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Multiparameter flow cytometry as a tool to evaluate antipsoriatic therapy

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Summary

Objective comparison of different antipsoriatic therapies requires quantitative assessment of disease severity. However, clinical assessment with the widely used Psoriasis Area and Severity Index (PASI) introduces inaccuracy. An alternative is the quantitative analysis of different epidermal cell parameters using multiparameter flow cytometry. Our aim in the present study was to compare the clinical and flow cytometric approach to monitor disease activity and to evaluate antipsoriatic efficacy. Clinical scores for erythema, induration and scaling were assessed and biopsies for flow cytometric analysis were obtained from the psoriatic plaques of 89 patients before and after treatment with different therapeutic regimens consisting of vitamin D₃ analogues and corticosteroids. In total, 219 epidermal cell suspensions were analysed using triple-labelling, with the simultaneous staining of markers for epidermal proliferation (DNA dye TO-PRO-3), differentiation (antikeratin 10), and inflammation (antivimentin).

Correlation analysis was performed on 166 paired values obtained from 83 patients. A highly significant correlation was observed between erythema and the percentage of vimentin-positive cells, between scaling and the percentage of keratin 10 positive keratinocytes, and between induration and the number of basal keratinocytes in the S- and G₂M phase, when all 166 biopsies were assessed. The correlation remained in the same range if the analysis was restricted to the 83 pretreatment biopsies. In contrast to the clinical scores, the flow cytometric analysis permitted a clear separation between the antiproliferative and anti-inflammatory or keratinization-enhancing effects of antipsoriatic treatment. The vitamin D₃ analogues proved to exert a mainly antiproliferative effect. The combination of calcipotriol and a topical corticosteroid improved all cell biological markers substantially, and clobetasol monotherapy had a powerful effect on these markers. In conclusion, multiparameter flow cytometry has been shown to be a sensitive tool to evaluate the growth inhibiting, anti-inflammatory and keratinization-enhancing effects of antipsoriatic therapies.

In the evaluation of antipsoriatic therapies, quantitative assessment of disease severity is essential. A popular and frequently used method for clinical assessment is the Psoriasis Area and Severity Index (PASI). In this scoring system, described by Fredriksson and Pettersson,¹ the percentage involvement and degree of erythema, induration and desquamation is estimated in four body areas. Using a formula, an ordinal value between 0 and 72 can be calculated. However, the authors stated that the PASI score should not to be regarded as an 'exact' numerical value, as the severity rating is subjective. Marks *et al.*² demonstrated a wide interobserver error of even well-designed clinical assessment techniques, especially with respect to the calculation of the involved areas. Various groups have emphasized the potential for reaching inaccurate conclusions when

clinical assessment alone is used.^{3,4} Therefore, there is clearly a need for objective measurements of psoriasis severity.

Flow cytometry permits quantitative analysis of different cell parameters and can be applied to epidermal single-cell suspensions prepared from normal and diseased skin.⁵ In analogy to the clinical signs which reflect the pathological changes in psoriasis, we developed a triple-labelling procedure with simultaneous staining of markers for epidermal proliferation (DNA content), differentiation (antikeratin 10), and inflammation (antivimentin).^{6,7} Recently, with the development of a dermo-epidermal separation method using thermolysin, it has become possible to perform this flow cytometric procedure on cell suspensions prepared from 3 mm punch biopsies.⁸

In the present study we have analysed 219 epidermal single cell suspensions prepared from keratome and punch biopsies of psoriatic plaques from 89 patients before and after treatment with different therapy regimens consisting of vitamin D₃ analogues and topical corticosteroids. Our aim in the study was to compare the clinical and flow cytometric approaches in monitoring disease activity and to establish whether flow cytometry is a valuable tool to evaluate and compare antipsoriatic therapies. We addressed in particular: to what extent the clinical scores for erythema, induration and desquamation correlate with the percentage of vimentin-positive cells, the percentage of basal keratinocytes in the S- and G₂M phase and the percentage of keratin 10 positive keratinocytes; to what extent the improvement of clinical parameters reflects a change in the cell biological markers; whether the method of preparation of cell suspensions influences the association of clinical and flow cytometric scores; and whether clinical and flow cytometric scores vary with different antipsoriatic therapies.

Materials and methods

Patients, treatment regimens and biopsy procedures

Data from 89 patients in total with psoriatic plaques were analysed. Patients' details have been described in our flow cytometric studies on the treatment of psoriatic plaques with 1 α ,24 dihydroxyvitamin D₃ ointment (Tacalcitol[®]), 4 μ g/g applied once daily for 8 weeks or placebo,⁷ calcipotriol, 50 μ g/g once daily, twice daily or in combination with the topical corticosteroids clobetasone 17-butyrate, 0.5 mg/g, and betamethasone 17-valerate, 1 mg/g, for 8 weeks,⁹ and after treatment with clobetasol 17-propionate (unpublished data). Before the wash-out period of 2 weeks, representative psoriatic lesions were selected. Clinical scores for erythema, induration and desquamation of these test lesions were assessed, on a five-point scale, before and after treatment. Scoring was performed by one investigator only, to avoid the interobserver error described by Marks *et al.*² Skin samples of the selected test were obtained simultaneously with clinical scoring. In this way 219 single-cell suspensions, derived from 152 keratome biopsies and 67 punch biopsies, were prepared for flow cytometric analysis.

