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Epidermal Langerhans' Cells in Patients with Pustulosis Palmoplantaris Treated with Etretinate or Etretinate + Methoxsalen Photochemotherapy

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Epidermal Langerhans' cells (LC) were studied in patients with pustulosis palmoplantaris (PPP) by utilizing the monoclonal antibodies anti-Leu 6 and anti-HLA-DR in combination with an immunoperoxidase technique. In non-pustular areas of the skin lesions in PPP, an increased number of epidermal LC was found compared with control subjects. No change in LC counts was observed following etretinate monotherapy for 2 weeks. Etretinate was then combined with PUVA treatment of one palm/sole with the contralateral side as a non-UVA exposed control. After 6–12 weeks of etretinate + PUVA treatment the PPP had resolved and the number of epidermal dendritic HLA-DR$^+$ and Leu 6$^+$ cells had normalized. On the contralateral side, etretinate treatment induced a marked clinical improvement and a reduction of HLA-DR$^+$ cells. The observation of an increased LC population in active PPP and a reduction during clinical improvement indicates a close relationship between LC and the activity of PPP. (Received November 9, 1987.)

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Pustulosis palmoplantaris (PPP) is a chronic recalcitrant skin disease characterized by erythema, desquamation and eruptive sterile pustules of the palms and soles (1). The cause of PPP is unknown but the existence of stratum corneum antibodies and chemotactic and phagocytic abnormalities of circulating polymorphonuclear leukocytes suggests an immunological pathogenesis (2, 3, 4). Despite the fact that Langerhans' cells (LC) play a crucial role in the afferent phase of the cutaneous cellular immune response by presenting foreign antigens to T-lymphocytes, the LC have not previously been studied in PPP (5).

Langerhans' cells are dendritic cells which constitute 2–4% of all epidermal cells. The number varies somewhat between different body regions, with relatively low values on palms and soles (6, 7). LC are the only epidermal cells regularly expressing class II antigens encoded for in the major histocompatibility complex. In addition, LC can be identified by monoclonal antibodies raised against the cell surface antigen T6 or by ATPase staining (8, 9).

Various treatment modalities which improve PPP are known to interact with the epidermal LC population. For example, photochemotherapy (PUVA) reduces the number of T6 expressing cells in the epidermis of normal as well as of psoriatic skin (10, 11). Furthermore, etretinate normalizes the number of LC in the psoriatic epidermis (12, 13). The stimulation of lymphocytes and the antichemotactic effect on granulocytes attributed to this drug may also contribute to the positive therapeutic effect of etretinate in PPP (14, 15).

With this background in mind, it is important to understand the role of the LC in the pathogenesis of PPP. In the present study we examined the number of T6 and class II antigen (HLA-DR) presenting LC in PPP and evaluated the effect of etretinate and etretinate + PUVA (Re-PUVA) on this cell population.

PATIENTS AND METHODS

PPP patients. Six men and 14 women with a mean age of 53 years (range 19–72 years) were studied. All patients had a symmetrical pustular eruption on palms and/or soles. The mean duration of the PPP was 9 years (range 0.5–26 years). Ten patients had or had had small patches of psoriasis vulgaris on the rest of the body.

Treatment. The PPP patients in this study participated concurrently in a therapeutic trial (16). Any topical or systemic treatment for PPP except emollients was discontinued 4 weeks before the start of this study.

Etretinate (Tigason®, Roche) was administered in a dose of 0.6 mg/kg body weight/day, throughout the trial. After 2 weeks of etretinate, one hand or foot received PUVA treatment three times a week for 6–12 weeks. 8-methoxypsoralen (Puvamet®, Draco) was given in a dose of 0.6 mg/kg body weight 90 min before irradiation (Waldmann 180+200). The UVA dose was increased at each treatment session by 5–10 kJ/m^2.

The severity of the disease was assessed by grading clinical parameters (erythema, scaling, pustulation, induration, itching and pain) from 0–3. The sum of these assessments was calculated for each hand and foot separately and used as a severity score. Following treatment, the PPP was further classified as cleared, much improved, somewhat improved, and unchanged/worse (16).

