Density of epidermal Langerhans cells in psoriasis patients treated with an aromatic retinoid (RO 10-9359). An immunoperoxidase study using anti-T6 and anti-Ia monoclonal antibodies

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Summary. Immunocytochemical techniques using antibodies to the specific T6 and Ia (Major Histocompability Complex, class 11, human HLA-Dr) antigens were used to determine the densities of epidermal Langerhans cells (LC) in psoriasis patients treated with the aromatic retinoid RO 10-9359. Fourteen patients were treated with the aromatic retinoid and were skin biopsied before, during and after therapy.

Two psoriasis patients receiving PUVA (systemic 8-metoxypsoralen + UVA irradiation) were included in the study. The results showed an increase in LC numbers during aromatic retinoid administration, which coincided with an improvement in the clinical severity of the lesions. At the end of retinoid administration the LC numbers were similar to those found in the initial psoriatic plaques. The density of Ia + LC, in comparison with T6 + LC in the epidermis of psoriatic plaques were significantly different. Dendritic and non-dendritic Ia + · cells were also observed in the dermis of the plaques. Unlike aromatic retinoid treated patients, PUVA treated patients showed a decrease of both T6+ and Ia + epidermal LC by the middle of therapy, a total absence of immunoreaction by the end of therapy, and a return to normal skin values a few weeks after treatment. This immunocytochemical study helps in distinguishing between dendritic and other possible Ia-expressing cells from the infiltrate that may penetrate the epithelium.

These results do not conclusively demonstrate the role of LC in the pathogenesis of psoriasis. Other factors, such as the interrelationship with other immune response cell types and alterations in the lymphokine cascade may be important.

Introduction

Psoriasis is a chronic skin disease of unknown etiology characterized by an epidermal hyperplasia with a subsequent hyperproliferation of keratinocytes. The pathogenic mechanisms involved, and the effects of different treatments on the course of the disease, are also unclear (Weinstein and Frost, 1968).

Langerhans cells (LC), which may be involved in such conditions, are probably the immunocompetent cells of the epidermis, and thus the first line of defense of the skin (Shelley and Juhlin, 1976; Silberberg-Sinaken et al., 1977; Stingl et al., 1980). These cells belong to the monocyte-macrophage series, and thus express many of their characteristics, which include: ATPase activity (Wolf, 1972), Fc-IgG and C3b receptors (Stingl et al., 1977). In addition, LC present antigen (Stingl et al., 1981; Braathen et al., 1984), express Ia antigens (Klareskog et al., 1977; Rowden et al., 1977) and produce Interleukin-1 (Sauder et al., 1984). Ultrastructurally, the most distinctive feature of LC is a trilaminar body known as the Birbeck granule (Birbeck et al., 1961). Recently, a monoclonal antibody (anti-T6) has been obtained, using thymocytes as the immunogen, recognizing specific determinants on the cell surface of LC (Fithian et al., 1981) and epidermal indeterminate cells (Murphy et al., 1982).

The aromatic retinoid RO 10-9359 (etretinate, Tigason®) is known to clinically improve keratinocyte disorders, by stimulating the activity of epidermal cell types, including LC (Orfanos, 1980).

The mechanism of action of retinoids has, however, not been elucidated. Similarly, PUVA (8 methoxypsoralen + ultraviolet light spectrum A) which has been demonstrated to induce a decrease in the number of epidermal LC, also reduces the severity of the dermatoses (Koulo et al., 1984). These observations suggest the hypothesis that LC may be the principal target of the therapeutic effect of photochemotherapy.

This study presents an immunoperoxidase study using anti-T6 and anti-Mayor Histocompatibility complex-class II antigen (Ia) (anti-human HLA-Dr) monoclonal antibodies to determine LC density in psoriatic patients after treatment with the aromatic retinoid RO 10-9359 and PUVA.

