Langerhans' Cells in Patients with Psoriasis: Effect of Treatment with PUVA, PUVA Bath, Etretinate and Anthralin

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Suction blisters were raised in lesions and normal appearing skin of patients with psoriasis. The blister roof which contains the epidermis separated at the dermal-epidermal junction was stained with ATPase, OKT-6 and anti-HLA-DR monoclonal antibodies. The technique permits the counting of the Langerhans' cells per mm^2. Their mean number varied between 888–987 cells per mm^2 in control subjects with the three staining procedures. In patients with psoriasis, the number of cells before treatment was between 1110–1179 in uninvolved skin and 521–1001 per mm^2 in the lesions as measured using both monoclonal antibodies and ATPase. However, the latter technique seemed to be inappropriate for lesional skin. After treatment with PUVA bath or oral PUVA with or without etretinate, fewer Langerhans' cells were seen in both lesions and normal appearing skin with the appearance of giant Langerhans' cells with long dendrites. In patients healed with anthralin + UV-B the Langerhans' cells appeared normal in number and size. *Key words: Langerhans' cells; Psoriasis; Psoralen + UVA treatment.* (Received September 18, 1984.)

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In patients with psoriasis, the Langerhans' cells have been studied by the ATPase method. Desquamating remains were seen in the stratum corneum (1). In lesional skin the Langerhans' cells were distributed irregularly in the epidermis and often clustered above the epidermal papillae (2). They were decreased in number and were almost absent in both plaques and normally appearing skin. The dendrites were often long. It was, however, difficult to count the cells since infiltrating white cells were also stained. Niebauer, who used the osmium-zinc-iodide method which stains both Langerhans' cells and melanocytes, mentions that in psoriasis there is a total increase of dendritic cells compared to normal skin, but their percentage was finally lower due to the marked increase of keratinocytes (3).

Clusters of Langerhans' cells were also seen with the HLA-DR antigen technique which also stains activated T cells and macrophages (4). A more selective marker of the Langerhans' cells was obtained with OKT-6 monoclonal antibody as a marker (5–6). With this technique Haftek et al. (7) reported reduced number of Langerhans' cells in psoriatic lesions where they were seen in clusters which became normalized after treatment with etretinate.

PUVA bath treatment of psoriasis is known to reduce the number of Langerhans' cells in normal appearing skin, with the appearance of abnormal looking giant Langerhans' cells (8).

The giant cells were seen in horizontal sheets of separated epidermis stained with ATPase but not in vertical sections with the immunological markers OKT-6 and anti-HLA-DR (9).

We now report on a technique which permits the counting of the Langerhans' cells per mm^2 in epidermal sheets from both normal and involved skin. Epidermis was separated in vivo by raising suction blisters and the blister roof was stained with ATPase, OKT-6 and

anti-HLA-DR antibodies. The aim was to study if some commonly used treatments can influence the number and morphology of the Langerhans' cells in lesional and non-lesional skin of patients with psoriasis.

PATIENTS AND TREATMENTS

Twenty men and 4 women (age 34–76 years) with typical psoriatic plaques and 4 non-psoriatic men have been studied. The patients were treated with PUVA bath 8-methoxypsoralen bath 2.2 mg/l + UV-A, 0.1–2.0 J/cm^2 in increasing doses in a Waldmann cabin with Sylvania PUVA lamps; PUVA bath + etretinate 25–50 mg daily; PUVA general 8-methoxypsoralen 0.6 mg/kg orally 2 hours before UV-A 1–20 J/cm^2 and anthraline 1 % in vaseline for 20 min followed by UV-B irradiation 0.01–2 J in a Waldmann cabin with 12 Sylvania UV-B lamps, 3–5 times weekly. The Langerhans' cell counting was performed 1–6 days after last treatment (4–7 weeks of treatment) on the practically healed lesional skin as well as on uninvolved skin.

METHODS

Suction blisters were raised on the abdomen in psoriatic lesions and normal appearing skin at room temperature by Kiistala's suction blister technique (10). The separation occurred in the epidermal-dermal junction in both normal and involved skin. The blister roofs were divided into the parts. One was stained directly with ATPase (11).

The other parts were fixed in acetone for 20 min at 4°C, washed in physiological saline solution for 1 hour and incubated for 16 hours at 4°C in 0.4 ml of monoclonal antibody solution. The antibodies used were OKT-6 (Ortho Pharmaceutical Corp., Raritan N.J., USA) and anti-HLA-DR (Becton Dickinson, Mountain View, Ca, USA) in a dilution of 1 : 50. As the second layer we used FITC-conjugated goat anti-mouse IgG (TAGO, Burlingame, Ca, USA) diluted 1 : 30 for 1.5 hours at 4°C. After washing the epidermal sheets were mounted in glycerol and examined under a fluorescent microscope.

The cell density was determined by random counts of 15 fields at 400× magnification with an occular grid of known area (0.038 mm^2) and expressed as number of Langerhans' cells per mm^2. Vertical sections of the stained blister roofs were also performed and investigated. Controls with second antibody alone were always included.

