 washes in PBS (5 min each), the sections were incubated with FITC-labeled F(ab′)2 fragments of goat anti-mouse IgG (Cappel, Malvern, Pennsylvania), diluted 1:40, under similar conditions. After 2 additional washes, the sections were mounted in p-phenylene diamine in buffered glycerol and examined under a Zeiss fluorescence photomicroscope (Carl Zeiss, New York, New York) equipped with epilumination and phase contrast. Structures in the hair follicles and surrounding tissue were identified under phase-contrast microscopy, and then both MNC infiltration and ectopic HLA-DR expression on hair follicle keratinocytes in each follicle present in the section were graded according to the number of cells positively stained by each one of the MoAbs as none, light, moderate, or heavy.

Indirect immunofluorescence was far more sensitive than H & E staining for the detection of MNC infiltrates, probably due to the much higher contrast of the IFL-positive cells against the dark background of unstained tissue, and to better preservation of antigenicity than morphology in the unfixed cryostat sections. Thus, some specimens without noticeable MNC infiltration when initially screened with H & E, showed a distinct IFL staining of light or even moderate perifollicular infiltrates.

Investigation of Associated Autoantibodies To investigate the presence of well-defined circulating autoantibodies, a blood sample was obtained from all 10 patients with AA at the time of the scalp biopsies. Detection of organ-specific (i.e. thyroid microsomal/microvillar [TMA], gastric parietal cell [GPCA], adrenocortical cell as well as pancreatic islet cell) autoantibodies and non-organ specific (i.e. nuclear [ANA], smooth muscle [SMA], mitochondrial, ribosomal, and reticulin) autoantibodies was performed by indirect IFL on cryostat sections of human thyroid, stomach, adrenal and pancreas, rat liver and kidney, and mouse stomach, as described above. Initial serum dilution for the tests was 1:4 on human tissues and 1:10 on animal tissues. FITC-labeled F(ab′)2 fragments of goat anti-human Ig G,M,A (Cappel), diluted 1:40, were used as the second antibody. Appropriate positive and negative control sera were also included in the tests.

Statistical Analysis The comparison of the proportions of hair follicles showing MNC infiltration and those showing ectopic HLA-DR expression on epithelial cells in each biopsy was performed using paired Student’s t tests. These were done separately for the affected and unaffected areas in each category (i.e., recent-onset

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Sex/Age</th>
<th>Episode</th>
<th>Duration (months)</th>
<th>Scalp Patches (n)</th>
<th>Associated Autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/19</td>
<td>1st</td>
<td>2</td>
<td>2</td>
<td>ANA 1:10; SMA 1:40</td>
</tr>
<tr>
<td>2</td>
<td>F/39</td>
<td>1st</td>
<td>5</td>
<td>1</td>
<td>SMA 1:20</td>
</tr>
<tr>
<td>3</td>
<td>F/32</td>
<td>1st</td>
<td>4</td>
<td>2</td>
<td>ANA 1:20; GPCA 1:20</td>
</tr>
<tr>
<td>4</td>
<td>F/22</td>
<td>1st</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M/34</td>
<td>1st</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Sex/Age</th>
<th>Episode</th>
<th>Duration (years)</th>
<th>Scalp Hair Loss (%)</th>
<th>Associated Autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>F/21</td>
<td>1st</td>
<td>1.5</td>
<td>51–75</td>
<td>ANA 1:20; GPCA 1:40</td>
</tr>
<tr>
<td>7</td>
<td>M/15</td>
<td>1st</td>
<td>1.5</td>
<td>26–50</td>
<td>SMA 1:40</td>
</tr>
<tr>
<td>8</td>
<td>M/28</td>
<td>1st</td>
<td>1.5</td>
<td>26–50</td>
<td>GPCA 1:80</td>
</tr>
<tr>
<td>9</td>
<td>M/25</td>
<td>2nd</td>
<td>5</td>
<td>26–50</td>
<td>ANA 1:20; GPCA 1:1280; TMA 1:160b</td>
</tr>
</tbody>
</table>

* This patient (as well as her father) had overt hypothyroidism.

