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A Clinical and Flow Cytometric Model to Study Remission and Relapse in Psoriasis

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The aim of the present study has been to analyse remission and relapse characteristics in psoriasis vulgaris. In 15 patients, two different psoriatic lesions (clinical and flow cytometric study) were treated with clobetasol propionate until clearance for maximally 23 days. In the clinical study only cleared lesions were divided into three test sectors with different post-clearance treatment: (1) alcoholic solution under occlusion, (2) occlusion only, and (3) no treatment. In the flow cytometric study, biopsies were taken from the test lesion before clobetasol therapy (i), at clearance (ii), and at relapse from both visibly affected and unaffected skin (iii, iv). Epidermal proliferation, differentiation and inflammation were quantified by multiparameter flow cytometry.

The clinical evaluation worked well and could discriminate between the different therapy modalities. After 28 days, 80% of untreated sectors showed a relapse. Occlusion decreased this percentage to 50%. Application of the alcoholic solution further decreased this percentage to 30%. The flow cytometric analysis demonstrated a very low proliferative activity of the basal compartment at clearance. This activity was higher in the visibly unaffected skin at relapse, whereas highest values were assessed in the affected skin at relapse. Interestingly, at relapse the proliferative activity in the suprabasal compartment of the visibly unaffected skin had increased to values identical to the affected skin.

The present model allows standardized comparison of different approaches for maintenance therapy in psoriasis vulgaris. We demonstrate that occlusion has an inhibitory effect on the tendency to relapse after successful treatment with clobetasol propionate. Quantitative information on remission and relapse of psoriasis can be obtained by multiparameter flow cytometry.

Key words: cytometry; hyperproliferation; psoriasis; relapse; therapy.

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Psoriasis is a chronic disease with exacerbations and remissions, often requiring long-term treatment. To be able to design optimal approaches for maintenance therapy, the study of early processes in the development of the psoriatic plaque is essential. It is well known that great differences exist between the acute and chronic psoriatic lesion. Early lesions are characterized by the presence of large numbers of polymorphonuclear leukocytes, monocytes and mast cells, whereas these cells appear only modestly in the chronic plaque (1–3). Furthermore, the advancing edge of the psoriatic plaque does not yet contain skin-associated antileukoproteinase, whereas the central zone of the plaque does (4). It is important to realize that the distinction between the acute and chronic stages of the psoriatic lesion is of therapeutic relevance. This is underlined by the fact that acitretin monotherapy is poorly effective in chronic plaque psoriasis but very effective in acute instable psoriasis (5).

However, a standardized model for studying remission and relapse characteristics of psoriasis is not available. Such a model would allow quantitative comparison of different antipsoriatic treatment modalities. Therefore, the aim of the present study was to provide a standardized clinical and flow cytometric model for analysis of relapse following corticosteroid induced clearing of a psoriatic lesion.

The clinical study consisted of relapse analysis of three post-therapy conditions, i.e. no treatment, occlusion or occlusion with an alcoholic solution. This alcoholic solution was chosen in preparation for a clinical study with a specific active agent to be administered in the alcoholic solution. The following questions were addressed: what are the dynamics of reoccurrence of erythema, induration and desquamation under each of the above-mentioned post-therapy conditions, using a semiquantitative 5-point scale? Can a relapse clearly be defined by clinical parameters and can the clinical evaluation be used to differentiate between the different post-therapy conditions?

In the flow cytometric study, quantitative parameters for epidermal proliferation, differentiation and inflammation were assessed in epidermal cell suspensions prepared from biopsies taken from the same patients before therapy, at clearance, and at relapse from pinpoint lesions, and from the visibly unaffected skin of the cleared lesion (6–8).