Materials and methods

Patients groups

The psoriasis patients (mean $\pm$ SD age = 46 + 12 years old) studied (n = 16; 13 severe generalized psoriasis in plaques, 2 pustular psoriasis and 1 psoriatic erythroderma) were grouped in the following manner:

a) Patients treated with aromatic retinoid RO 10-9359 (n = 14) received the drug orally 1 mg/kg/day for 4 weeks, after which the doses were progressively decreased until total cessation at the 14th or 15th week.

b) Patients treated with PUVA (n = 2) received 8-methoxypsoralen (8-MOP) at doses of 0.6 mg/kg, and 2 hours later were exposed to UV light, spectrum A (320-400 nm) using a Waldman 6001 photochemotherapy chamber with 40 Tubes 180 cm in length.

The 2 patients were classified as type III, and the irradiation was given, according to Stern (1984). PUVA was applied 4 times a week, reaching a maximal dose of 9 J/cm²/day.

In addition, uninvolved skin from psoriasis patientes (n = 16) was included as control.

Complete laboratory and clinical examinations were carried out in all patientes to evaluate any possible side effects due to the aromatic retinoid treatment. PUVA patients were ophthalmologically examined before and after therapy.

Clinical Severity Scoring

Initial body surface involved (IBSI) in all psoriasis patients varied from 30-90%. Clinical improvement was scored assuming that the IBSI was 0, using the criteria of Fredriksson and Pettersson (1978).

Skin Biopsies

Punch biopsies of 5 mm in diameter were taken from psoriatic plaques under local anesthesia (2% xylocaine) from either the forearm or abdomen. The group of patients receiving aromatic retinoid treatment were biopsied before, then on the 1st, 2nd, 3rd, 4th, 5th and 6th week of treatment, as well as after treatment (16 weeks).

Patients receiving PUVA were biopsied before therapy, in the middle of therapy (session No. 16) and at post-treatment (6 weeks after, session No. 30).

For the immunoperoxidase studies, the skin biopsies were embedded in OCT compound (Lab. Tek, Miles Labs., lnc., U.S.A.), snap frozen in liquid Nitrogen and stored at -20" C until sectioning.

Monoclonal Antibodies

Monoclonal antibody OKT6 (Ortho Diagnostics, lnc., U.S.A.) diluted 1:50 in a modified phosphate-buffered saline (PBS) (Hofman et al., 1982) and anti-HLA-Dr (Becton Dickenson, lnc., U.S.A.) diluted 1:60 in PBS were used in all immunoperoxidase staining.

Immunoperoxidase Staining Procedure

Frozen sections (5 µm) were cut with a cryostat and air-dried overnight before the immunostaining procedure.

The immunostaining was carried out using Avidin-Biotin (ABC) immunoperoxidase (Hsu et al., 1981; Hofman et al., 1982) with the sections treated as follows: 1). Fixation in fresh acetone, 10 min.; 2). PBS, 5 min.; 3). Primary monoclonal antibody (optimal dilution), 15 min.; 4). PBS, 5 rnin.; 5). Biotinylated horse anti-mouse IgG (Vector Labs., Inc., U.S.A.) 1:30 in PBS (50 µg/ml), 15 min.; 6). PBS, 5 rnin.; 7). ABC (Vectastain Kit, Vector Labs, lnc., U.S.A.) 1:100, 15 min.; 8). PBS, 5 min.; 9). Developing for 10 min. with 90 µM H_2O_2 and 3-amino-9-ethyl-carbazole (final concentration 0.88 mM) which was dissolved in 50 mM N, N-dimethylformamide in 0.1 M acetate buffer, pH 5.2; 10). Rinse in water; 11). Counterstaining with Mayer's Haematoxylin and mounting in glycerin-gelatine.

Langerhans Cell Quantification

Cell counting was carried out using a light microscope with a millimetered reticule (Carl Zeiss, Germany) on an eyepiece calibrated to determine the number of cells/mm2 of epidermis. Only dendritic cells showing red immunostaining and a visible nucleus were counted as positive.

Statistical Analysis

Analysis of the data was carried out using Student's t-test, with $p < 0.05$ being taken as statistically significant.

