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INVESTIGATIVE REPORT

Selective Persistence of Dermal CD8+ T Cells in Lesional Plaque Psoriasis after Clobetasol-17 Propionate Treatment

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In psoriasis, T-cell infiltration and epidermal hyperproliferation are key phenomena which are closely related. Our aim was to investigate the dynamics among T-cell subsets in relation to epidermal proliferation and clinical severity in psoriasis during treatment with an ultra-potent corticosteroid. Seven psoriasis patients were treated twice daily for 14 days with clobetasol-17 propionate ointment. Punch biopsies were taken at day 0, 3, 7 and 14. Epidermal proliferation marker Ki-67 and CD4+, CD8+, CD45RO+, CD2+ T cells were quantified by immunohistochemical techniques and image analysis. The clinical score declined significantly (60%; $p < 0.01$) and a 47% reduction of Ki-67+ nuclei was observed after only 3 days ($p < 0.01$). In the epidermis all investigated T-cell subsets were significantly reduced at day 14 ($p < 0.05$). In the dermis, treatment resulted in a significant decrease of CD4+, CD45RO+ and CD2+ T cells, but dermal CD8+ T cells persisted. In psoriasis, reduction of clinical severity and epidermal proliferation during the early phase of topical corticosteroid therapy cannot primarily be the result of decreased T-cell subsets. Furthermore, selective persistence of dermal CD8+ T cells was observed, which might be associated with disease relapse.
Key words: psoriasis; T-cell subsets; epidermal proliferation; corticosteroids.

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Key phenomena in the psoriatic plaque are accumulation of T-cell subsets and epidermal hyperproliferation with premature keratinization (1–3). In the past, the keratinocyte was believed to be an initial factor in the pathogenesis of psoriasis (4). However, in the last two decades it has been recognized that infiltration of the skin, mainly by T cells, precedes the changes in epidermal proliferation and differentiation (5–7). Biological treatments specifically targeted at T cells are effective in psoriasis, which demonstrates that T-cell involvement is not just an epiphenomenon (8–12).

It has been shown that CD4+ T cells (helper T cells) outnumber CD8+ cells (cytotoxic T cells) in the dermis, whereas CD8+ T cells are more abundantly present in the epidermis of the psoriatic plaque (13, 14). The majority of T cells are of the memory effector subset (CD45RO+) and, upon activation, these cells strongly express upregulated amounts of co-stimulatory molecule CD2 (15, 16). T-cell subsets, in particular CD8+, CD45RO+ and CD2+, appear in the early phase of the evolving psoriatic plaque, well before epidermal proliferation is disturbed (1, 17). However, the dynamics of changes in T-cell subsets and epidermal proliferation in response to treatments remain less well understood.

It has been proposed that hyper-responsiveness of psoriatic keratinocytes in reply to growth-promoting signals from T cells in inflamed skin could result in a hyperproliferative state of the epidermis (18, 19). It has been shown that psoriatic hyperproliferation results from increased recruitment of cycling epidermal cells and not from a decreased cell cycle time (20). Immunohistochemical assessment of cycling epidermal cells is possible with the mononuclear antibody against a nuclear epitope in proliferating keratinocytes (Ki-67). Nuclear staining with this antibody in the suprabasal layers indicates progression through the cell cycle (21).

Topical corticosteroid treatment is a first-line treatment for plaque psoriasis. Corticosteroids are believed to act through interference with gene transcription by binding the glucocorticoid receptor and certain DNA response elements, which account for the anti-inflammatory, immunosuppressive and anti-mitotic properties of these drugs in a broad sense: in general when given systemically and on diseased skin tissue when applied topically. Actions of glucocorticosteroids on inflammatory skin disease are numerous: effects on growth, differentiation and function of lymphocytes, inhibition of cytokine production, suppression of fibroblast, Langerhans' cells and endothelial cell function, inhibition of leucodiapedesis and an inhibitory effect on mitotic activity of keratinocytes have all been reported (22–31).

In order to gain more insight in the role of T cells in relation to epidermal proliferation in the pathogenesis of psoriasis, we set out a study to clarify the dynamics of corticosteroid-induced changes in the psoriatic plaque. In particular, we asked the question whether changes of relevant T-cell subsets anticipated or followed reduction of epidermal hyperproliferation and clinical severity.

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PATIENTS AND METHODS

Patients

Seven Caucasian patients with mild to moderate chronic plaque psoriasis participated in the present study. Approval by the ethics committee was obtained by the national Ethical Committee and all patients gave informed consent. Inclusion criteria were: no topical therapy for 2 weeks, no systemic anti-psoriatic therapy for 4 weeks, and the existence of one or more active psoriatic plaques with a diameter of at least 3 cm. Furthermore, patients did not use drugs that could interfere with the course of psoriasis (e.g. systemic corticosteroids, beta-blockers, anti-malaria agents or lithium). Patients did not have relevant co-morbidity and patients with psoriasis inversa were excluded.