Skin biopsies. Informed consent was obtained from all patients. Three-mm punch biopsies were taken under local anesthesia (Xylocain® 10 mg/ml, adrenalin 5 µg/ml) from involved but non-pustular skin before treatment in all 20 patients (palms $n=5$ and soles $n=15$). The thenar or hypothenar area of the palms and the medial or lateral parts of the soles were biopsied. After the initial 2 weeks of etretinate treatment, 8 patients agreed to new biopsies. This biopsy was obtained from involved non-pustular skin at least 1 cm from the previous biopsy site. On conclusion of the treatment, 5 patients agreed to new biopsies, on both the Re-PUVA-treated hand/foot and the etretinate-treated hand/foot.

Control subjects. Control biopsies were taken from palms ($n=5$) and soles ($n=5$) of 10 healthy control persons (6 women and 4 men) with a mean age of 38 years (range 27–47 years).

Immunohistochemical staining

Avidin–biotin–peroxidase staining was performed on 6 µm vertically cryostat-sectioned specimens (17). The sections were fixed in 50% acetone for 30 s, +4°C, and then in 100% acetone for an additional 3 min at the same temperature. To avoid endogenous peroxidase activity, air-dried sections were incubated in 0.3% hydrogen-peroxide in the dark for 15 min. Subsequently, every incubation step was followed by extensive washing in phosphate-buffered saline (PBS). Non-specific binding of antibodies was inhibited by pre-incubation of the specimens with non-immune porcine serum dissolved in 4% bovine serum albumin. The sections were incubated with the primary antibodies anti-HLA-DR (1:128) or anti-Leu6 (1:32) (Becton Dickinson Corp, Sunnyvale, Calif, USA) in a moist chamber at room temperature for 30 min.

Biotinylated horse-anti-mouse IgG (1:800, Vector laboratories, Burlingame, Calif) was used as a secondary antibody, and the incubation was performed for another 30 min. The specimens were further incubated with an unsaturated peroxidase-conjugated biotin–avidin complex (1:80, Vector laboratories, Burlingame). To visualize the peroxidase activity, the specimens were kept in 6 ml DMSO buffered to pH 5.5 which contained 10 mg of 3-amino-9-ethyl-carbazole and 4 µl 30% hydrogen-peroxide. Finally, the specimens were counterstained with Mayer's hematoxylin and mounted in a gelatin–glycerin solution. To test the specificity of the staining, the primary antibodies were omitted. No background or other unspecific staining was detected.

For comparative purposes, consecutive sections were stained with hematoxylin-eosin.

Quantitation of Langerhans' cells

At the end of the trial all slides were coded and counts were done by two of the authors. There was no significant difference in counts between the two investigators. Several vertical sections were screened and LC along the entire epidermal length of a representative central section of the 3 mm punch biopsies were counted with an objective ×40. LC with a dendritic morphology and with a nucleus visible as negatively stained were counted. The number of LC was expressed as cells per linear mm of surface epidermis.

Wilcoxon's rank sum test and Wilcoxon's signed rank test for paired comparisons were used for statistical calculations.