Single-cell suspensions and staining

Epidermal cell suspensions from keratome biopsies

were prepared using a trypsin separation method. The cell isolation procedure on punch biopsies consisted of dermo-epidermal separation with thermolysin and subsequent incubation with trypsin. In flow cytometric studies of epidermis, the quality of cell suspensions is essential. As enzymatic and mechanical methods are used to separate the cells, membrane antigens are easily damaged. Therefore, we decided to use a multiparameter technique with simultaneous quantification of the DNA content and of the intermediate filaments. These filaments are resistant to breakdown and antibodies are well-studied. Several keratins are highly expressed in the epidermis. Keratin 10 is a well-known epidermal differentiation marker. Vimentin is expressed in all epidermal mesenchymal cells, i.e. dendritic cells and (especially in psoriatic skin) inflammatory infiltrate cells. A detailed description of both cell isolation methods and the staining procedure for multiparameter flow cytometric analysis have been published.⁸ After isolation, cells were fixed in 70% w/v ethanol until required for staining.

Multiparameter flow cytometry by the simultaneous measurement of fluorescein-isothiocyanate (FITC), phycoerythrin (PE) and propidium iodide (PI) is considered difficult because of the large spectral overlap of PE and PI. Hoechst 33342 and 7AAD have been proposed as alternatives to PI in multiparameter flow cytometry. However, in our hands, 7AAD gave unacceptably high coefficients of variation (CVs). Hoechst 33342 staining requires an expensive ultraviolet laser, which is usually not present in commercial flow cytometers. On the contrary, many research flow cytometers are equipped with a low-power HeNe laser (633 nm), which excites TO-PRO-3 iodide (TP3) (peak absorbance at 642 nm). The quality of staining with TP3 has been extensively studied by our group.⁶ At a concentration of 1 μ mol/L, typical cell cycle histograms of peripheral blood lymphocytes showed a CV of 3.6%. The determination of human and mouse cell lines S-phase fractions with TP3 and PI showed a good correlation. Combined with FITC and PE, TP3 was superior to PI as a DNA stain in multiparameter flow cytometry.

Spectral overlap of the fluorochromes used proved to be minimal. After a first incubation for 30 min with anti-vimentin (Vim3B4, mouse IgG2a, Novocastra Laboratories Ltd, Newcastle upon Tyne, U.K.) and antikeratin 10 (RKSE60, mouse IgG1, Department of Molecular Biology, University of Maastricht, The Netherlands), a second incubation for 15 min with the fluorochromes FITC and PE conjugated to monoclonal goat antibodies against mouse IgG2a and mouse IgG1 (Southern

Biotechnology Associates, Birmingham, AL, U.S.A.), respectively, was performed. After a third washing step, DNA staining was performed by addition of 300 μ L TP3 (1 μ mol/L in phosphate-buffered saline (PBS)) (Molecular Probes, Eugene, OR, U.S.A.) and 50 μ L RNase (1 mg/mL in PBS) (Sigma, St Louis, MO, U.S.A.).

Flow cytometric analysis

The flow cytometric analysis of triple-stained cell suspensions has been described before.^{7,8} From each sample, 5000–10,000 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, the percentages of vimentin-positive cells, differentiated keratinocytes (vimentin negative and keratin 10 positive) and basal keratinocytes (vimentin negative and keratin 10 negative) were calculated. Using Multicycle[™] software (Phoenix Flow Systems, San Diego, CA, U.S.A.) the percentages of basal keratinocytes in the S- and G₂M phases of the cell cycle (proliferation) were calculated from DNA histograms.

Statistical analysis

Analysis of the correlation between clinical and flow cytometric scores was performed by calculation of the Pearson correlation coefficient. In the placebo-controlled 1 α ,24 dihydroxyvitamin D₃ study four biopsies were obtained per patient and in the corticosteroid monotherapy study three biopsies were obtained per patient. Therefore, to avoid within-person variability, correlation analysis was restricted to paired values, i.e. before and after treatment, with omission of the values of the placebo-treated lesions. This resulted in 166 paired values obtained from 83 patients. To compare the flow cytometric scores before treatment for both cell isolation procedures, the paired *t*-test for means (two-tail) was used.