MATERIALS AND METHODS

Subjects and test lesions

Nine male and six female patients aged 18–70 years (mean 54 years) with chronic plaque psoriasis for 4 to 60 years were recruited after written informed consent was obtained. The patients had to have at least two lesions with a minimal size of 10 cm^2 not directly located above the elbow or knee. The score for erythema, thickness, and scaling had to be at least grade 2 (assessment on a 5-point scale: 0 = not present, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). Exclusion criteria were pregnancy or lactation and known allergy to the test medications: clobetasol 17-propionate, the alcoholic solution or to the hydroactive dressing. Systemic treatment with antipsoriatic or immunosuppressive drugs and UVB therapy were not allowed.

A clinical test lesion and a flow cytometric test lesion were selected in each patient. Both lesions were treated twice daily with clobetasol 17-propionate lotion (Glaxo Wellcome, Nieuwegein, The Netherlands) under hydroactive occlusion (Cutinova®Thin, Beiersdorf, Hamburg, Germany) until clearance or for maximally 23 days. Clearance was defined as no induration, no desquamation and no or only a mild

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erythema (grade 1). Patients whose lesions did not clear by day 23 were excluded from further participation.

Clinical study

The study design was an open intraindividual comparison. For the clinical evaluation, three sectors – each 1.8 cm^2 in size – were marked in the area of the healed test lesion. Sector 1 was treated once daily with an alcoholic solution (Schering AG, Berlin, Germany) containing 5.0 g isopropylmyristat and 74.0 g isopropanolol in 100 ml solution under hydroactive occlusion, sector 2 with hydroactive occlusion and sector 3 was left untreated and unoccluded. Patients visited the department on days 7, 11, 14, 17, 20, 23, 26, and 29 (end of study) after clearance for evaluation of clinical scores, itching, burning and adverse events. Relapse was defined as an at least grade 2 erythema with additionally at least grade 2 thickness and/or scaling. Photographic documentation was performed at recruitment, at clearance, at relapse and at the end of study.

Flow cytometric study

From the test lesion chosen for the flow cytometric study, 3-mm punch biopsies were obtained (*i*) before treatment with clobetasol 17-propionate, (*ii*) at clearance, (*iii*) at relapse from pinpoint lesions, and (*iv*) from the visually unaffected skin of the former lesion for flow cytometric analysis. After healing, the cleared lesion was left untreated and unoccluded and observed for a period of 35 days. The observation period in the flow cytometric study was 1 week longer compared to the clinical study in order to permit a further development of initial psoriatic lesions in this experimental group. Relapse was defined as the clinical occurrence of pinpoint lesions in the area of the cleared lesion. Clinical scores were assessed before treatment and at clearance.

Epidermal single cell suspensions were prepared shortly after biopsying using the combined thermolysin-trypsin separation method as described previously (9) and stored in 70% ethanol at $-20°C$ until staining. Approximately $1–2 \times 10^5$ cells were simultaneously labelled with the DNA fluorochrome TO-PRO-3 iodide (TP3, Molecular Probes, Eugene, U.S.A.) and mouse antibodies directed against intermediate filaments vimentin (Vim3B4, IgG_{2a}, Novocastra Laboratories Ltd., Newcastle upon Tyne, U.K.) and keratin 10 (RKSE60, IgG_1, Department of Molecular Biology, University of Maastricht, The Netherlands). Emitting fluorochromes were TP3 in combination with fluorescein-isothiocyanate (FITC) and phycoerythrin (PE), which were conjugated to subtype-specific monoclonal goat antibodies against mouse IgG_{2a} and mouse IgG_1, respectively (Southern Biotechnology Associates, Birmingham, U.S.A.).

From each sample 5,000–10,000 gated cells were measured and analysed using an EPICS® Elite flow cytometer (Coulter, Luton, U.K.) equipped with a dual laser system. PE and FITC were excited with an air-cooled argon ion laser (15 mW, 488 nm). TP3 was excited with a HeNe laser (10 mW, 633 nm). Fluorescence was measured using bandpass filters of 520–530 nm (green, FITC), 555–595 nm (orange, PE), and 670–680 nm (red, TP3). The area/peak ratio of the red signal (DNA) was used to discriminate between doublets of diploid cells (clumps) and real single tetraploid cells (10). After setting appropriate gates with the EPICS® Elite software, percentages of vimentin positive and keratin 10 positive cells were calculated. Using Multicycle™ software (Phoenix Flow Systems, San Diego, U.S.A.) the percentages of basal (vimentin negative and keratin 10 negative) and suprabasal keratinocytes (vimentin negative and keratin 10 positive) in S- and G_2M phase (proliferation) of the cell cycle were calculated from DNA histograms.