* This IFL titer is roughly equivalent to a titer of 1:25,000 by hemagglutination [20].

ANA, antinuclear antibodies; SMA, smooth muscle antibodies; GPCA, gastric parietal cell antibodies; TMA, thyroid microsomal/microvillar antibodies.
and longstanding cases). Binomial tests were also used to assess whether the proportions of patients in each subgroup showing a prevalence of ectopic HLA-DR expression lower than that of perifollicular MNC infiltration differed from chance.

RESULTS

Table II contains the quantitative data from all 20 biopsies examined and their statistical significance. Results from the five patients in each group (i.e., recent-onset and long-standing cases) have been combined. When considered individually, all 20 biopsies showed a proportion of hair follicles with MNC infiltration which was higher than the proportion of follicles having ectopic HLA-DR expression on keratinocytes.

Recent-Onset Cases Biopsies from the affected area of recent-onset cases displayed perifollicular and/or intrapapillary MNC infiltrates (Fig 1a) in a significant proportion (34.9%) of hair follicles, although in most cases (40 of 43 follicles) the infiltration was only light or moderate in intensity. Moreover, the two biopsies in this group having occasional follicles with heavy MNC infiltration were from patients with the longest duration of the disease within this group (5 and 4 months, respectively). All the infiltrated MNC were positively stained by the HLE-1 anti-human leucocyte MoAb (Fig 1b), and the vast majority of these cells also expressed HLA-DR antigens on the cell surface (Fig 2). In contrast with the strong IFL staining obtained on perifollicular/perivascular MNC infiltrates with HLE-1 MoAb, only occasional intraepithelial Langerhans cells were clearly labeled by this MoAb.

In these biopsies HLA-DR expression on the hair follicle epithelium was almost completely restricted to Langerhans cells (Fig 2) and only a minor proportion (2.5%) of follicles displayed a focal ectopic expression, of light or moderate intensity, of these class II antigens on keratinocytes in the vicinity of MNC infiltrates. The difference between the prevalence of hair follicles having MNC infiltration and that of follicles with ectopic HLA-DR expression on keratinocytes (34.9% vs. 2.5%) was highly significant (p < 0.003). Here again, the biopsy from the patient with the longest duration of disease within this group (#3) had 2 of the 3 hair follicles where ectopic HLA-DR expression on keratinocytes was detected in the entire group.

Biopsies from unaffected areas of the same cases, on the other hand, showed a low prevalence (10.6%) of hair follicles with infiltrated MNC, and in all instances these infiltrates were light in intensity (scattered MNC around the follicle, either singly or in small clusters). This difference was sufficient to allow the correct identification of the site of the biopsy examined (i.e., whether from the affected or unaffected area) in all five patients, at the time of the screening of H & E-stained sections. Furthermore, in biopsies from the unaffected area of these 5 early AA cases, none of the 130 hair follicles examined showed any ectopic HLA-DR expression on keratinocytes.

Longstanding Cases Immunofluorescence stainings with HLE-1 MoAb on biopsies from both affected and unaffected areas of longstanding AA cases showed significant proportions of hair follicles with various degrees of perifollicular MNC infiltration (50.8% and 24.8% of the examined follicles, respectively). Furthermore, 33/173 (19.0%) of the hair follicles in biopsies from affected areas and 15/217 (6.9%) of those from unaffected areas had either moderate or heavy MNC infiltrates (Table II). This histopathologic involvement of clinically unaffected (or, at least, less affected) areas prevented identification of the site from which some of the biopsies had been obtained, during the initial blind screening with H & E.

In this group, ectopic HLA-DR expression by follicular keratinocytes having either light, moderate or heavy intensity (Fig 3) was detected in 20/164 (12.1%) of hair follicles in biopsies from affected areas and in 7/178 (3.9%) of those from unaffected areas. Here again, these prevalences were significantly lower than the fractions of follicles having some degree of MNC infiltration in the same biopsies (p < 0.009 for biopsies from affected areas and p < 0.076 for those from unaffected areas).