Results

In all the 219 flow cytometric samples investigated, the

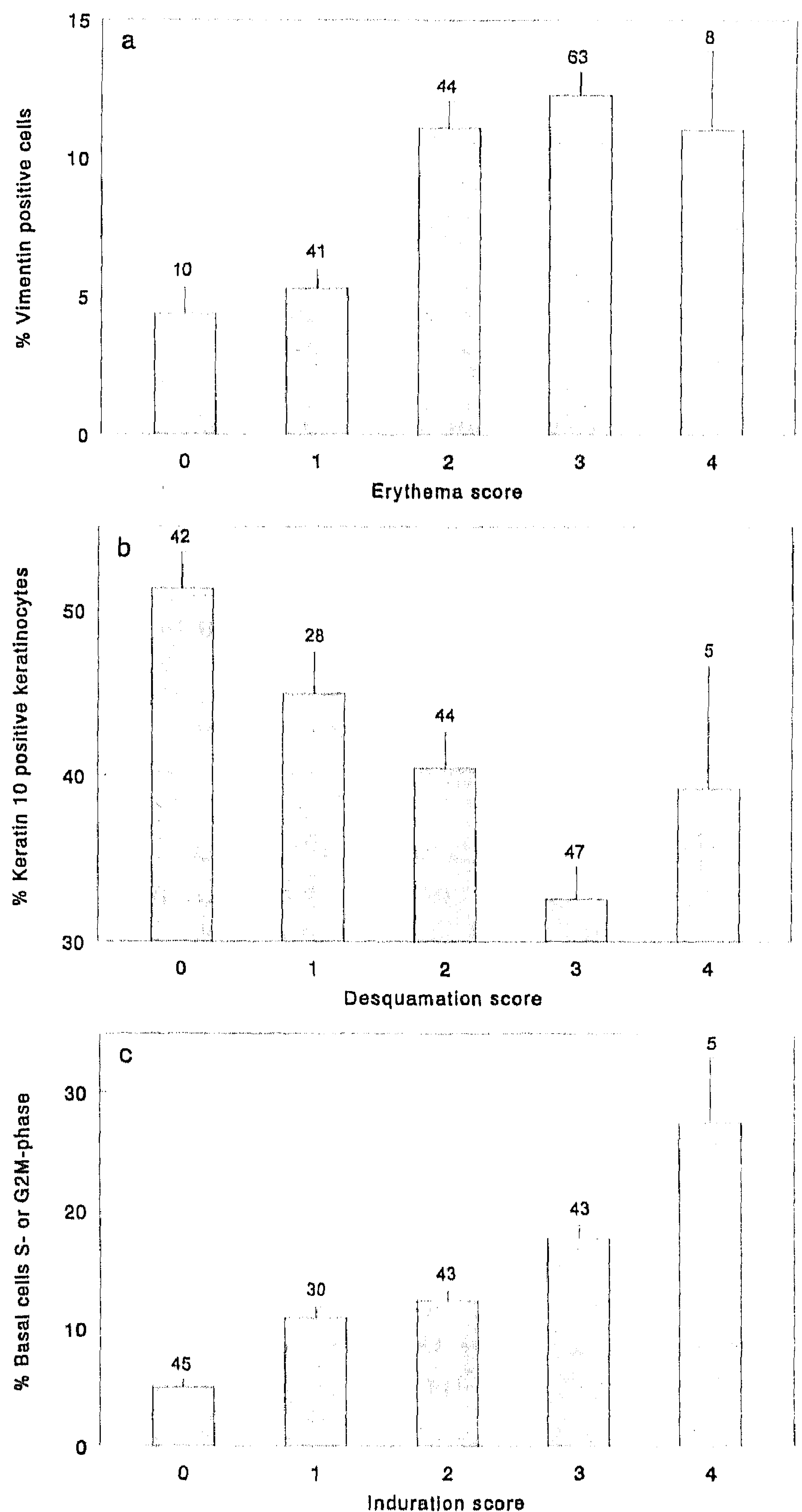


Figure 1. Correlation of clinical scores and flow cytometric values (mean \pm SEM) of 166 test lesions. (a) The erythema score vs. the percentage of vimentin-positive cells; (b) the desquamation score vs. the percentage of keratin 10 positive keratinocytes; and (c) the induration score vs. the percentage of basal keratinocytes in the S- and G₂M phase. The number of biopsies is indicated above the error bars.

number of intact cells was at least 30% (without debris and clumps). Coefficients of variation of the G₁ peaks were \leq 10. In Figure 1 we show the results of the correlation analysis of clinical and flow cytometric scores, which was restricted to 166 paired values of 83 patients. It can be seen that high clinical scores for

Table 1. Correlation coefficients of clinical and flow cytometric scores (relative percentage of cells) of all psoriatic lesions ($n = 166$) and psoriatic lesions before treatment ($n = 83$)

	% Vimentin- positive	<i>P</i>	% Keratin 10-positive	<i>P</i>	% basal cells in S- or G ₂ M-phase	<i>P</i>	Erythema	<i>P</i>	Induration	<i>P</i>
<i>n</i> = 166										
% Vimentin	1									
% Keratin 10	-0.38	<0.0001	1							
% S- or G ₂ M phase	0.37	<0.0001	-0.13	0.087	1					
Erythema	0.41	<0.0001	-0.32	<0.0001	0.62	<0.0001	1			
Induration	0.44	<0.0001	-0.40	<0.0001	0.66	<0.0001	0.84	<0.0001	1	
Desquamation	0.37	<0.0001	-0.44	<0.0001	0.61	<0.0001	0.79	<0.0001	0.87	<0.0001
<i>n</i> = 83										
% Vimentin	1									
% Keratin 10	-0.19	0.086	1							
% S- or G ₂ M-phase	0.084	0.45	0.25	0.021	1					
Erythema	0.22	0.044	0.16	0.14	0.44	<0.0001	1			
Induration	0.35	0.0010	-0.012	0.91	0.52	<0.0001	0.54	<0.0001	1	
Desquamation	0.093	0.40	-0.19	0.080	0.32	<0.0036	0.23	0.036	0.52	<0.0001

erythema correlate with high percentages of vimentin-positive cells (Fig. 1a; correlation coefficient $r = 0.41$, $P < 0.0001$), that the desquamation score inversely correlates with the number of keratin 10 positive keratinocytes (Fig. 1b; $r = -0.44$, $P < 0.0001$) and that the induration of a psoriatic plaque correlates with its proliferative activity (Fig. 1c; $r = 0.66$, $P < 0.0001$). High correlation coefficients were shown between the clinical scores of the psoriatic plaques: 0.79 (erythema and desquamation), 0.84 (erythema and induration), and 0.87 (induration and desquamation) with $P < 0.0001$ (Table 1). However, the coefficients of the flow cytometric values were -0.38 , $P < 0.0001$ (vimentin and keratin 10); 0.37 , $P < 0.0001$ (vimentin and SG₂M); and -0.13 (not significant) (SG₂M and keratin 10).