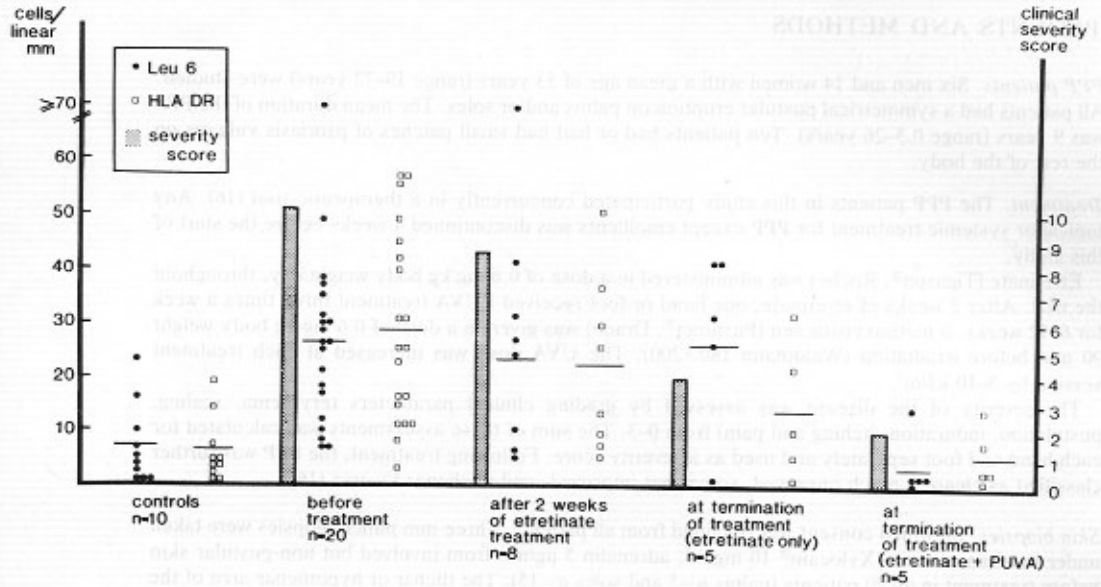

Fig. 1. Number of Leu 6$^+$ and HLA-DR$^+$ dendritic epidermal cells in palms/soles of control subjects and patients with pustulosis palmoplantaris treated with etretinate and PUVA. Individual and mean values are given. A clinical severity score is included (staples).

RESULTS

In the untreated 20 PPP patients, a mean number of 26 (range 7–70) Leu 6 positive (Leu 6$^+$) epidermal cells was observed per mm surface of the epidermis. In the control group the corresponding mean number was 7 (range 1–23) ($p<0.001$). The mean HLA-DR positive (HLA-DR$^+$) dendritic cell count in PPP was 29 (range 3–57) compared with 6 (range 1–19) in the control group ($p<0.001$) (Fig. 1). There was no systematic difference between counts from palms and soles in either group. No correlation was observed between the initial number of Leu 6$^+$ or HLA-DR$^+$ cells and the initial severity score. No differences were found in the mean numbers of Leu 6$^+$ or HLA-DR$^+$ counts between patients with a history of psoriasis and those without.

The mean severity score for all patients before treatment was 10.3. The corresponding score for the 8 patients who agreed to a second biopsy was 11.1, decreasing after 2 weeks of etretinate treatment to 8.6 (Fig. 1). When individual scores were paired, this reduction was not statistically significant. The clinical improvement observed after 2 weeks was due mainly to a reduction of pustulation and scaling. The 5 patients biopsied at the conclusion of treatment had a reduced mean severity score, from 11 at the start to 2 on the Re-PUVA-treated side and to 4 on the etretinate-treated side (Fig. 1). On the Re-PUVA side, four of the five hands/feet were cleared. On the etretinate-treated side, two hands/feet were cleared, two considered much improved and one somewhat improved. The reduction in severity of the disease was virtually identical in the biopsied and in the non-biopsied patients.

After 2 weeks of etretinate treatment the 8 biopsied patients had a reduced mean number of Leu 6$^+$ cells in the epidermis, from 27 (range 7–49) to 23 (range 0–91). The mean HLA-DR$^+$ cell number decreased from 29 (range 3–57) to 22 (range 5–50). When individual counts were paired, the reductions were not statistically significant.