RESULTS

The control subjects had 888–987 cells per mm^2 evenly distributed with the three techniques used (Fig. 1 A and B). In psoriatic lesions the Langerhans' cells were often seen to aggregate in cluster and in some areas of the epidermis no fluorescent cells were seen (Fig. 2 A and B). Their numbers per mm^2 before treatment were similar in uninvolved skin and lesions (Table I).

In patients with anthralin + UV-B irradiation the number of Langerhans' cells was within normal limits. After treatment with PUVA bath alone or combined with etretinate

Table I. *Number of OKT-6, HLA-DR and ATPase positive cells in the epidermis of normal subjects (controls) and in psoriatic epidermis fron uninvolved (UINV) and involved (INV) skin*

Subjects	OKT-6 (+)/mm^2	HLA-DR (+)/mm^2	ATPase/mm^2
Controls (4)[b]	987±137[a]	910±211	888±161
Psoriatic (5)			
UINV	1 179±261	1 146±216	1 110±310
INV	943±357	1 001±311	521±211

[a] Mean number and standard deviation.
[b] Number of cases studied.

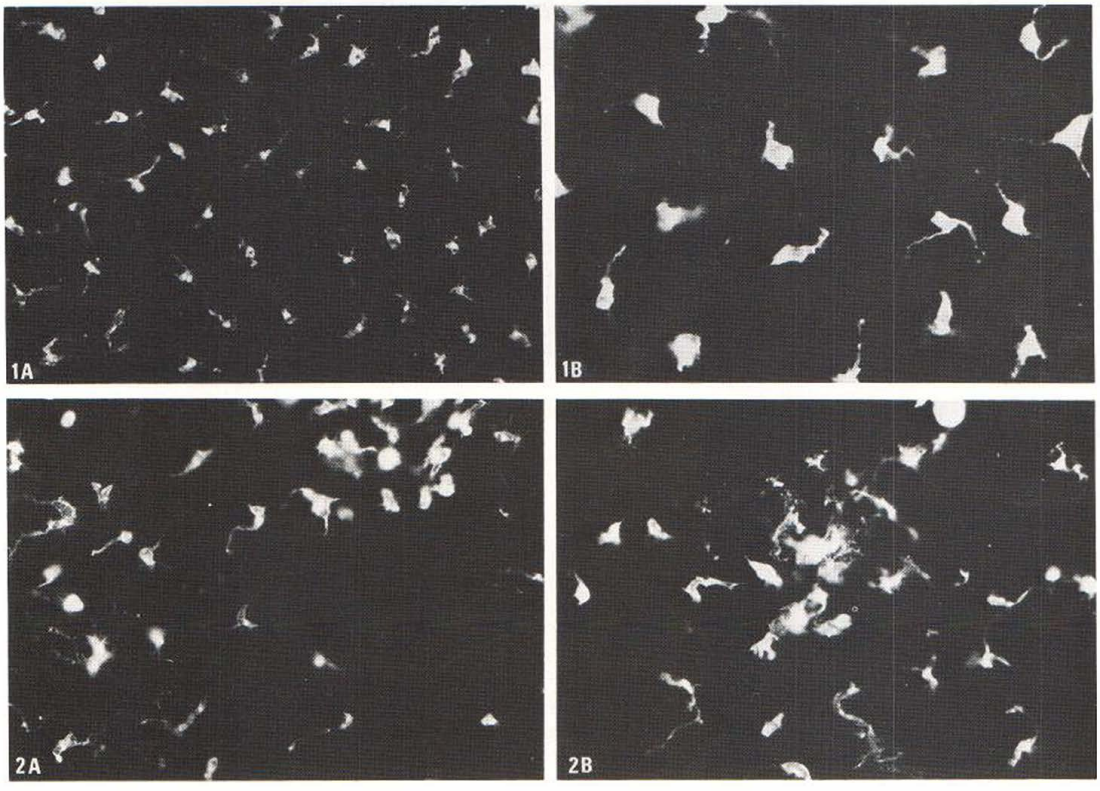


Fig. 1. Normal human epidermis. (A) OKT-6 staining (×100), (B) anti-HLA-DR staining (×250).

Fig. 2. Psoriatic epidermis—involved skin. (A) Anti-HLA-DR staining (×160), (B) OKT-6 staining (×160).

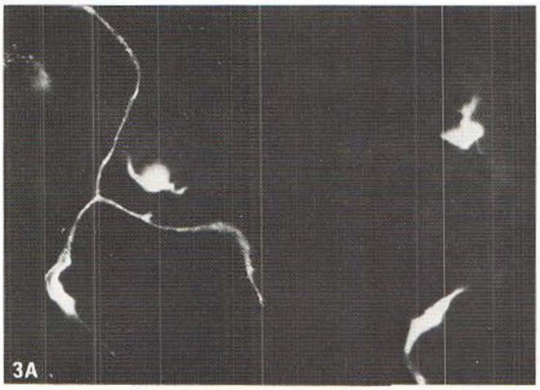
fewer Langerhans' cells were seen in both lesions and normal appearing skin. Oral PUVA also decreased the number of Langerhans' cells (Table II).