To exclude a possible effect of the treatment regimen on the degree of correlation, analysis of coefficients was also performed on values from biopsies obtained before treatment ($n = 83$). Table 1 shows that the correlation coefficients of clinical scores of untreated lesions only were reduced to 0.23 ($P < 0.05$, erythema and desquamation), 0.54 ($P < 0.0001$, erythema and induration), and 0.52 ($P < 0.0001$, induration and desquamation), whereas the correlations between the flow cytometric values remained below the level of statistical significance or strongly changed. Furthermore, it can be seen from Table 1 that, compared with the assessment on all lesions, the correlation in untreated lesions between basal cells in S- or G₂M phase, and induration, remained in the same range ($r = 0.52$; $P < 0.0001$). The association between erythema/vimentin and desquamation/

keratin 10 was reduced to the lower values of 0.22 ($P < 0.05$) and -0.19 (not significant), respectively. To find out whether a change in clinical scores was reflected in changes in the flow cytometric scores, correlation analysis was performed on the differences of these scores before and after treatment. Figure 2 clearly shows that some degree of correlation exists. Quantitative analysis revealed correlation coefficients of $r = 0.14$ (not significant, Fig. 2a), $r = -0.28$ ($P < 0.01$, Fig. 2b) and $r = 0.19$ ($P = 0.08$, Fig. 2c).

In the present study, epidermal single-cell suspensions were prepared according to two different methods. Cell isolation of dermatome biopsies (area 1 cm² thickness 0.4 mm) was obtained after a one-step trypsin incubation method. Three millimetre punch biopsies were processed using a two-step thermolysin-trypsin separation method. The question was addressed as to whether the cell isolation procedures influence the flow cytometric results. Therefore, in Table 2, the mean pretreatment flow cytometric values of each clinical score are given for both methods of cell isolation. It can be seen that all corresponding flow cytometric parameters (except for psoriatic plaques with an induration score of 1 and very scaly lesions) were significantly lower in epidermal cell suspensions prepared from punch biopsies, compared with dermatome biopsies.

The results of the flow cytometric analysis, comparing both absolute and relative changes after different anti-psoriatic therapy regimens and after placebo therapy, are given in Figure 3. With respect to epidermal proliferative activity (Fig. 3a, percentage basal keratinocytes