Procedure and clinical outcomes

One solitary plaque of at least 3 cm in diameter was selected as target lesion. All patients were treated with clobetasol-17 propionate 0.5 mg/g (0.05%) ointment (Dermovate®, Glaxo, UK) for 2 weeks, twice daily, on all lesions except the face, scalp and intertriginous areas. In each patient, four 4 mm punch biopsies were taken from representative sites in the target lesion at 1 cm within the edge of the psoriatic lesion, at baseline (t=0), after 3 days (t=3), 1 week (t=7) and 2 weeks (t=14) of treatment, respectively. Prior to biopsy, local anaesthesia was given (xylocaine/adrenaline 1:100,000). Skin defects were closed with one suture. At each visit the severity scores (PASI for overall severity and SUM-score for the severity assessment of the target lesion) were assessed and signs of irritation were recorded. The SUM-score comprises the total score for erythema (0–4), scaling (0–4) and induration (0–4), whereas the Psoriasis Area and Severity Index (PASI) takes total body area involvement into account as well. PASI scores range from 0 to 72. Photographs of the target lesions were taken at baseline and at the end of the study.

Immunohistochemical staining

Biopsies were embedded in Tissue Tek OCT compound (Miles Scientific, Naperville, USA), instantly frozen in liquid nitrogen and stored at −80°C until use. Staining of the following T-cell subsets was performed: CD4+, CD8+, CD45RO+ and CD2+. The Ki-67+ nuclei were stained to assess epidermal proliferation. Sections were sliced 6 μm thick, air-dried for 30 min and then fixed in cold acetone for 10 min. Ki-67 sections were fixed in acetone-ether. After blocking endogenous peroxidase using 0.2% sodium azide for 5 min, they were washed in phosphate-buffered saline (PBS) for 15 min. Subsequently, the sections were incubated with the primary antibodies for 1 h. The following primary antibodies (mouse anti-human) were used, diluted in 1% bovine serum albumin (Sigma, St Louis, USA)/PBS: anti-CD2 (1:200) (clone MT910), anti-CD4 (clone MT310) (1:200), anti-CD8 (clone DK25) (1:200), anti-CD45RO (clone UCHL1) (1:100) and Ki-67 (clone MIB-1) (1:200), all obtained from DAKO (Copenhagen, Denmark).

Sections were washed in PBS for 15 min. Secondary IgG-labelled polymer, HRP anti-mouse EnVision+ (DAKO) was added for 30 min. The sections were washed again for 15 min in PBS. To visualize the staining we used AEC + High Sensitivity Substrate Chromogen for 10 min (DAKO). Counterstaining was performed with Mayer's haematoxylin (Sigma). The sections were washed in tap-water and dried. Finally, they were mounted in glycerol gelatin (Sigma).

In addition, a section from each patient was stained with haematoxylin-eosin. After dehydration in alcohol and histo-safe, these sections were mounted in Permount.

Quantification

Quantification of T-cell subsets was performed at ×200 magnification; CD4, CD8, CD45RO and CD2 positive cells in the epidermis were counted from the basement membrane up to the stratum corneum across the whole section (4 mm). Cells in the dermis were counted from the basement membrane down to 100 μm under the basement membrane and also across the whole section. Quantitative cell counts were expressed as 'positive cells per mm skin length'.

Image analysis

To analyse Ki-67+ cells, three representative digital photographs were taken at ×100 magnification. Each photograph was analysed using IP-lab software. A line, with known length and following the stratum basale, was drawn after choosing a representative 'region of interest' (ROI). All positive cells above this line were counted and expressed in the unit 'positive cells per mm length of basement membrane'.

Statistical analysis

All analyses were performed using Statistica® statistical software, version 6.0. To compare psoriasis severity scores and the number of positive cells at four different moments in time we performed one-way analysis of variance (ANOVA). If significant, Duncan's post hoc comparison was carried out. A p value of $<5\%$ denoted the presence of a statistically significant difference.

RESULTS

Patient population

Six men and one woman participated in this study. One of these patients had a wound infection at the site of the first biopsy. The data for this patient were not further analysed. Of six evaluable patients, the age was 51 ± 6 (mean $\pm$ SEM) years and reported history of psoriasis was a 31 ± 7 years.

Clinical response

The PASI at baseline was 10.7 ± 1.2, declining to 5.0 ± 1.0 after 2 weeks of therapy. SUM-scores of the target lesion were 8.8 ± 0.5 and 3.5 ± 0.4 at baseline and after therapy, respectively, reflecting a SUM-score reduction of 60%. PASI scores had declined significantly after 7 days ($p < 0.05$), whereas SUM-scores had already decreased significantly after 3 days (−23%; $p=0.01$).

