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ORIGINAL PAPER

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Multiparameter flow cytometric characterization of epidermal cell suspensions prepared from normal and hyperproliferative human skin using an optimized thermolysin-trypsin protocol

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Abstract Reliable flow cytometric analysis of normal and diseased skin requires pure epidermal single-cell suspensions. Several methods to separate the dermis from the epidermis are available. The proteolytic enzyme thermolysin separates the epidermis from the dermis at the lamina lucida and therefore permits reliable dermoepidermal separation. In the present study an optimized cell isolation procedure using thermolysin and trypsin is described, which is particularly suitable for punch biopsies. A 16–20-h (overnight) incubation of biopsies taken from normal and hyperproliferative skin with thermolysin (0.5 mg/ml) at 4°C produced a selective separation of the dermis and epidermis. After a 30-min trypsin incubation (0.25 mg/ml) at 37°C a cell suspension was produced which was characterized by minimal cell damage (cellular debris and clumps), a high recovery of basal cells and high quality DNA histograms. Furthermore, dermal contamination was very low. The thermolysin–trypsin separation methodology followed by triple-labelling flow cytometry provided a precise quantification of the percentage of keratin 10-positive cells, vimentin-positive cells and cells in S and G₂M phases. Proliferative activity was selectively measured in the basal, the suprabasal and the non-keratinocyte compartment at various time intervals during epidermal regeneration after adhesive tape stripping. In contrast to the non-keratinocytes, the percentage of cells in S and G₂M phases in the basal keratinocytes and in the suprabasal compartment increased 44–48 h after stripping. The increased proliferation following tape stripping was paralleled by an increased invasion of vimentin-positive cells into the epidermis and preceded by a decreased number of keratin 10-positive cells. Thermolysin–trypsin separation

followed by three-colour flow cytometry permits a highly selective characterization of normal and hyperproliferative epidermis.

Key words Separation method · Thermolysin–trypsin · Flow cytometry · Human epidermis · Tape stripping

Introduction

Flow cytometry is a valuable approach for quantifying the behaviour of normal and diseased epidermis under various conditions [2, 5, 9]. A prerequisite for accurate and reliable quantification is a high quality of epidermal single-cell suspensions. In this respect it is essential that the biopsy specimens obtained for preparation of these suspensions always contain the whole epidermis. Therefore, in hyperproliferative skin diseases such as psoriasis, dermatome (0.4 mm depth) or preferably punch biopsies are required. However, contamination with dermal cells is inevitable if dermoepidermal separation is not part of the cell isolation procedure.

In the past, dermoepidermal separation has been achieved using different methods such as suction blisters [16] (mechanical), sodium chloride [30] (chemical) and proteolytic enzymes, such as trypsin [13], dispase [17] and thermolysin [29]. Enzymological methods are preferred as they are site-specific, and less time consuming. Until recently we have used incubation with trypsin in combination with dithioerythritol (DTE) to obtain dermoepidermal separation and dissociation of the keratinocytes [12–14]. A limitation of this method is the fact that the level of separation is dependent on factors such as thickness of the biopsy, purity of the preparation, incubation time and temperature [10, 25]. Dispase is often used to obtain samples free from dermal contaminants [2, 17, 18]; however, changes in the basal compartment and impaired viability have been described [23, 24]. Thermolysin appears to produce a selective separation of the dermoepidermal junction, providing an epidermal sheet with a complete basal layer [29]. Subsequent incubation with

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trypsin provides single-cell suspensions suitable for flow cytometric analysis [10]. Various incubation times and temperatures have been proposed by different authors [10, 25, 29, 30]. So far, thermolysin–trypsin separation has not been validated in hyperproliferative skin.