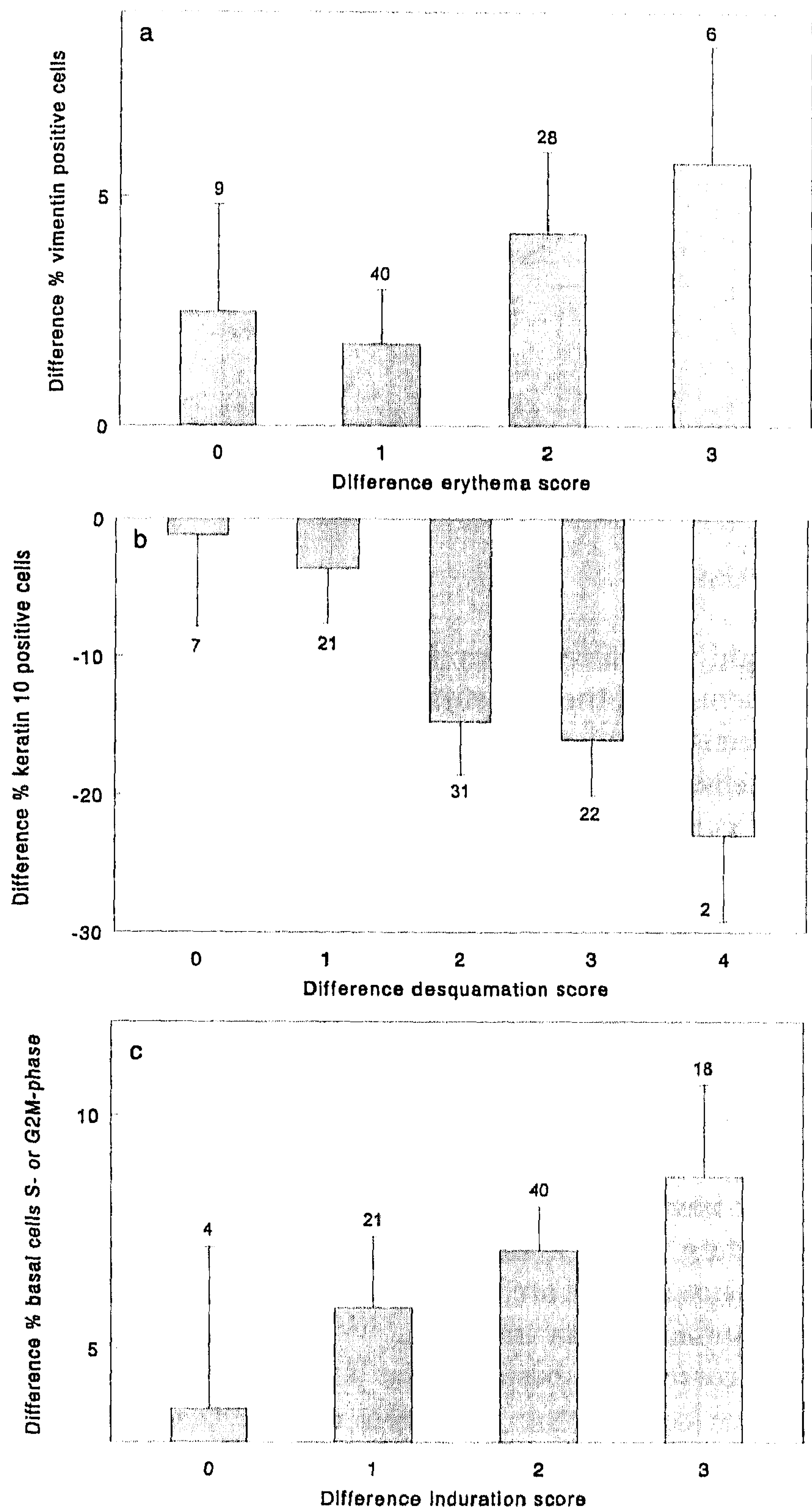


Figure 2. Correlation of differences before and after treatment of clinical and flow cytometric parameters (mean \pm SEM) of 83 test lesions. (a) The erythema score vs. the percentage of vimentin-positive cells; (b) the desquamation score vs. the percentage of keratin 10 positive keratinocytes; and (c) the induration score vs. the percentage of basal keratinocytes in the S- and G₂M phase. The number of biopsies is indicated above the error bars.

in the S and G₂M phase), 8 weeks of placebo treatment resulted in a reduction of 15% compared with the pre-treatment situation. Both calcipotriol cream (50 μ g/g, applied twice daily) and 1 α ,24 dihydroxyvitamin D₃ ointment (4 μ g/g, applied once daily) resulted in a reduction in proliferative activity of 34%. The application of calcipotriol cream (50 μ g/g once daily), however, resulted