T-cell infiltration

Mean T-cell counts per mm section length are depicted in Fig 1. The dermis of untreated psoriatic skin showed counts of CD4+, CD45RO+ and CD2+ T cells of 110 ± 18, 145 ± 40 and 127 ± 41, respectively. In contrast, the count of CD8+ T cells in untreated dermis of psoriatic skin was only 27 ± 9 per mm.

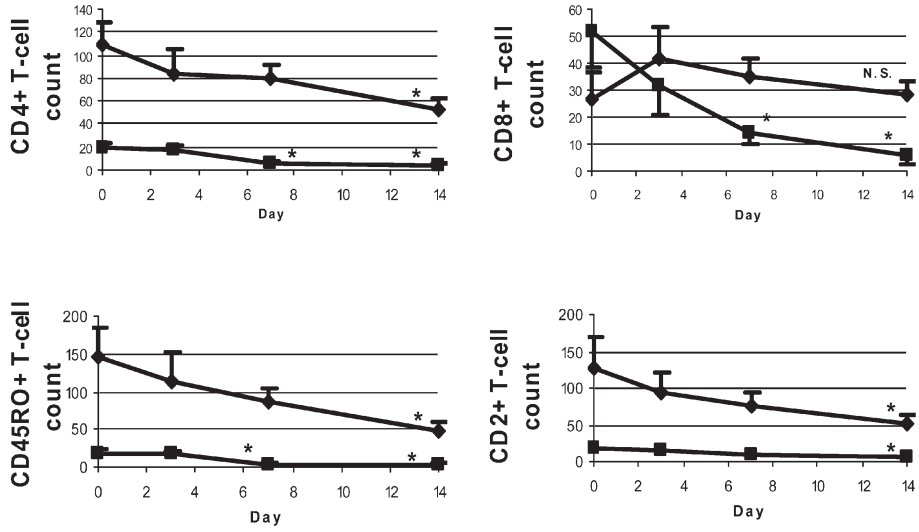

Fig. 1. T-cell counts (CD4+, CD8+, CD45RO+ and CD2+) per mm section length (mean ± SEM) in dermis (◆) and epidermis (■) during 2 weeks of topical treatment with clobetasol-17 propionate ointment, twice daily. *Statistically significant difference compared to baseline; NS, not significant.

In the epidermis of untreated psoriatic skin, however, the CD8+ T-cell count was high (52 ± 13 per mm), whereas the CD4+, CD45RO+ and CD2+ T-cell counts were 20 ± 4, 19 ± 5 and 19 ± 5 per mm section, respectively.

In the dermis, CD4+, CD45RO+ and CD2+ T cells showed a substantial reduction, reaching 53%, 67% and 59% of their baseline values after 14 days of treatment. All these reductions were statistically significant ($p < 0.05$). In contrast, CD8+ cells in the dermis did not show any tendency to reduce during treatment, as illustrated by representative histology in Fig. 2. At 3 and 7 days of treatment none of the dermal T-cell subsets showed a statistically significant decrease.

In the epidermis, all the T-cell subsets showed a substantial decrease after 14 days' treatment, reaching 79% (CD4+), 89% (CD8+), 82% (CD45RO+) and 69% (CD2+), respectively, of the baseline values. After 7 days' therapy CD4+, CD8+ and CD45RO+ T cells had already reached a statistically significant reduction, but at 3 days' treatment no T-cell subset showed a statistically significant change as compared to baseline.

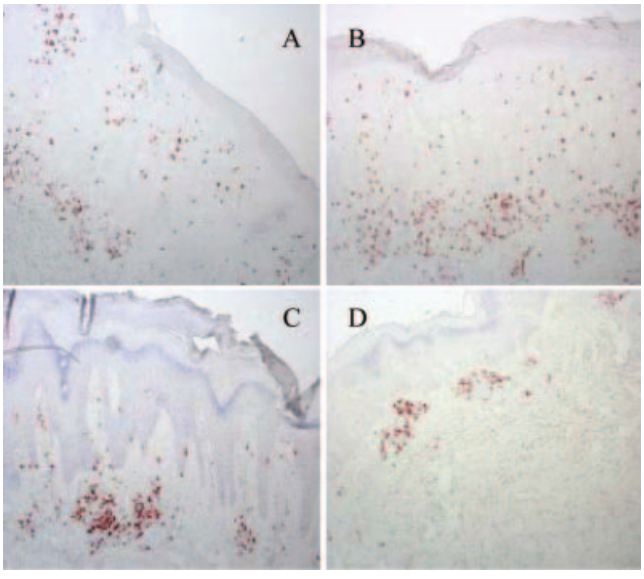

Fig. 2. Staining for CD8+ T cells in skin biopsies after 0 (A), 3 (B), 7 (C) and 14 (D) days treatment with clobetasol-17 propionate ointment, showing selective persistence of CD8+ T cells in the dermis.