The presence of moderate and/or heavy ectopic HLA-DR expression on follicular keratinocytes proved to be a more discriminating indicator than perifollicular MNC infiltration to distinguish between the biopsies originating from affected versus unaffected areas within this group. Thus, whereas 11/164 (6.7%) of the follicles showed either moderate (7 follicles) or heavy (4 follicles) ectopic HLA-DR expression in the biopsies from affected areas, only 2/178 (1.1%) of the follicles showed moderate expression in those obtained from unaffected areas (p < 0.039). Moreover, this increased ectopic HLA-DR expression in the biopsy from the affected area was observed in all five individual patients.

Ectopic Expression of HLA-DQ Antigenic Determinants by Hair Follicle Keratinocytes Neither HLA-DR - negative nor HLA-DR - positive hair follicle keratinocytes showed any significant IFL staining with Leu 10 MoAb, and only those follicles with a heavy HLA-DR expression in biopsies from longstanding AA cases displayed a faint reactivity on the epithelial cell-surface (Fig 4). The fact that perifollicular lymphoid infiltration and ectopic HLA-DR expression by epithelial cells correlated with each other (Table II).
Figure 1. Consecutive cryostat sections of a scalp biopsy obtained from the affected area of a recent-onset AA (#2) case. a, Hematoxylin-eosin staining, showing a moderate mononuclear cell (MNC) infiltrate between 2 hair follicles. b, Similar field to that in (a), stained by indirect immunofluorescence (IFL) with the monoclonal antibody (MoAb) anti-human leukocytes (HL-1, 20 μg/ml), followed by fluorescein-labeled F(ab')2 fragments of goat anti-mouse IgG. All the infiltrated MNC show a positive IFL staining. Original magnification X 200.

Figure 2. Indirect immunofluorescence (IFL) with anti-HLA-DR MoAb (20 μg/ml) on a cryostat transverse section of a scalp biopsy obtained from the affected area of a recent-onset AA (#2) case. a, Phase-contrast microscopy of a hair follicle (left), showing the hair root (HR), Huxley's (Hu), and Henle’s (He) layers of the inner root sheath, the outer root sheath (ORS), and the connective tissue sheath (CT). The perifollicular MNC infiltrate is on the right. b, Same field as in (a), showing positive IFL staining on the infiltrated MNC and one Langerhans cell (arrow) in the outer root sheath of the hair follicle. Follicular epithelial cells are negative. Original magnification X 300.

shown to be similar to that of HLA-DR molecules expressed by immunocompetent cells [25]. Moreover, ectopic HLA-DR molecules on thyroid epithelial cells have been reported to be able to provide the required signal for recognition by, and activation of, MHC-restricted T inducer/helper lymphocytes specific for foreign antigenic determinants associated with these HLA-DR molecules on the cell surface [26]. In this context, it has been proposed that ectopic HLA-DR expression by endocrine and epithelial cells [5,27] might be an early event triggering autorecognition and the immune-mediated attack against self constituents on the cell surface [6,28,29]. According to this hypothesis, the sequence of events would be as follows: (1) a viral or other environmental agent provokes the local production and release of gamma interferon by T cells sensitized to that particular agent, at the site of encounter; (2) gamma interferon, in turn, induces the ectopic expression of HLA-DR antigens on normally HLA-DR negative epithelial cells; (3) these cells are now able to present their own self antigens to preexisting autoreactive T lymphocytes and thus to initiate the cascade leading to autoimmune tissue damage. The alternative possibility would be that ectopic HLA-DR expression is, rather, a consequence of the autoreactive MNC infiltration and local release of gamma interferon [14,30].

Alopecia areata, an organ-specific disorder thought to involve autoimmune mechanisms and characterized by the presence of peri-

Circulating Autoantibodies. Low or moderate titers of organ-specific and non-organ-specific autoantibodies were detected in the serum of several patients in both recent-onset and longstanding AA groups (Table 1). In addition, one of the longstanding patients (#10) showed clinical and serologic evidence of organ-specific autoimmune disease. She, as well as her father, had hypothyroidism associated with high titers of TMA (1:160 by IFL, which is roughly equivalent to 1:25,000 by the standard hemagglutination test [20]) and GPCA (1:1,280 by IFL).