Statistical analysis

Paired values were analysed with the paired two-sample *t*-test for means. For unpaired data the two-sample *t*-test assuming equal variances was used. With ANOVA (single-factor) durations of remission were compared. ANOVA (two-factor) and Duncan's multiple range test were performed to analyse clinical scores.

RESULTS

Clinical study

In 10 out of the 15 patients the test lesions could be cleared within the 23 days allowed for the clobetasol treatment. The mean clearing time was 16.4 ± 1.8 days (range 8–23 days). The cleared lesion was divided into three sectors and monitored over a period of 28 days. One patient did not appear for observation after day 23. This patient had a relapse in all three sectors on day 11 and the results were included in the evaluation. In the observation period of 28 days the relapse percentage was 30% (3 patients) in sector 1 (alcoholic solution with occlusion), 50% (5 patients) in sector 2 (occlusion only), and 80% (8 patients) in sector 3 (no treatment). Intraindividual comparison of the three sectors showed that relapse always occurred earlier or at the same time in sector 3 compared to sector 2, and earlier or at the same time in sector 2 compared to sector 1.

Fig. 1a–c summarizes the assessment of clinical scores for erythema, induration and desquamation during the study. Occlusion has an inhibitory effect on erythema, induration and desquamation. However, the effect is more pronounced with respect to induration and desquamation compared to erythema. Comparing the alcoholic solution under occlusion with occlusion alone, there is only a significant difference for the desquamation score at the end of the study.

Flow cytometric study

Thirteen of 15 patients participated in the flow cytometric study. One patient preferred not to be biopsied, and in the other patient no suitable test area was left for the flow cytometric study. Clearance was reached in 11 of the 13 test lesions (mean time 18.1 ± 1.9 days; mean ± SEM). In the observation period of 35 days, 7 (64%) of the 11 cleared test lesions relapsed. The mean time until relapse was 17.9 ± 4.4 days (mean ± SEM). Although the durations of the remission periods of the untreated test lesions from both the clinical and the flow cytometric study differed substantially in some patients (up to 23 days), no significant differences for the whole groups existed (ANOVA single-factor analysis).

The results of the flow cytometric analyses on 36 samples are summarized in Fig. 2a–d. At the beginning before treatment with clobetasol the psoriatic lesions were characterized by a high percentage of vimentin-positive cells, a low number of keratin 10 positive keratinocytes and a high number of basal keratinocytes in S- and G_2M phase. At clearance, a marked and significant improvement of all markers was found. In the basal and suprabasal compartments proliferation was substantially decreased.

At relapse from each test lesion two biopsies were obtained: one from the pinpoint lesions and one from the visibly unaffected skin of the cleared lesion. The flow cytometric analysis of the pinpoint lesions revealed a return to the psoriatic phenotype for all markers. No significant differences existed between the psoriatic lesions before treatment and the pinpoint papules at relapse. Significant differences were observed between the percentage of keratin 10 positive keratinocytes at clearance and the visibly unaffected skin of the