At the conclusion of Re-PUVA treatment, 5 patients were biopsied. For this group the

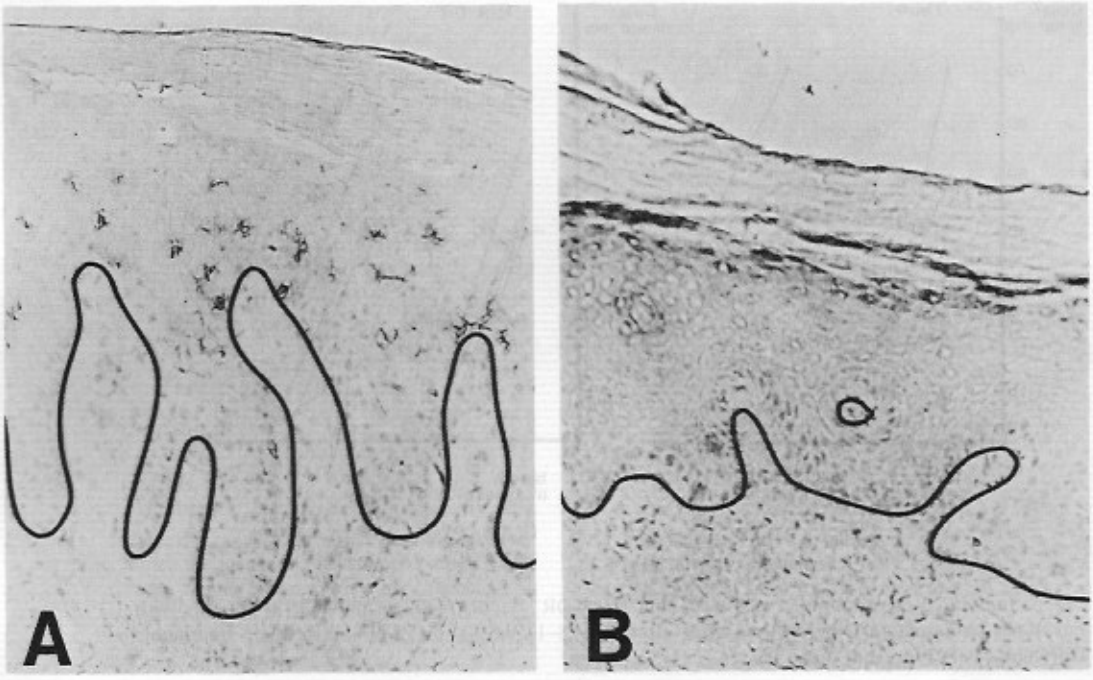

Fig. 2. Leu6$^+$ dendritic epidermal cells in untreated (*A*) and Re-PUVA treated (*B*) pustulosis palmoplantaris. Biopsies from lesional skin of the sole. ×90.

mean Leu6$^+$ cell count was reduced from 40 at the start (range 8–70) to 3 on the PUVA-treated (range 0–14) ($p<0.05$) and to 28 on the non-UVA-exposed side (range 1–41) (n.s.). The mean HLA-DR$^+$ count was reduced from 40 at the start (range 16–57) to 6 on the UVA-irradiated (range 1–14) ($p<0.01$) (Fig. 2B) and to 14 on the non-UVA-exposed side (range 1–32) ($p<0.05$) (Figs. 1–2). Individual changes in cell counts are shown in Fig. 3. Due to the fact that only a few patients agreed to post-treatment biopsies, statistical analysis of paired differences could not be done.

HLA-DR expression on keratinocytes was not noticed in any patient. This antigen was frequently expressed by cells in the dermal infiltrate, while only occasional Leu6$^+$ cells were present. The inflammatory infiltrate gradually diminished during treatment.

DISCUSSION

There are several difficulties involved in estimating epidermal LC in an acanthotic, inflamed and pustulating skin. For example it is difficult to count the LC in split skin due to the irregular thickness of the epidermis. Moreover, it is impossible to achieve an exact separation along the basal lamina. Therefore one might lose LC from the epidermis or unintentionally add positive cells from the dermis to the counts by using a split-skin technique. Instead we have studied vertical sections and the LC were expressed per mm length of the skin surface to reach comparable counts, irrespective of differences in acanthosis. It might be suggested that the decrease in the number of LC following treatment could be explained by the treatment-associated reduction in acanthosis. However, the marked decrease in LC surface markers following Re-PUVA with a concurrent