Multiparameter flow cytometry enables simultaneous measurement of different cell parameters [9]. Until now three-colour flow cytometry combining dual immunophenotyping with quantitative DNA analysis has been impeded by a considerable spectral overlap of the fluorochromes used. Recently, we have demonstrated that this problem can be overcome by using the new DNA dye TO-PRO-3 iodide (TP3) in combination with the fluorochromes phycoerythrin (PE) and fluorescein isothiocyanate (FITC) [15]. In the present study the contamination with dermal cells and the relative numbers of basal cells after dermoepidermal separation were quantified using double labelling flow cytometry. In epidermal cell suspensions prepared from normal and hyperproliferative skin, a triple-labelling procedure was applied with simultaneous quantification of basal and differentiated keratinocytes, cells of mesenchymal origin and the DNA content of these epidermal subpopulations.

The aim of the present study was to validate a combined thermolysin–trypsin method in normal skin and hyperproliferative skin. Psoriatic lesions and recovery from tape stripping were studied using flow cytometry as conditions characterized by epidermal hyperproliferation. In particular the following issues were addressed: (1) optimization of the thermolysin–trypsin separation procedure in normal and psoriatic skin; (2) quantification of epidermal cell characteristics in biopsies taken from normal skin following both separation methods using triple-labelling flow cytometry; and (3) analysis of epidermal hyperproliferation induced by tape stripping using triple-labelling flow cytometry following thermolysin–trypsin separation.

Materials and methods

Subjects

Skin samples were obtained from 26 healthy volunteers (16 males, 10 females, age range 18–29 years) without signs or history of skin diseases and from the lesional skin of 6 psoriasis vulgaris patients (3 males, 3 females, age range 30–64 years). All subjects had given informed consent prior to biopsy.

Biopsy and tape stripping procedure

Dermatome biopsies were taken from normal and lesional psoriatic skin [12]. After induction of local anaesthesia with ethyl chlo-

ride spray (Medica, 's Hertogenbosch, The Netherlands), a small dermatome (Coriotome 6B333, Aesculap, Tuttlingen, Germany) with a metal guard was used to obtain skin samples with an area of 1 cm² and a thickness of 0.2 mm (normal skin; *n* = 10) or 0.4 mm (psoriatic skin; *n* = 6). Punch biopsies (3 mm) were taken from normal skin (*n* = 7), from lesional psoriatic skin (*n* = 6) and in the tape stripping experiment (*n* = 28). Local anaesthesia was induced with Xylocaine/adrenaline 1 : 100 000.

Tape stripping was carried out on the back of seven healthy volunteers [8]. By repeated applications of Sellotape adhesive tape the stratum corneum was removed from four test areas (± 2 cm²) until the surface was glistening. In total, five punch biopsies were obtained from each volunteer, before tape stripping and after 8, 24, 44 and 48 h. At each visit erythema, induration and desquamation were assessed clinically using a five-point scale (0 = not present, 1 = slight, 2 = moderate, 3 = severe and 4 = very severe).

Cell isolation procedure

The trypsin cell isolation procedure has been described previously [12]. In brief, after a one-step incubation of the skin specimen in phosphate-buffered saline (PBS) containing 0.25 mg/ml trypsin (Sigma T-8253, St. Louis, Mo., USA) and 3.0 mg/ml DTE (Sigma, D-8255) for 30 min at 37°C, the dermis was separated with forceps in PBS containing 10% heat-inactivated newborn calf serum (HINCS, Life Technologies, Paisley, UK). The remaining epidermis was then gently mixed on a vortex to separate the keratinocytes, resulting in a single-cell suspension. The stratum corneum was discarded. A limitation of this method is that it is not applicable to punch biopsy specimens.

The two-step thermolysin–trypsin separation procedure was tested under different concentrations, incubation times and temperatures for both thermolysin (Sigma P-1512) and trypsin as described in the Results section. After thermolysin incubation (0.5 mg/ml dissolved in PBS with Ca²⁺ and Mg²⁺; Seromed, Berlin, Germany), the dermis and epidermis were separated using fine forceps. This separation was visualized in normal and psoriatic skin by routine HE staining. Subsequent trypsin–DTE incubation provided single-cell suspensions.