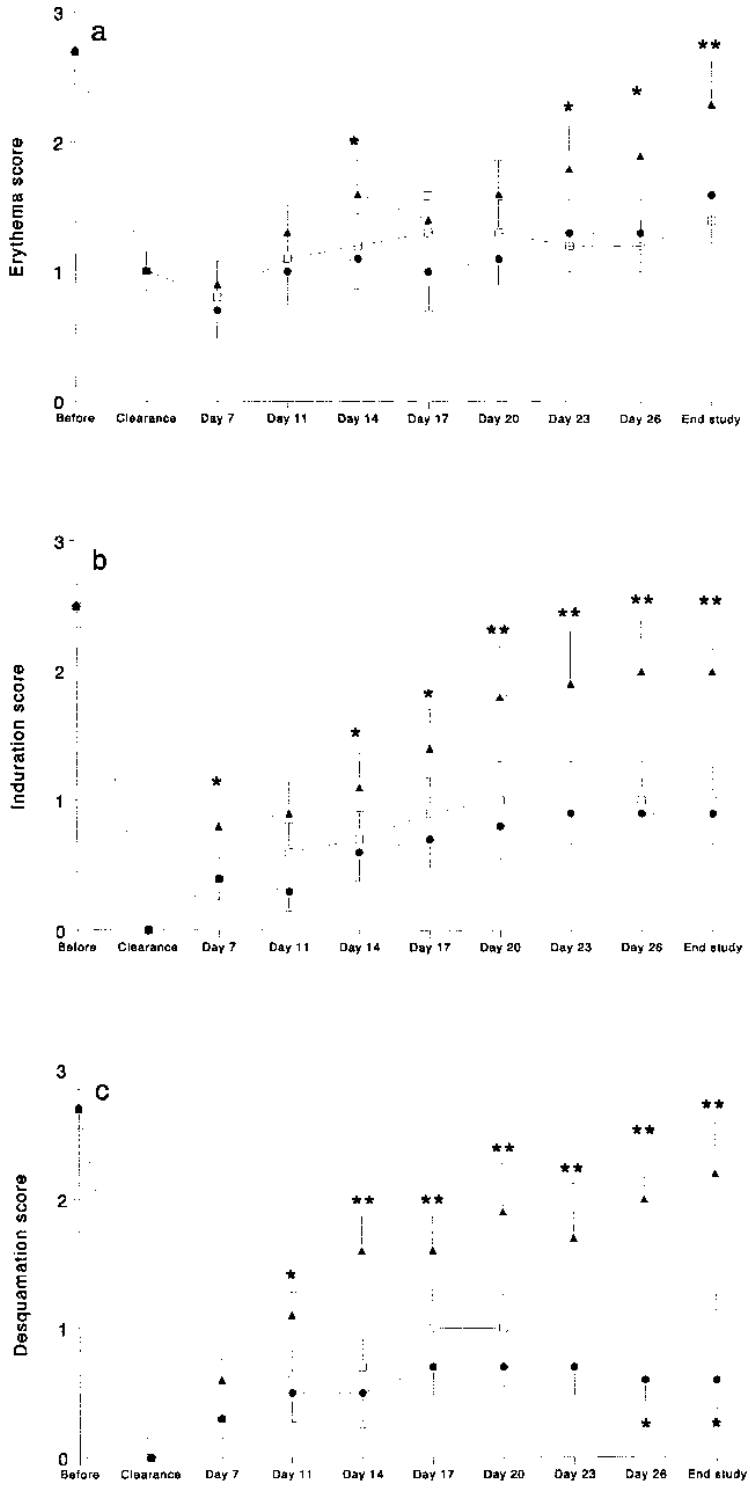

Fig. 1a–c. Clinical scores (mean ± SEM) assessed for erythema (*a*), induration (*b*), and desquamation (*c*) of 10 psoriatic lesions during treatment and remission period at different test sectors: sector 1, alcoholic solution and occlusion (●); sector 2, hydroactive occlusion only (□); sector 3, no occlusion (▲). Significance is expressed as compared to occlusion only: *($p < 0.05$) and **($p < 0.01$).