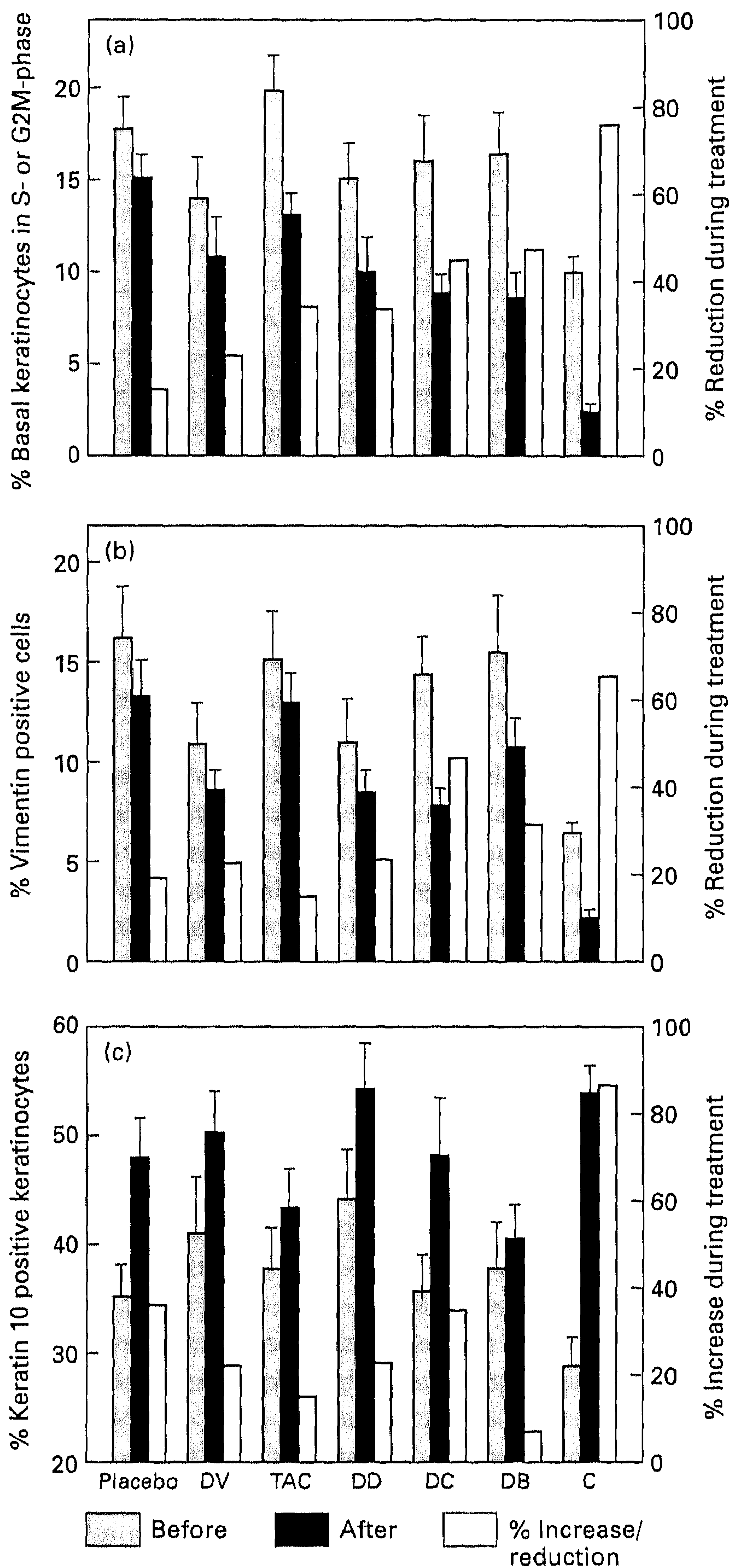


Figure 3. Comparison of the absolute (mean \pm SEM) and relative (%) changes of flow cytometric parameters after different antipsoriatic treatments with respect to the percentage of basal keratinocytes in the S- and G₂M phase (a), the percentage of vimentin-positive cells (b), and the percentage of keratin 10 positive keratinocytes (c). DV, calcipotriol once daily and vehicle once daily; TAC, Tacalcitol[®] (1 α ,24 dihydroxyvitamin D₃) ointment once daily; DD, calcipotriol twice daily; DC, calcipotriol once daily and clobetasone butyrate once daily; DB, calcipotriol once daily and betamethasone valerate once daily; C, clobetasol 17-propionate.

Clinical score		Flow cytometric score	Dermatotome	Punch	Δ (%)	P
Erythema	2	Vimentin (%)	17.4	6.4	63	<0.001
	3		14.2	7.1	50	<0.01
Induration	1	S- or G ₂ M-phase (%)	9.8	10.3	-5.1	NS
	2		14.2	9.3	35	<0.01
	3		19.6	12.4	35	<0.05
Desquamation	1	Keratin 10 (%)	50.3	29.8	41	<0.05
	2		45.4	32.1	29	<0.05
	3		35.2	26.6	24	<0.05
	4		36.8	26.6	28	NS

Table 2. Comparison of pretreatment flow cytometric values (relative percentage of cells) of punch and dermatotome biopsies prepared by different cell isolation procedures ($n = 102$). Δ implies the difference of values obtained by punch biopsies and dermatotome biopsies as a percentage of the value in dermatotome biopsies.

$$\left(\Delta = \frac{[\text{Dermatotome}] - [\text{Punch}]}{[\text{Dermatotome}]} \times 100\% \right)$$

in a reduction of only 23%. The combination of topical corticosteroids and calcipotriol further increased the effect on epidermal proliferation. The reduction of proliferative activity in psoriatic plaques, treated for a maximum of 3 weeks with clobetasol 17-propionate, proved to be 76%. With respect to the number of vimentin-positive cells (Fig. 3b), placebo treatment and monotherapy with the vitamin D₃ analogues resulted in reductions of less than 23%. This is in contrast to treatment regimens comprising corticosteroids (combination or monotherapy), where reductions of 31–65% were observed. In Figure 3c, we indicate the effect of different antipsoriatic therapies on the number of keratin 10 positive keratinocytes (differentiation marker). Monotherapy with the vitamin D₃ analogues 1 α ,24 dihydroxyvitamin D₃ and calcipotriol (once daily or twice daily) resulted in an increase of 15–23%. A combination of calcipotriol and topical steroids did not seem to increase this percentage substantially. In contrast, the monotherapy of psoriatic plaques with clobetasol 17-propionate increased the number of keratin 10 positive keratinocytes by 87%, compared with an increase of 36% for placebo.

In Figure 4 we summarized the effect of the different antipsoriatic therapies on the clinical scores of the test lesions. Therapy regimens using topical corticosteroids proved to have a more pronounced effect on erythema compared with monotherapy with vitamin D₃ analogues. The effect of placebo on the reduction of the erythema score was only 15% (Fig. 4a). All therapies showed a substantial reduction (50% or more) with respect to the induration score, in contrast to placebo (28%, Fig. 4b). The mean desquamation score was reduced by 42% after treatment with placebo, which implies that this parameter is placebo-sensitive. Mono or combination therapy using vitamin D₃ analogues showed reductions in desquamation values of 58–81% (Fig. 4c).

Discussion

A highly significant correlation was observed between erythema and the percentage of vimentin-positive cells, between scaling and the percentage of keratin 10 positive cells, and between induration and the percentage of basal cells in the S- and G₂M phase, if all 166 biopsies are considered. The latter correlation remained equally pronounced, whereas the other two associations were less prominent, if the analysis was restricted to 83 biopsies taken from untreated skin.

The percentage of vimentin-positive cells consists, to a large extent, of inflammatory cells. Although erythema, i.e. vasodilatation, is part of cutaneous inflammation, the appearance of inflammatory cells in the epidermis might have a different course. In the marginal zone of spreading psoriatic plaques, significant changes in the microvasculature are visible well before the appearance of an inflammatory infiltrate.^{11,12} Therefore, the incomplete correlation between the percentage of vimentin-positive cells in the epidermis and erythema can be explained by the partly independent changes of the microvasculature and the appearance of an infiltrate in the epidermis. Induration of the psoriatic plaque is supposed to be related to epidermal proliferation. Indeed, the high correlation between the percentage basal cells in the S- and G₂M phase, and the induration score, is in line with this commonly held belief. As the formation of scale in the psoriatic plaque represents impaired differentiation, the distribution between the number of basal (keratin 10 negative) and suprabasal (keratin 10 positive) keratinocytes was presumed to be indicative of the desquamation score. However, because of the reduction of keratin 10 at some sites in the psoriatic lesion, this differentiation marker does not seem to visualize all suprabasal cells.¹³ We also should reconcile the observation that keratin 10 is not a marker which includes all aspects of differentiation.