DISCUSSION

In the normal human epidermis the presence of HLA-DR antigens is restricted to bone marrow-derived Langerhans cells [21]. Epidermal keratinocytes, however, are also able to synthesize and express these cell surface determinants in abnormal situations characterized by mononuclear cell (MNC) infiltration and cell-mediated immune processes [10,12,22–24]. Although the role that ectopic class II HLA antigens may play in these conditions is not clear as yet, the structure of the molecules bearing those determinants has been
folicular/perivascular MNC infiltrates, was recently shown to provide another example of ectopic HLA-DR expression by the presumptive cell target of the immune-mediated process \[4,31\]. The purpose of this study was, therefore, to determine the sequence in which perifollicular MNC infiltrates and ectopic expression of HLA-DR on follicular keratinocytes appear in AA lesions. Results presented here strongly suggest that the perifollicular infiltration by lymphocytes and macrophages precedes the ectopic HLA-DR expression on hair follicle keratinocytes. In recent-onset cases, ectopic HLA-DR expression was found only occasionally (in 3 out of 247 follicles examined), and only in the two patients with the longest duration of disease within this group. Furthermore, it was restricted to the biopsies from the affected areas, in which a much higher proportion (34.9%) of the follicles already showed

**Figure 3.** Indirect immunofluorescence with anti-HLA-DR MoAb (20 \( \mu g/ml \)) on cryostat sections of scalp biopsies obtained from the affected area of three longstanding AA (#8, 7, and 10) cases. \( a \), Phase-contrast microscopy, showing a hair follicle cross-sectioned at the papilla level; the papilla (PAP) is surrounded by the presumptive cortex (PC) and outer root sheath (ORS). The perifollicular MNC infiltrate is on the right. \( b \), Same field as in \( a \), showing positive IFL staining on MNC in the perifollicular infiltrate and within the papilla. In this case, epithelial cells in the layers adjacent to the MNC infiltrate also display cell-surface IFL staining, due to (light) ectopic HLA-DR expression. \( c \), Phase-contrast microscopy, showing a hair follicle cross-sectioned at the suprapapillary level with occasional MNC (arrows) infiltrating the presumptive cortex. \( d \), Same field as in \( c \), showing (moderate) ectopic HLA-DR expression on the surface of epithelial cells in the presumptive cortex. \( e \), Phase-contrast microscopy, showing a hair follicle cross-sectioned approximately midway between epidermis and papilla. The hair root (HR), inner root sheath (IRS), and outer root sheath (ORS) are seen. \( f \), Same field as in \( c \), showing (heavy) ectopic expression of HLA-DR antigens on the surface of most epithelial cells in the ORS. Original magnification: \( a-d \times 500; e, f \times 300 \).
26% and 50% of the scalp, also had overt hypothyroidism associated with extremely high titers of thyroid microsomal and gastric parietal cell autoantibodies. The biopsy from the affected area of this patient’s scalp showed ectopic HLA-DR expression by keratinocytes in only 2 of 22 (9%) hair follicles, whereas perifollicular MNC infiltrates were present in 13 of 24 (54%) follicles. Moreover, in the biopsy from an unaffected area, none of 29 follicles screened displayed any ectopic HLA-DR on keratinocytes.