Results

Dendritic T6 positive cells were counted in the epidermis active plaques of psoriasis patients. The mean ± SD density (Table 1) of T6+ cells in pretreatment uninvolved skin was 125 + 44 cells/mm², distributed mainly in the suprabasal region, and absent in the dermis (Fig. I). In the psoriatic plaques the T6+ density was 203 + 61 cells/mm² (p< 0.001), located primarily in the suprabasal region, with a few cells being found in the papillar dermis (Fig. 2).

At the end of the 1st week of treatment an initial decrease of 12% from pretreatment values was demonstrated and an increase of up to 64% (p< 0.001) in LC numbers occurred during the 5th week of aromatic retinoid treatment. Postreatment values were however not significantly different from the pretreatment levels (p> 0.05). Despite these changes in LC density, clear clinical improvement of the lesions was observed (Fig. 3). Histologically, the skin of patients under retinoid therapy showed a decreased acanthosis and parakeratosis, as well as the appearance of a moderate histiocytic-lymphocytic infiltrate in the dermis (Figs. 4a, b).

The number of Ia positive LC in the epidermis of psoriatic plaques was significantly lower (p< 0.2) than that found using anti-T6 (Table 1, Fig. 5). In the psoriatic plaques, before and during the course of aromatic retinoid treatment, Ia+ cells were observed in the dermis, forming clusters in the papillar dermis, perianexial and perivascular infiltrates (Fig. 6).

PUVA treated patients showed a marked decrease of both T6+ and Ia+ epidermal and dermal dendritic cells by the middle of therapy (session No. 16, average cumulative dose = 40 J/cm²), and these cells were totally absent by the end of treatment (70 J/cm²) (Fig. 7). However, normal skin values of LC were found at 6 weeks postreatment.

The decreased values in LC numbers coincided with a clinical improvement, which was about 60% in session No. 16 and 100% in session 30.

Table 1. Density values of T6+ and Ia+ cells (Mean + SD) in the epidermis of uninvolved skin and psoriatic plaque obtained during and post-retinoid administration

CELLS	US	PP	AROMATIC RETINOID TREATMENT						PR
			1st	2nd	3rd	4th	5th	6th	
T6+	125±44	203+61	180+37	258±42	320+85	336+80	318±80	280+95	244+78
Ia+	101+38	174+48	139+44	189+43	257+87	251+97	265±103	260+85	188+68
Statistical significances between T6+ and Ia+ cells	p< 0.001	p< 0.2	NS	p< 0.001	p< 0.05	p< 0.01	NS	NS	NS

US: uninvolved skin
PP: psoriatic plaque before treatment
PR: post-aromatic retinoid treatment
NS: non-statistical significant