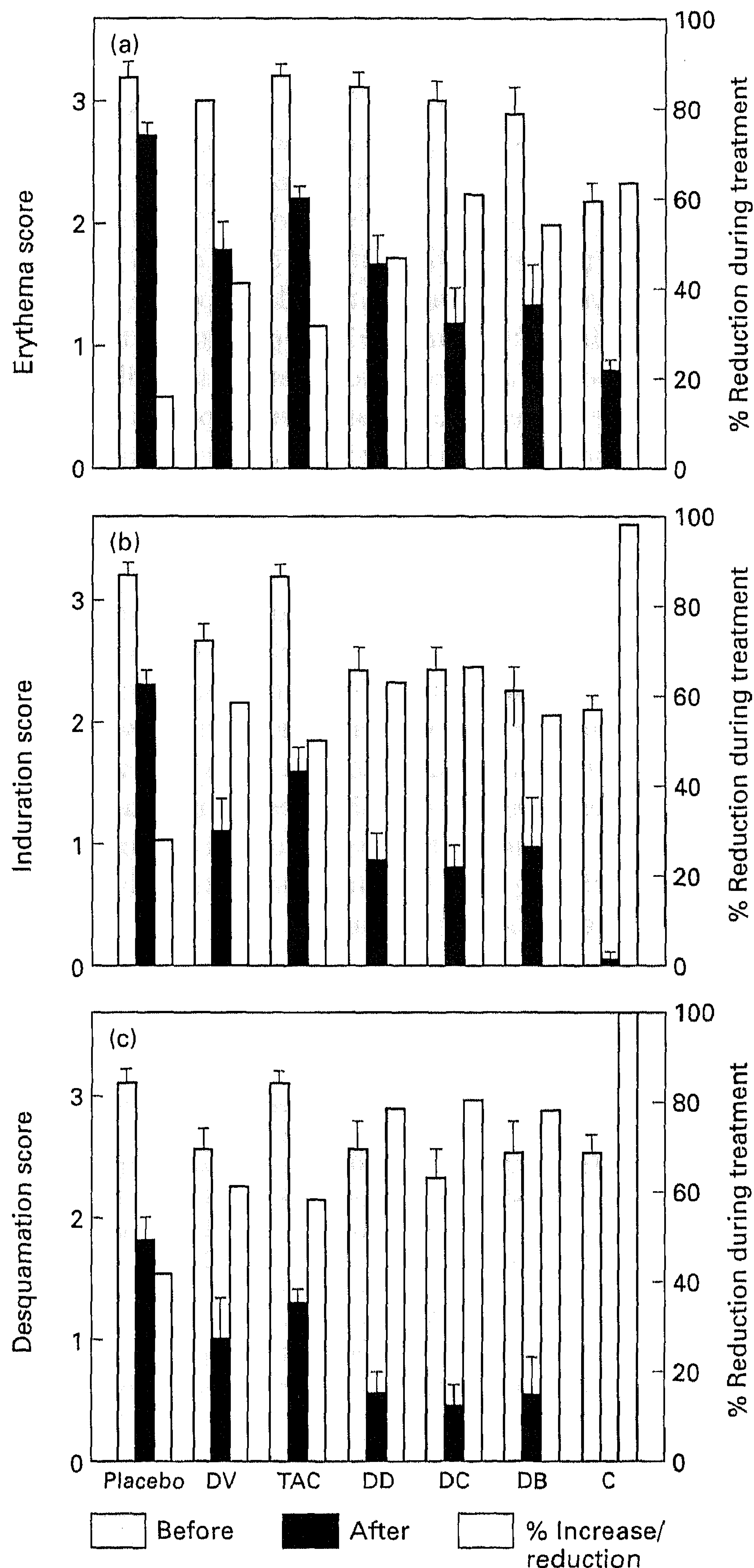


Figure 4. Comparison of the absolute (mean \pm SEM) and relative (%) changes of clinical scores after different antipsoriatic treatments with respect to erythema (a), induration (b), and desquamation (c). DV, calcipotriol once daily and vehicle once daily; TAC, Tacalcitol[®] ($1\alpha,24$ dihydroxyvitamin D_3) ointment once daily; DD, calcipotriol twice daily; DC, calcipotriol once daily and clobetasone butyrate once daily; DB, calcipotriol once daily and betamethasone valerate once daily; C, clobetasol 17-propionate.

Therefore, the correlation between scaling and keratin 10 expression has to be an incomplete one.