Taken together, these findings argue against the notion of a primary role for the ectopic expression of HLA-DR antigens on hair follicle keratinocytes in triggering the putative autoimmune response in AA and suggest that this ectopic expression is the consequence of the lymphoid infiltration and the local release of lymphokines, as seen in experimentally induced contact hypersensitivity lesions [34]. Furthermore, it has recently been reported that ectopic HLA-DR expression by keratinocytes is a common feature in a wide variety of dermatoses characterized by infiltration of activated T lymphocytes [35]. In that study, comprising 52 different skin disorders (AA was not included), 38 of which showed some degree of ectopic HLA-DR expression, HLA-DR+ keratinocytes were never observed in the absence of, or preceding, the dermal infiltrates [35]. This sequence of events represents the in vivo counterpart of the recently reported in vitro induction of HLA-DR expression on human keratinocytes cocultured with mitogen-activated MNC [25], and of our own findings concerning the induction of HLA-DR expression on human thyroid epithelial cells from normal and autoimmune glands when these cells are cocultured with autologous MNC from peripheral blood or from the intrathyroidal infiltrate, respectively [36]. Studies in vitro have shown that gamma interferon is the lymphokine responsible for the induction of HLA-DR expression on both epidermal keratinocytes [25,37,38] and thyroid epithelial cells [39]. Previous reports [40,41] and our own unpublished results indicate that the large majority of the MNC in the perifollicular infiltrates of AA lesions are activated T lymphocytes, which constitute the main cellular source of gamma interferon [42].

The ectopic HLA-DR expression by keratinocytes in the precoronal matrix and presumptive cortex of the hair bulb in AA lesions has been interpreted to suggest that these cells are the primary target in the disease [31,43]. Although this may well be the case, an alternative possibility is that ectopic HLA-DR expression is the nonspecific result of the local diffusion of gamma interferon from the lymphoid infiltrate. This would be supported by our relatively frequent finding of ectopic HLA-DR expression on keratinocytes in the outer root sheath, which is a direct continuation of the malpighian layer of the epidermis [44], provided that perifollicular MNC infiltrates were present in the vicinity. Furthermore, the presence of ectopic HLA-DR antigens on hair follicle keratinocytes in a case of discoid lupus erythematosus [24] suggests that keratinocytes might be induced to a greater or lesser extent according to the concentration of gamma interferon [25] at that site and their degree of inducibility, irrespective of whether or not they are the specific target of the cell-mediated immune response.

As previously reported in other skin disorders characterized by ectopic HLA-DR expression on keratinocytes [23], HLA-DR+ follicular keratinocytes in the AA lesions showed no significant expression of specific HLA-DQ antigens. A differential expression of HLA-DR, -DQ and -DP antigens by human keratinocytes has also been found after experimental induction of contact hypersensitivity and irritant contact dermatitis [45].

Regardless of the mechanism and timing of its induction, ectopic HLA-DR expression by hair follicle keratinocytes might be expected to enable them to function as (auto) antigen-presenting cells and to activate MHC-restricted T helper lymphocytes, especially in view of the capacity of keratinocytes to produce the second signal for T cell activation (i.e., interleukin-1-like epidermal cell-derived thymocyte activating factor [ETAF; 46,47]). This, in turn, would enhance and perpetuate the putative autoimmune response or, for that matter, any cell-mediated immune response against foreign antigenic determinants displayed on the epithelial cell surface, which might be operating in AA. In a recent study, however,
Breathnach and associates [48] have shown that ectopic expression of IA (the murine counterpart of HLA class II antigens) by epidermal keratinocytes from mice undergoing GVHD does not confer to those IA* keratinocytes antigen-presenting capacity for either allo- specific cell-surface determinants, soluble antigens or antigenic peptide fragments. Moreover, that reported failure of IA* keratinocytes to activate alloreactive lymphocytes or antigen-specific T cell lines and hybridomas was not a consequence of inactivity by the epidermal cells to secrete IL-1/ETAF.

On the other hand, ectopic IA expression on keratinocytes of nude BALB/c mice, induced by the adoptive transfer of mononuclear leukocytes from normal semisynthetic animals, has been correlated with the infiltration of donor Langerhans cells into the host epidermis [49]. In the present study, a precise quantification of Langerhans cells within and around hair follicles was not attempted, since the MoAbs systematically employed in the immunostaining, directed against HLA class II and leucocyte common determinants, were therefore not specific for Langerhans cells. A noticeable hyperplasia of intraepithelial Langerhans cells, however, was detected in the hair follicles of one of the longstanding cases (#9), confirming previous observations of occasionally increased numbers of Langerhans cells in this condition [31,41].

The skilful technical assistance of Ms. Eileen L. Wong is gratefully acknowledged. We also thank Dr. Alan Bestrom for statistical analysis.

REFERENCES