Epidermal proliferation

The number of Ki-67 positive nuclei per mm basement membrane was 247 ± 26 at baseline. After 3, 7 and 14 days the number of Ki-67+ nuclei was 132 ± 28, 115 ± 21 and 31 ± 11, respectively (all values $p < 0.01$ compared to baseline).

CD8+ T cells and SUM-scores

The dermal T-cell count and individual SUM-scores of the target lesion did not show a significant correlation (r = 0.19). In contrast, the number of epidermal CD8+ T cells, together with the other T-cell markers, correlated much better with the SUM-score value (r = 0.66).

DISCUSSION

The present study describes the time course of T-cell subset changes in relation to epidermal proliferation during a 2-week treatment course with clobetasol-17 propionate ointment.

After 2 weeks' treatment six evaluable patients had a mean PASI decrease of 54% and mean reduction of the

SUM-score of 60%. This was accompanied by an 87% reduction in Ki-67+ cells in epidermis, 53–67% reduction of CD4+, CD45RO+ and CD2+ T cells in the dermis and 69–89% decline in CD4+, CD8+, CD45RO+ and CD2+ T cells in the epidermis.

These findings confirm and extend the results of previous studies. For example, De Jong et al. (31) found a significant PASI reduction and a significant reduction in epidermal proliferation, measured by Ki-67+ nuclei, after 1 week's treatment with budesonide ointment. However, no significant reduction of T cells was observed. In another study, patients who were treated with betamethasone dipropionate showed significant reductions of SUM-score, epidermal proliferation (Ki-67) and a non-significant tendency to a decrease of T-cell subsets (32). Although betamethasone dipropionate and clobetasol-17 propionate are both topical corticosteroids, one is class III and the other is class IV. In the study by Vissers et al., all T-cell subsets declined and statistical significance was almost reached (32). In our opinion, the differences between the present study and the previous studies are explained by differences in corticosteroid potency, patient populations, individual variation in response to corticosteroids, and possibly the sites of biopsy.

While significant reductions of the SUM-scores (−23%) and number of Ki-67+ nuclei after 3 days was already 47%, the declines after 3 days in epidermal and dermal CD4+, CD8+, CD45RO+ and CD2+ T-cell counts were minor and not statistically significant from baseline.

The highly significant reduction in SUM-score and epidermal proliferation (Ki-67+ nuclei) in the early phase of clobetasol-17 propionate treatment thus cannot be the result of a reduction in T-cell subsets. Instead, various studies have demonstrated a direct inhibitory effect of corticosteroids on mitotic activity of keratinocytes *in vitro* and *in vivo* (29, 30). Therefore, combination therapies with topical corticosteroids and drugs that exclusively block T-cell activation, such as alefacept, might be very effective in the treatment of psoriasis (8–12).

We found CD8+ T cells in the epidermis of untreated psoriatic plaques to be predominant, whereas other subsets were mainly found in the dermis. This finding is a reconfirmation of other studies (1–3, 13, 14). A remarkable observation, however, was the persistence of CD8+ T cells in the dermis after 2 weeks of therapy. To the best of our knowledge this observation has not been reported earlier. It is attractive to speculate that the persistence of CD8+ cells in the dermis may be related to the relatively rapid relapse that may occur after cessation of treatment with ultra-potent topical corticosteroids.

An earlier study showed a pronounced reduction of dermal CD8+ T cells and CD45RO+ T cells after treatment with calcipotriol ointment (32). These differences between clobetasol-17 propionate and calcipotriol, with respect to the effect on different T cells, may explain the superb efficacy of combined treatments of topical corticosteroids and vitamin D_3 derivatives in psoriasis (33–35).

In the past, experiments with transgenic (SCID) mice showed that CD4+ T cells (but not CD8+ T cells) injected into human skin explants induced psoriatic lesions (36). Recently, it has been postulated that CD4+ T cells may be necessary for providing critical inductive and helper signals, while CD8+ T cells act as principal effector cells in the pathogenesis of psoriasis (2). In fact, it has been shown that depletion of regulatory T cells (CD4+CD25+) induces a remarkable clonal expansion of CD8+ cells in the skin (2, 37). It might therefore be interesting to study the role of regulatory T cells, and especially their interaction with the CD8+ T cells, during treatments for plaque psoriasis.

In conclusion, in lesional psoriatic skin, the reduction of clinical activity and epidermal proliferation in the early phase of topical corticosteroid therapy cannot primarily result from a reduction of T cells. Furthermore, the specific persistence of dermal CD8+ T cells after corticosteroid therapy might account for a relatively rapid relapse after cessation of ultra-potent corticosteroid therapy.

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