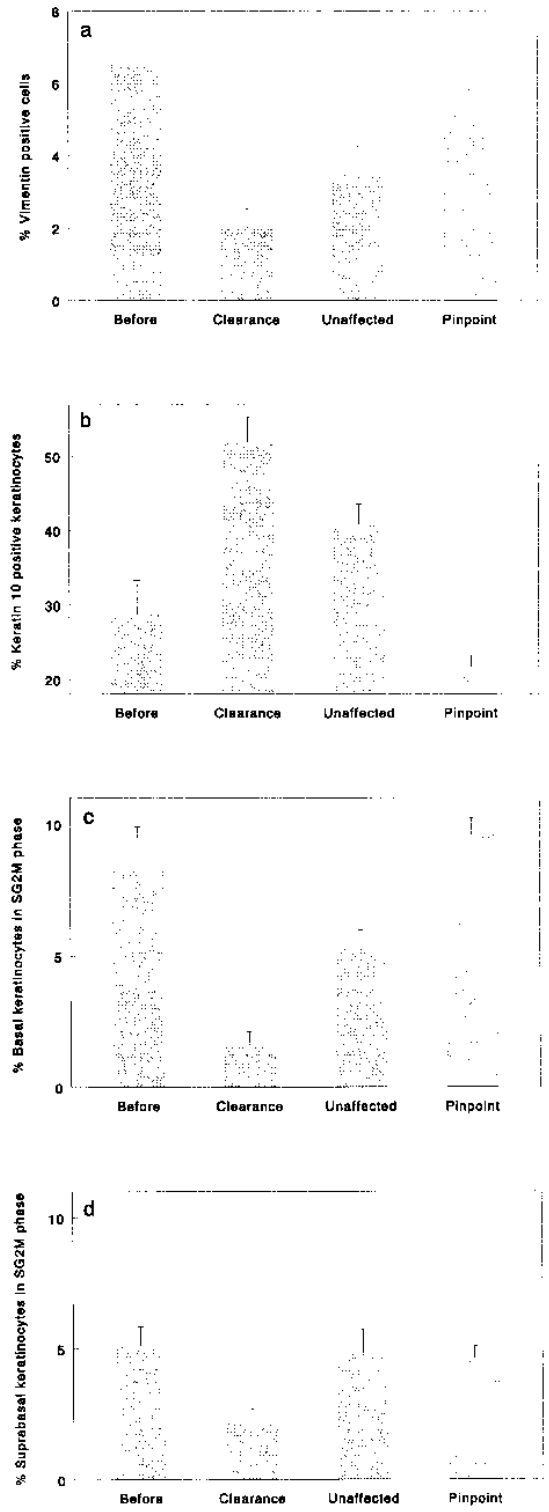

Fig. 2a–d. Flow cytometric analysis (mean ± SEM) of markers for percentage vimentin-positive cells (*a*), percentage keratin 10 positive keratinocytes (*b*), and percentage keratinocytes in S- and G_2M phase in basal (*c*) and suprabasal (*d*) compartments.

cleared lesion at relapse ($p<0.05$). In the visibly unaffected skin of the cleared lesion at relapse the percentage of keratinocytes in S- and G_2M phase was significantly higher compared to clearance in the basal ($p<0.01$) and in the suprabasal ($p<0.05$) compartment. Also, with respect to the relative number of vimentin-positive cells in the visibly unaffected skin of the cleared lesion at relapse showed values that were intermediate between the situation at clearance (N.S.) and relapsed skin ($p<0.05$). All flow cytometric parameters, including the proliferative activity in the basal compartment, were significantly different between the involved and the visibly unaffected skin of the cleared lesion at relapse. Remarkably, at relapse the proliferative activity in the suprabasal compartment in both the pinpoint and the visibly unaffected skin was comparable. In Fig. 3 we compared clinical features of inflammation (erythema), impaired differentiation (desquamation) and hyperproliferation (induration) with the cell biological markers of these processes. It can be seen that in all cases a strong correlation exists between clinical and flow cytometric parameters (Fig. 3a, erythema score versus percentage vimentin-positive cells: $r=0.76$, $p<0.0001$; Fig. 3b, desquamation score versus percentage keratin 10 positive keratinocytes: $r=-0.73$, $p<0.0001$; Fig. 3c, induration score versus percentage basal keratinocytes in S- and G_2M phase: $r=0.74$, $p<0.0001$).