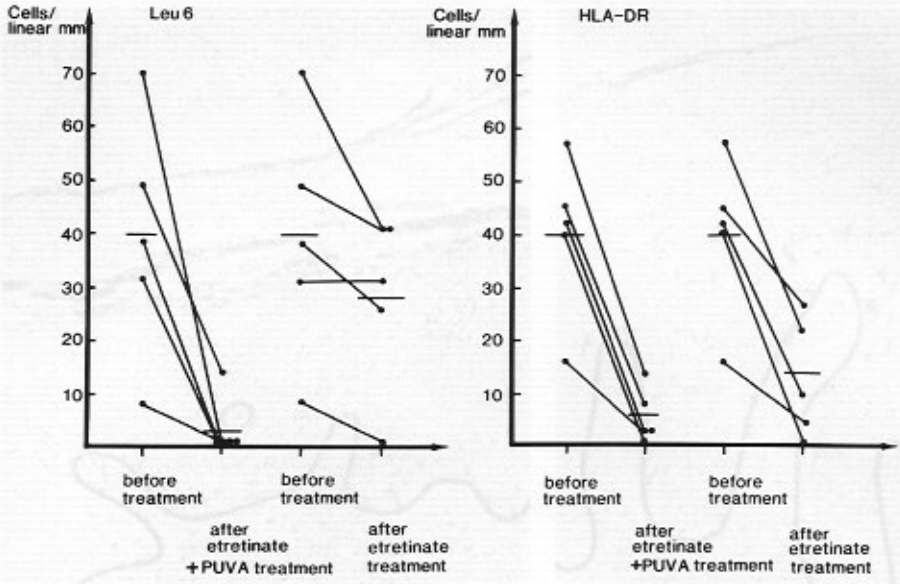

Fig. 3. Individual paired number of Leu 6$^+$ and HLA-DR$^+$ dendritic epidermal cells in patients with pustulosis palmoplantaris before and after etretinate (8–14 weeks) or PUVA + etretinate treatment. Horisontal bars represent mean values.

moderate reduction in acanthosis argues against a change in epidermal thickness as being the major explanation for the reduced LC number.

In active PPP, the number of Leu 6$^+$ and HLA-DR$^+$ dendritic epidermal cells was significantly increased, compared with control subjects. Almost the same number of cells expressed Leu 6 and HLA-DR antigens in PPP. It might be argued that the large number of epidermal cells in PPP expressing class II antigen does not signify an increased LC population, but is instead due to an invasion of activated T-cells from the inflammatory infiltrate in the dermis (12). This is less likely, however, since only dendritic cells were counted and the same ratio of cells expressing the two surface markers was present even after the dermal infiltrate was reduced, following clearing of the disease. Whether the numerical increase in LC is a primary event in the pathogenesis of PPP or due to a secondary stimulation in inflamed skin cannot be determined from our observations.

After 2 weeks of etretinate treatment there was a non-significant reduction in the total severity score and the LC counts. The observed improvement in pustulation and scaling might be due to non-LC-dependent mechanisms such as the antichemotactic effect of etretinate on granulocytes (15).

The combined treatment with etretinate and PUVA resulted in a superior clinical effect as well as a pronounced LC reduction. In addition, there was a parallel decrease in Leu 6 and HLA-DR antigen expressing cells. This speaks in favour of a direct cytotoxic effect on the LC rather than a specific suppression of the HLA-DR membrane antigen following PUVA treatment (18). It should be noticed that in psoriatic epidermis a pronounced fall in numbers of T-helper lymphocytes preceded clearing of lesions while disappearance of LC seemed to occur somewhat later (19).

On the etretinate-treated side (non-UVA exposed) there was a gradual clinical improvement and a reduction of HLA-DR$^+$ LC (Fig. 3). This reduction might be explained by etretinate alone and/or a systemic effect of the PUVA treatment. The fact that the initial 2

weeks of etretinate treatment had no influence on the LC count supports the idea that PUVA treatment has both local and distant immunomodulating effects.

The observation of an increased LC population in active PPP and a reduction during clinical improvement indicates a close relation between LC and the activity of PPP.

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