Fig. 1. Non-involved psoriatic skin. Note numerous T6+ Langerhans cells in the suprabasal region and absence in the dermis. x 460, scale bar = 5 μm.
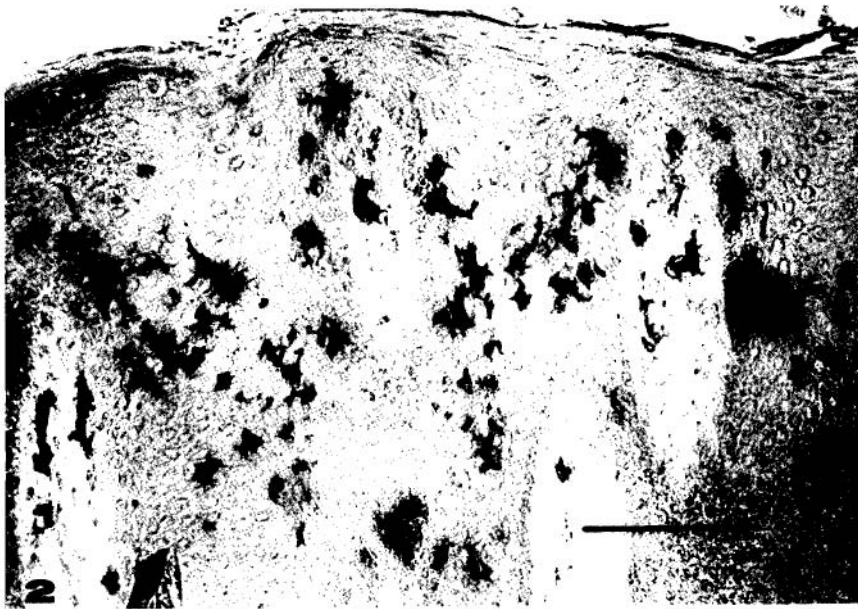
Fig. 2. Psoriatic plaques showing an abundant number of T6+ Langerhans cells located mainly in the suprabasal epidermis. x 460, scale bar = 5 µm.
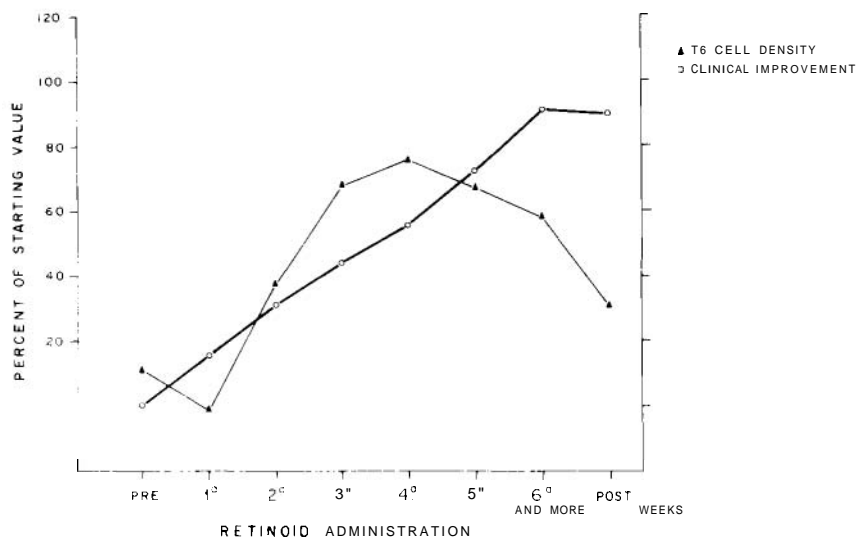

Fig. 3. Clinical improvement index and epidermal T6+ Langerhans cell density before (PRE), during (1-6 weeks) and after (POST) aromatic retinoid administration.

