

Reduced Density of T6-positive Epidermal Langerhans' Cells in Uninvolved Skin of Patients with Psoriasis

A. OXHOLM,¹ P. OXHOLM² and B. STABERG³

¹Department of Dermatology, and ²Laboratory of Medical Immunology TA, Rigshospitalet, University Hospital and ³Department of Dermatology, Gentofte Hospital, Copenhagen, Denmark

Oxholm A, Oxholm P, Staberg B. Reduced density of T6-positive epidermal Langerhans' cells in uninvolved skin of patients with psoriasis. *Acta Derm Venereol (Stockh)* 1987; 67: 8-11.

The Langerhans' cell (LC) density is known to be reduced in skin lesions as compared to uninvolved skin in patients with psoriasis. It is, however, still unsettled whether the LC density in uninvolved psoriatic skin differs from the density in normal skin. We have enumerated epidermal LC in uninvolved skin from 15 patients with stable psoriasis and in 15 healthy subjects. Punch biopsies from non-sunexposed skin from the buttock were taken. Epidermal sheets were separated by EDTA and LC then stained with an indirect immunoperoxidase technique using the mouse monoclonal antibody ●KT6. The LC density was significantly reduced in uninvolved skin of patients with psoriasis (mean±SD: 375±37/mm²) as compared to healthy controls (544±168/mm²) ($p<0.01$). A reduced number of LC in uninvolved psoriatic skin is in accordance with previous reports demonstrating an impaired DNCB reactivity in patients with psoriasis. Whether the reduction in LC density is of pathogenic importance for psoriasis is unknown. *Key words: Uninvolved psoriatic skin; Epidermal sheets.* (Received June 6, 1986.)

A. Oxholm, Department of Dermatology, Rigshospitalet, DK-2100 Copenhagen, Denmark.

The involvement of epidermal Langerhans' cells (ELC) in both immunologic processes and keratinization has been suggested by various observations (1, 2). An inverse relationship seems to exist between proliferative events in the skin and the number of ELC as well as between the degree of parakeratosis and ELC density (3, 4, 5). Recent studies have demonstrated that the number of ELC is reduced in psoriatic skin lesions as compared to uninvolved skin (6, 7). Only few observations of ELC changes in uninvolved psoriatic skin have been reported (6, 8) and the conflicting results are probably caused by the different methods applied.

Because psoriasis is a condition in which genetic factors are important, the entire epidermis is involved in the disease. Evaluation of ELC in uninvolved skin may uncover clues to the cause of the disease. We have found it important to assess the ELC density in patients with moderate, stable psoriasis by the most accurate and specific method available.

MATERIAL AND METHODS

Patients

A selected group of 15 patients with psoriasis participated in the study. All patients had stable, chronic psoriasis involving less than 30% of the body surface. The extent of the skin involvement was assessed by "the rule of nines". Mean age was 52 years (range 26-76). None of the patients had been exposed to UV radiation on their body at least two months before the investigation. None had received systemic treatment with either glucocorticosteroids, cytostatics or retinoids. Ten of the patients did not receive any local treatment, two were treated with coal-tar on plaques, one had dithranol and two had topical steroid.

Fifteen healthy sex matched persons, mean age 47 years (range 28–85) served as controls.

Informed consent to have a skin biopsy performed was obtained from both patients as well as controls.

Skin biopsies

Punch biopsies (4 mm) were removed from uninvolved, non-sun-exposed skin from the upper buttock. The biopsies were taken as far away as possible from skin lesions (>10 cm), in which distance light microscopic examination (hematoxylin-eosin) did not reveal any dermal or epidermal changes characteristic of psoriasis.

The skin was anaesthetized with ethyl chloride spray and the biopsies were transported in Histocon® (Histolab, Göteborg, Sweden), frozen as soon as possible and stored at -70°C.

Light microscopical enumeration of Langerhans' cells

Fat-trimmed biopsies were washed in phosphate-buffered saline (PBS) (pH 7.4, free of Ca^{2+} and Mg^{2+}) for 3×5 min and immersed in 1.0 mM EDTA for 120 min at 37°C. The epidermis was subsequently teased off the dermis with fine forceps and washed in PBS (3×5 min) prior to immunoperoxidase staining.

The density of LC within each epidermal sheet was enumerated with a light microscope (Leitz Dialux 20 EB) at ×1 000 magnification and oil immersion. The slides were read without knowledge of clinical status. Ten randomly chosen, non-overlapping fields (0.0255 mm²/field containing 5–15 OKT6 positive cells) were examined, and the number of LC per mm² counted.