DISCUSSION

In the present study we provide a model for studying the dynamics of the fast relapsing psoriatic lesion after successful treatment with clobetasol applied twice daily under occlusion. Flow cytometric analysis of the samples permitted quantitative measurement of the epidermal changes with respect to proliferation, differentiation and inflammation.

Defining clinical clearance as maximally grade 1 erythema, no induration and no desquamation, we observed a mean clearing time of 17.3 days (range 7–28 days, $n=21$). Other studies describe clinical healing after 6–10 days (3) and 12 days (11) after treatment with clobetasol propionate under occlusive dressings. In one study (3), however, clearance criteria allowed a grade 3 erythema and in the other study (11) these criteria were not mentioned. In most studies the clearance percentage after treatment with clobetasone propionate under occlusive dressings has been reported to be 100%. In contrast, the present study revealed a percentage of clearing in only 75% after 3 weeks of treatment with clobetasone propionate twice daily under occlusive dressings.

All markers for epidermal proliferation, differentiation and inflammation significantly decreased during remission induction. Epidermal proliferation was even totally blocked in some patients, as also shown by Goodwin (12). This implies that the situation at clearance was substantially different from the situation before therapy and constituted a suitable starting point for studying the process of change towards the psoriatic phenotype. In comparison to treatment with clobetasol under occlusive dressings, treatment of psoriatic plaques with the vitamin D_3 analogues Tacalcitol® (ointment 4 μg/g) once daily (8) or calcipotriol (cream 50 μg/g) twice daily (13) interfered predominantly with epidermal proliferation, resulting in a reduction of the number of basal keratinocytes in S- and G_2M phase by 34%. Vitamin D_3 analogues affected epidermal differentiation and inflammation less vigorously.