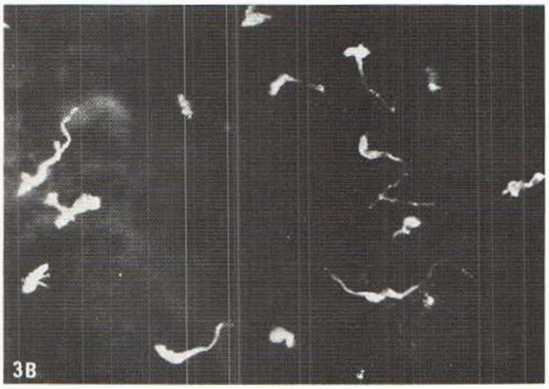
In all patients receiving PUVA, PUVA-bath, with or without etretinate, the Langerhans' cells were often much larger than normal, with long dendrites (Fig. 3 A and B).

Table II. *Number of OKT-6 and HLA-DR positive cells in psoriatic epidermis from uninvolved (UINV) and involved (INV) skin after various treatments*

Treatment	OKT-6 (+)/mm^2	HLA-DR (+)/mm^2
PUVA-bath (7)[a]		
UINV	204±63	234±71
INV	347±134	385±284
PUVA-general (4)		
UINV	315±101	289±131
INV	251±91	297±161
PUVA-bath + Etreinate (4)		
UINV	270±130	281±113
INV	378±151	392±201
UV B + anthralin (4)		
UINV	918±205	891±225
INV	863±305	860±331

[a] Number of cases studied.





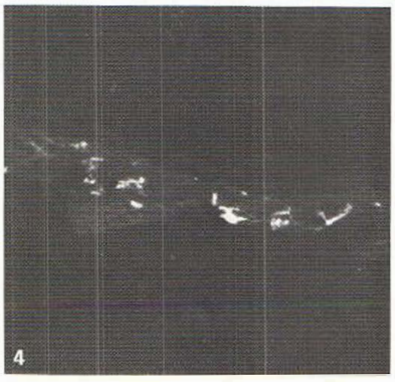


Fig. 3. Giant Langerhans' cells. (A) Involved psoriatic skin after PUVA-bath treatment. OKT-6 staining (×250). (B) Non-involved psoriatic skin after PUVA + Etretinate treatment. OKT-6 staining (×160).

Fig. 4. Psoriatic involved skin after PUVA treatment. Vertical cross-section of blister roof previously stained with anti-HLA-DR antibody.

The same number was as a rule counted with the two immunological markers used to visualize the Langerhans' cells whereas the ATPase technique often gave a lower number of cells. A clustering of cells was seen in lesions and decreased after the various treatments but could still to some extent be seen in healed or almost healed lesions.

In vertical sections the Langerhans' cells were seen extending from the granular layer to the basal layer, both in normal skin and in the thickened psoriatic lesions (Fig. 4). A decrease in number of Langerhans' cells after psoralen + UV-A treatment was evident also here, but more difficult to measure. No giant Langerhans' cells were seen in these sections.

DISCUSSION

Counting the number of Langerhans' cells in vertical sections is problematical since often only a part of the cell is observed. By using suction blister roofs, it was possible to count the cells more exactly also in diseased skin, where the epidermis is thicker and papillomatous. By slightly focusing up and down, one is sure that deeper situated Langerhans' cells in psoriatic lesions were, as reported earlier, sometimes seen in clusters (2, 4, 7).

The Langerhans' cell number per mm^2 in non-treated psoriatic lesions was similar to that observed in skin of normal appearance as well as in skin from control subjects. However, the epidermis of the lesional psoriatic skin is much thicker than uninvolved or healthy epidermis and this may well explain the low Langerhans' cell number as reported for the epidermal surface of vertical skin sections (7, 12).

It was, however, clear that general or local PUVA treatment, with or without etretinate,

decreases the number of Langerhans' cells as reported by several authors (8, 9, 13–17). The malformed giant Langerhans' cells have previously only been found with the ATPase staining in horizontal sheets (9, 13). We have now been able to demonstrate them also with immunofluorescent markers. They are extending horizontally and could not be recognized in vertical sections. Their nature and function remain obscure.

The ATPase technique gives excellent staining in normal skin but in pathological conditions it often fails to demonstrate the Langerhans' cells, which probably depends on the fact that it is an in vivo staining requiring active ATPase and good penetration of reacting chemicals. The immunofluorescent technique stains dead cells and is therefore less vulnerable to pathological processes. This was evident in some of our treated patients, where the number of the ATPase positive cells was lower than the number of those stained with the two very similar immunofluorescent techniques.

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