A description of the improvement in psoriatic plaques can be made clinically. The PASI score has been popular and practical, and adequate for comparative analysis. However, the interobserver variability is large.² It has been suggested by van de Kerkhof⁴ that the scores of erythema, induration and scaling should be considered separately, as these markers provide information on the anti-inflammatory vs. the antiproliferative capacity of treatment. The present study, however, suggests that the three scores are, to a large extent, correlated, which implies that the sum of these scores might be a more appropriate reflection of the psoriatic process. Such a sum might be the combined result of the cell biological denominator of the three processes. But we have to consider the possibility that this correlation, at least to some extent, is introduced by the unblinded investigator who, biased by a severe erythema, scaling or induration, might overinterpret the remaining signs. An important aspect of flow cytometric assessment during experimental treatment of psoriasis is the discrimination between antiproliferative, keratinization-enhancing and anti-inflammatory effects *in vivo*. As demonstrated by correlation studies, the clinical parameters do not adequately permit this separation.

Vimentin can be used as a marker for epidermal infiltrate, showing higher values in psoriatic compared with normal skin. In Table 2, we compared pretreatment flow cytometric values of both separation methods in psoriatic skin, with equal erythema scores. As the epidermal infiltrate is assumed to be equal, and interference by treatment is excluded, the difference in the numbers of vimentin-positive cells may be accounted for by dermal infiltration. The data in Table 2 show that the biopsy and preparation procedure has a clear impact on the flow cytometric values reached. The objective for developing the dermo-epidermal separation method, using thermolysin on punch biopsies, was to obtain high-quality cell suspensions with low dermal contamination and a high yield of basal cells.⁸ Indeed, for each erythema score, the number of vimentin-positive cells is significantly lower in cell suspensions prepared from punch biopsies compared with dermatome biopsies, indicating low dermal contamination. The suprabasal compartment (keratin 10 positive) is significantly smaller in cell suspensions prepared from punch biopsies, indicating the presence of a larger quantity of basal cells. It seems likely that a 0.4 mm dermatome biopsy does not always contain all epidermal cells. Furthermore, in contrast to thermolysin, enzymatic separation with

trypsin sometimes cleaves above the dermo-epidermal junction, resulting in a loss of basal cells. A lower proliferative activity in the basal compartment was found in the cell suspensions prepared from punch biopsies. This might be the result of less clumping in the cell suspension, as clumps mimic cells with a tetraploid DNA content. Another explanation for the lower percentage of basal cells in the S- and G₂M phase might be the yield of a larger quantity of non-proliferating or slowly proliferating stem cells, which have been suggested to be located at the tips of the rete ridges.^{14,15}

Although a comparison of different studies is hazardous due to preselection bias and subjective factors in clinical scoring, we have summarized the flow cytometric and clinical assessments of the efficacy of several topical antipsoriatic treatments in Figures 3 and 4. As different preparation methods were used, it is important to consider relative improvement only and to refrain from a comparison of absolute values. The placebo (the application of a bland emollient) had a substantial effect on the percentage of keratin 10 positive keratinocytes (a 36% increase in average counts). This effect on cell biology is in line with the tendency of the placebo to improve desquamation (by 42%), whereas induration and erythema are improved by 28% and 16%, respectively. Previously, we have demonstrated a reduction in the suprabasal expression of involucrin and the restoration of filaggrin expression during treatment with a hydrocolloid.¹⁶ In the present study, we again suggest that improvement in the suprabasal compartment results from the artificial restoration of the skin barrier by an ointment.

The flow cytometric quantification reveals a comparable effect of calcipotriol, once or twice daily, with respect to the percentage of keratin 10 positive keratinocytes and the percentage of vimentin-positive cells. However, the reduction of basal cells in the S- and G₂M phase was comparable for 1 α ,24 dihydroxyvitamin D₃ ointment (Tacalcitol[®]) once daily and calcipotriol twice daily, whereas once daily calcipotriol had a smaller effect on this proliferation marker. A comparison of clinical markers suggests that calcipotriol once and twice daily, and 1 α ,24 dihydroxyvitamin D₃ ointment once daily, induce an improvement well above that seen with placebo, with 1 α ,24 dihydroxyvitamin D₃ ointment tending to be slightly less effective. The twice daily application of calcipotriol resulted in a greater improvement in scaling. Monotherapy with vitamin D₃ analogues had a small effect on the percentage of vimentin-positive cells and the percentage of keratin 10 positive keratinocytes, at a level comparable with placebo. Interestingly, the

addition of a topical corticosteroid to calcipotriol substantially enhanced the reduction of the percentage of vimentin-positive cells and, to a lesser extent, compensated for the abnormally low number of keratin 10 positive keratinocytes. Therefore, flow cytometric analysis suggests a powerful antipsoriatic effect for combination treatment of calcipotriol and a topical corticosteroid. This is, to some extent, expressed at the clinical level by the erythema score (see Fig. 4a). Clinical practice, however, has shown that the combination of calcipotriol and a topical corticosteroid has a major effect on recalcitrant psoriatic plaques. Treatment with a potent corticosteroid, such as clobetasol 17-propionate, is the most powerful topical antipsoriatic treatment available. Its effect on the percentage of basal keratinocytes in the S- and G₂M phase, the percentage of vimentin-positive cells and the percentage of keratin 10 positive keratinocytes, is enormous. This effect is clearly seen clinically.

In conclusion, the clinical severity scores of psoriatic plaques are correlated to such an extent that they do not permit a separation between the effects on hyperproliferation, inflammation and differentiation by antipsoriatic treatment. Triple-labelling flow cytometry permits the demonstration of antipsoriatic effects beyond the clinical scores, as assessed by the investigator, and further permits differentiation between the anti-inflammatory, growth inhibiting and keratinization-enhancing capacity of antipsoriatic treatments.

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