Fig. 4. a. Numerous T6+ Langerhans cells in a psoriatic plaque at the beginning (2 weeks) of aromatic retinoid treatment. x 460, scale bar = 5 pm. b. Post aromatic retinoid treatment skin (5 weeks) showing decreased parakeratosis and acanthosis. Note the scarce number of T6+ Langerhans cells. x 1,150, scale bar = 2.5 μm.
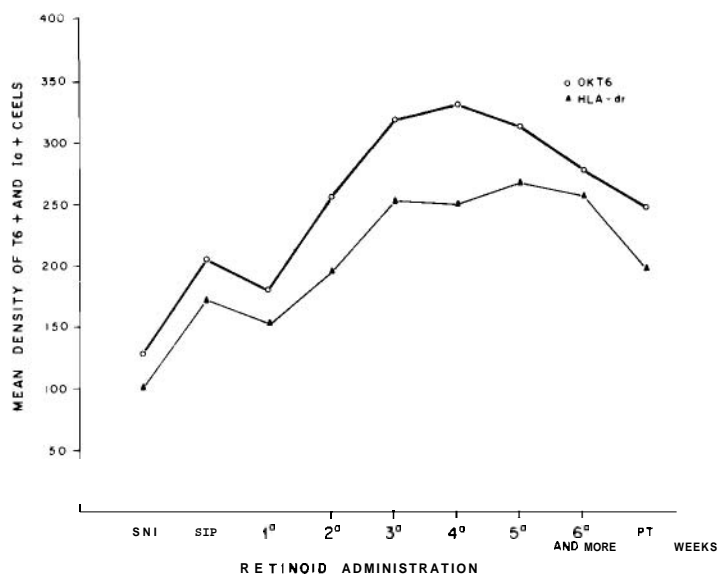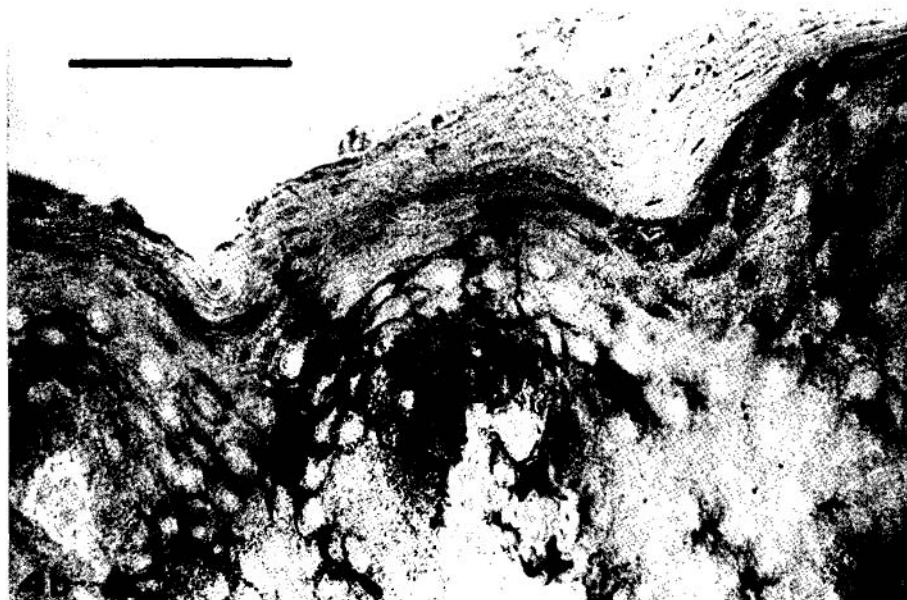

Fig. 5. Diagram showing Ia+ (HLA-Dr) and T6+ epidermal Langerhans cell density during aromatic retinoid administration. SNI: non-involved, SIP: psoriatic plaque, PT: postreatment.
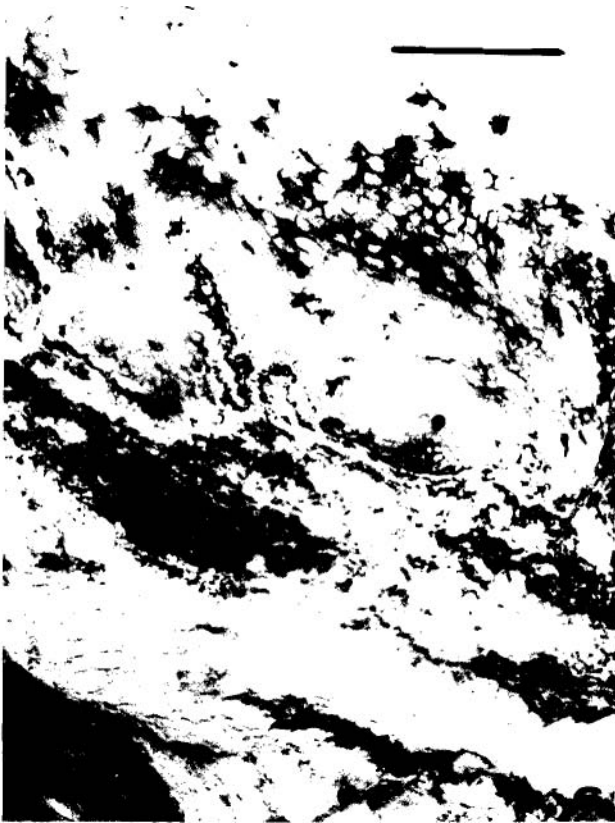
Fig. 6. Ia + cells in the epidermis and dermis of a psoriatic plaque (5th week of aromatic retinoid treatment). Note the presence of Ia+ cells forming clusters in the papillar dermis and perivascular infiltrate. x 460, scale bar = 5 µm.
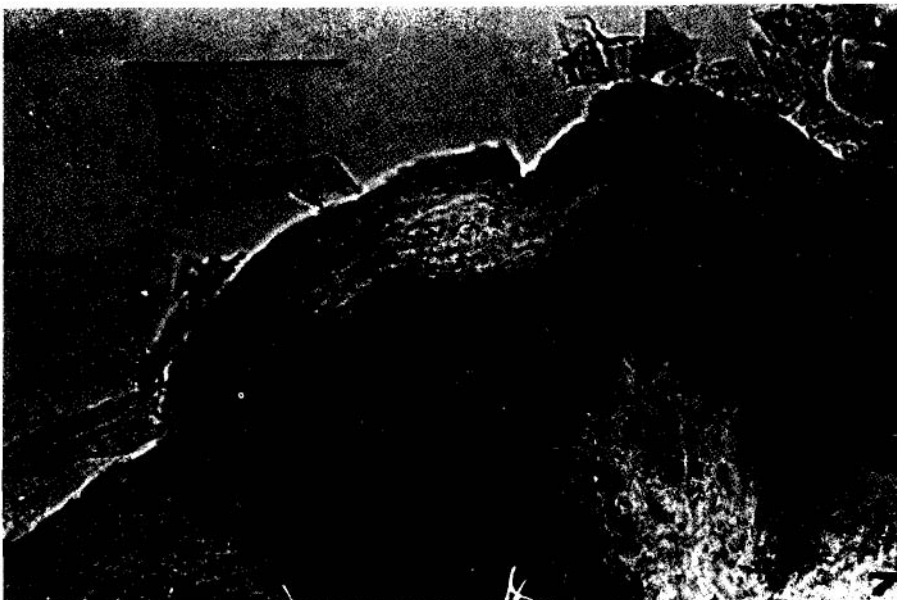