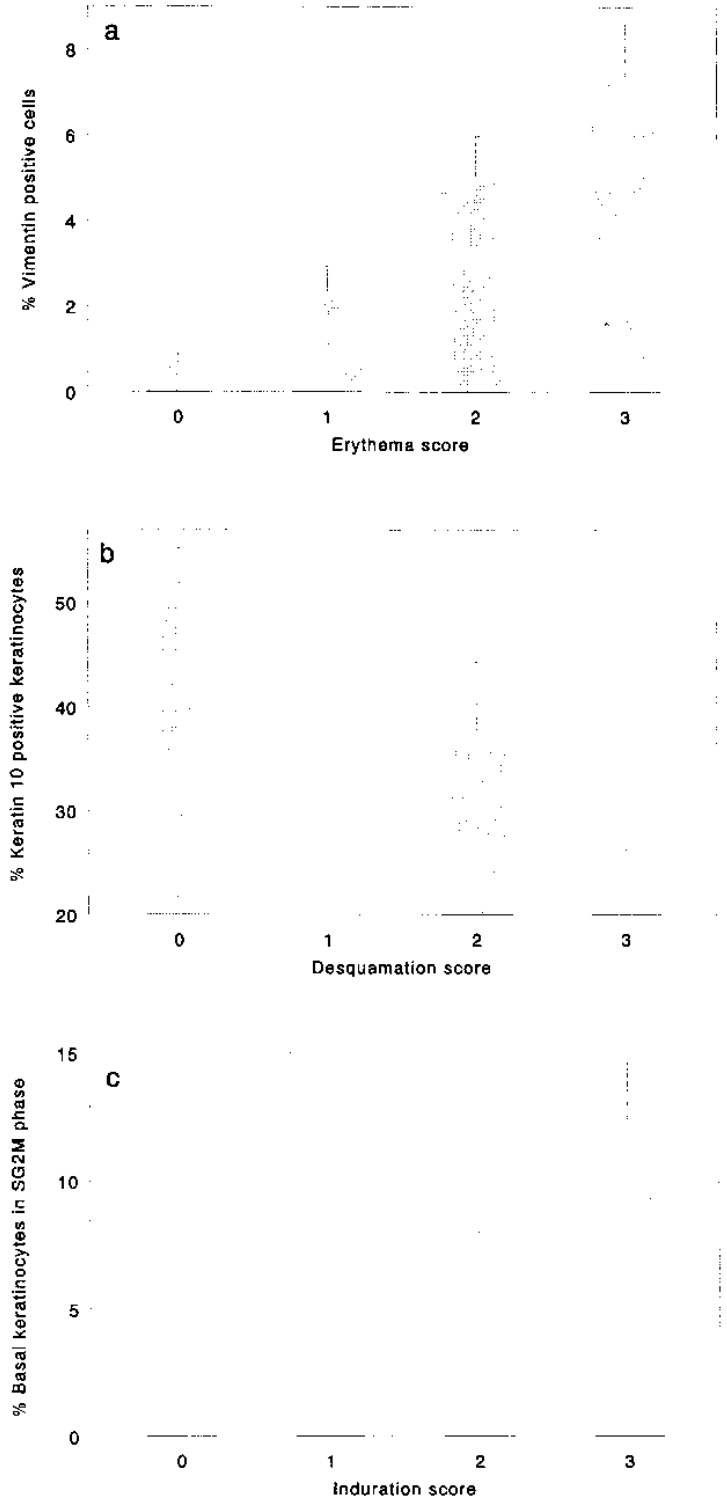

Fig. 3a–c. Correlation between erythema and percentages of vimentin-positive cells (*a*), desquamation and percentages of keratin 10 positive keratinocytes (*b*), and induration and percentages of basal keratinocytes in S- and G_2M phase (*c*) in psoriatic lesions before and after clearance with clobetasol propionate lotion under hydroactive occlusion.

The 28-day observation period in the clinical part of the study proved to be appropriate in showing differences between different treatment modalities. The occlusion not only reduced the number of relapses but also had significant influence on the clinical scores for erythema, induration and desquamation. The combination of the alcoholic solution with hydroactive occlusion further reduced the number of relapses compared to occlusion only. In particular, the effect on desquamation of the lesion was marked (Fig. 1c). No adverse events were encountered in association with the use of the alcohol solution. Furthermore, no influence on burning or itching of the lesion was reported. Therefore, the alcohol solution seems a safe vehicle for future research with active agents. The relapse periods of the untreated sectors of the cleared lesion from the clinical part and the cleared lesion from the flow cytometric part showed no significant intraindividual variation, which implies that within-patient comparison of topical treatment is justified.

To study the early development of psoriatic lesions different aproaches have been used in the past. Early lesions such as pinpoint papules, the margins of spreading plaques and relapsing psoriasis have been studied as models for investigating this process. Influxes of polymorphonuclear leukocytes, monocytes and mast cells have been described as early events in developing lesions (1–3). In spreading plaques and pinpoint lesions, dermal or vascular changes have been described to precede epidermal changes (14–16). One report, however, showed epidermal differentiation to be altered prior to vascular changes in the spreading psoriatic plaque (17).

The present evaluation provides a new approach for studying an early age of the psoriatic lesion: analysis of the visibly unaffected skin of a healed lesion adjacent to newly developed pinpoint lesions. The number of non-keratinocytes in the ''unaffected skin at relapse'' is not significantly different compared to the situation at clearance. In this ''unaffected stage of relapsing skin'' the percentage of cells in S- and G_2M phase in the basal cell population is intermediate between percentages at clearance and percentages of lesional skin. However, the percentage of suprabasal keratinocytes in S- and G_2M phase in unaffected relapsing skin was already substantially increased with values comparable to lesional skin. This model shows that the proliferative activity in the suprabasal compartment is an early aspect of epidermal proliferation in the pathogenesis of psoriasis.

In summary, we present a clinical and flow cytometric model by which to analyse clearance and relapse characteristics of the psoriatic lesion. It is attractive to speculate that this model will provide a new approach for comparing different antipsoriatic maintenance therapies *in vivo* under standardized circumstances. Occlusion of psoriatic plaques with hydroactive dressings delays the process of relapse. Multiparameter flow cytometry provides quantitative information on these processes with respect to epidermal proliferation, differentiation and inflammation.

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