Indirect immunoperoxidase technique for demonstration of Langerhans' cells

Epidermal sheets were washed in PBS (3×5 min) and incubated with OKT6 at 37°C using a 1:100 working dilution. Following incubation for 60 min, the epidermal sheets were washed in PBS (3×10 min), incubated with horseradish peroxidase labelled rabbit Ig against mouse Ig (Dakopatts), absorbed with human serum, using a 1:10 working dilution. After incubation for 60 min the epidermal sheets were washed in PBS (3×10 min) followed by 0.038% 3-amino-9-aethylcarbazol (Sigma, St. Louis, USA) and 0.014% hydrogen peroxide for an additional 10 min. The specimens were then washed in water (3×10 min) and mounted with Gurr® (BDH Chemicals, Poole, England).

Control epidermal sheets stained without adding primary layer antibody (OKT6) were negative.

Statistics

The Mann-Whitney test was used in the statistic analyses. Level of significance, 5%.

RESULTS

Counting of ELC in sheets (Fig. 1) revealed a significantly reduced number in the patients with psoriasis (mean±SD: 375±37/mm²) as compared to healthy controls (544±168/mm²) ($p<0.01$). Differences in morphology and distribution of ELC were not observed. No correlation was found between number of ELC and the kind of topical treatment in those patients who received treatment of the psoriatic plaques. There was likewise no correlation between the ELC density and age or disease duration.

DISCUSSION

Assessment of ELC density in EDTA-separated epidermal sheets is known to be an accurate method used on uninvolved skin (9). OKT-6 is furthermore considered the most specific marker of ELC (9).

Our finding of a reduced number of ELC in uninvolved, non-sun-exposed skin from the buttock of patients with psoriasis using the above mentioned technique is in accordance with the results of Haftek et al. (6) using different methods. In the latter study a reduced number of OKT-6 and HLA-DR positive cells was found in vertical sections as well as ATP-ase positive cells in EDTA-separated epidermal sheets taken from uninvolved skin of the extensor surface of the forearm.



Fig. 1. Langerhans' cells demonstrated in an epidermal sheet from a patient with psoriasis using an indirect immunoperoxidase technique. $\times 900$.

Czernielewski et al. (8) recently reported that assessment of ELC in sheets obtained by suction blister technique revealed no difference when uninvolved skin from psoriatic patients was compared with skin from healthy controls. The divergence from the results reported by Czernielewski et al. on one side and by Haftek et al. and by us on the other side may be caused by the different methods used for dermoepidermal separation. The suction blister technique might be a less suitable procedure to obtain epidermal sheets for ELC quantification, since the time necessary for epidermal separation (and thereby the time of suction) differs considerably from patient to patient and this might influence the ELC assessment.

Given the appropriate stimulus any skin site can develop lesions in psoriatics (10). Thus, biochemical, physiological and immunohistological examinations carried out on lesion-free skin may uncover clues to the cause of the disease. Several studies have revealed abnormalities in uninvolved psoriatic skin as e.g. elevated DNA synthesis and cell proliferation (11), increased cutaneous blood flow (12) and impaired contact hypersensitivity (13). Our findings of a reduced ELC number in uninvolved psoriatic skin support these investigations.

A substantial amount of information on the physiological and pathophysiological role of LC has been gained during the last few years (14). ELC seem to play an important role in cutaneous immune responses. Thus there is considerable evidence indicating that ELC have capacity for antigen presentation, as well as being capable of synthesizing immunomodulatory products such as interleukin-1 (14).

Since LC are involved in antigen presentation in contact hypersensitivity, our finding

might explain the observation made by Moss et al (13) of a significantly less responsiveness to DNCB in psoriatic patients as compared to controls.

ELC density has been found markedly decreased in anergic sarcoidosis patients in uninvolved skin as well as in their cutaneous lesions (15). Some investigators have studied the relationship between malignant skin disorders and ELC density and found an increased number in patients with basal cell carcinoma (16, 17) and mycosis fungoides (18, 19). LC are considered to participate in the immunological "surveillance function", and a decreased number of ELC in patients with psoriasis might therefore give rise to an increased chance of developing skin tumours. However, it has previously been suggested that patients with psoriasis may be less likely to develop skin and other cancers (20, 21). At present the pathogenetic background for these associations is unknown. Similarly, it is still unclear whether a decreased density of ELC in uninvolved skin of patients with psoriasis is of pathogenic significance for the disease.

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