Fig. 7. Scarce T 6 + cells in the suprabasal epidermis of a patient treated with PUVA at the end of treatment. x 1,150. scale bar = 2.5 µm

Discussion

In the present study, two well-established monoclonal antibodies (OKT6 and Ia) were used to study, by an immunoperoxidase staining procedure, the density and distribution of dendritic LC in psoriatic plaques. The LC density was evaluated before, during and after treatment with aromatic retinoid RO 10-9359 and PUVA.

The results confirmed previous studies (Haftek et al., 1983; Koulo et al., 1984) which suggest that after both types of therapy the LC numbers, which are altered in the disease state, tend to return to normal skin values.

This study also demonstrates a higher density of T6+ cells than Ia ɩ cells in psoriatic epidermal skin. In addition, a progressive increase of T6+ and Ia+ cells occurs during aromatic retinoid administration. The opposite occurs using PUVA, where LC numbers diminish during the photochemotherapy. It could be suggested that PUVA either affects LC surface membranes, resulting in a decrease or lack of antigen expression (Iacobelli et al., 1983), or the irradiation forces the LC to migrate from the epidermis (Ree, 1982).

The exact mechanisms of action of aromatic retinoids is unknown, although there is evidence to suggest that they stimulate different epidermal cell types (Orfanos, 1980). Our results do not, however, conclusively demonstrate that LC are the major cell type involved in the pathogenisis of psoriasis. Other factors, such as the interrelationship with other immune cell types and alterations in the lymphokine cascade, may be important. These posibilities require further examination.

The use of the immunoperoxidase staining method helps in distinguishing between dendritic and other possible Ia-expressing cells from the exocytic infiltrate that may penetrate the epithelium. In contrast to other pathological conditions (Tjerlund, 1980, 1982; Modlin et al., 1985) we have observed that the keratinocytes in psoriatic plaques do not express Ia antigens, thus agreeing with previous studies on this disease (Bjerke and Matre, 1983; Haftek et al., 1983). There is some evidence that keratinocyte proliferation is altered in psoriasis, thus the lack of Ia expression in psoriatic plaque keratinocytes may be due to alterations, such as defects in the production of Interleukin 1-like factors by keratinocytes.

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