Cells were fixed in 1–3 ml ice-cold ethanol (70% v/v) and kept at –20°C. To be able to compare the percentage of intact cells (without debris and clumps) the suspensions were not filtered before staining and flow cytometric analysis.

Flow cytometric analysis

Flow cytometric validation of the separation method consisted of double-labelling procedures. To obtain information on contamination with dermal cells, propidium iodide (PI; Calbiochem, San Diego, Calif., USA) staining was combined with staining with a monoclonal antibody against the intermediate filament vimentin (Vim3B4, Novocastra Laboratories, Newcastle upon Tyne, UK; see Table 1). The relative amount of basal and suprabasal cells present in the suspensions was quantified by simultaneous staining with the monoclonal mouse antibodies RCK102 and RKSE60 (gifts from Prof. F.C.S. Ramaekers, Department of Molecular Biology, University of Maastricht, The Netherlands) as primary antibodies. Rabbit antimouse IgG conjugated with fluorescein isothiocyanate (RAM-FITC, Dakopatts, Copenhagen, Denmark) was used as secondary antibody in these experiments. Cell suspensions were prepared from dermatome and punch biopsies from normal

Table 1 Primary antibodies used in flow cytometric analysis

Primary antibody	Directed against	Present in	Dilution	Source	References
Vim3B4	Vimentin	Mesenchymal cells	1 : 50	Novocastra	20
RCK102	Keratins 5 and 8	Basal keratinocytes	1 : 30	Pathology Department	28
RKSE60	Keratin 10	Suprabasal keratinocytes	1 : 15	Pathology Department	9

and psoriatic skin with different separation conditions. From each suspension two samples containing about 1×10^5 ethanol-fixed cells were washed in PBS, centrifuged (5 min, 3000 rpm) and resuspended in 500 μ l PBS containing either VIM3B4 (1:50 dilution) or RCK102 (1:30 dilution) and RKSE60 (1:15 dilution). After 30 min incubation in the dark at room temperature the cells were washed in PBS containing 1% HINCS, centrifuged and resuspended in 500 μ l PBS containing RAM-FITC and HINCS (both 1:50 dilution). After 15 min incubation at 5°C the cells were washed and centrifuged as before and finally resuspended in 500 μ l PBS containing 40 μ g/ml PI and 0.1% RNase (Sigma, R-4875) and kept for 10 min in the dark.

A triple-labelling procedure was used to quantify epidermal cell characteristics in normal and hyperproliferative skin. In preliminary experiments specificity and optimal concentrations of the antibodies were tested. We have described the staining procedure previously [12]. The DNA content was measured using the new DNA fluorochrome TP3 (Molecular Probes, Eugene, Ore., USA). TP3 intercalates with double-strand DNA and permits the measurement of the proliferative activity of cells by quantification of the percentage of cells in S and G₂M phases. As TP3 also binds to RNA to some extent, it was used in combination with RNase. Primary intermediate filament antibodies were RKSE60 (mouse, IgG₁) and Vim3B4 (mouse, IgG_{2b}). The second step of the indirect immunofluorescent staining was performed with monoclonal goat antibodies against mouse IgG₁ and IgG_{2b}, conjugated with PE and FITC, respectively (Southern Biotechnology Associates, Birmingham, Ala., USA). Usually suspensions containing about $1-2 \times 10^5$ cells were used for flow cytometric analysis.

From each sample 5000 gated cells were measured and analysed using an EPICS Elite flow cytometer (Coulter, Luton, UK) equipped with a dual laser system. PE, FITC and PI 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) and a longpass filter of 630 nm (orange-red, PI). The area/peak ratio of the red signal (DNA) was used to discriminate between doublets of diploid cells and real single tetraploid cells [4]. After setting appropriate gates with the EPICS Elite software, the percentages of cells expressing vimentin, and keratins 5, 8 and 10 were calculated. Using Multicycle software (Phoenix Flow Systems, San Diego, Calif., USA) the percentages of basal and suprabasal keratinocytes and of non-keratinocytes in S and G₂M phases of the cell cycle were calculated from DNA histograms.

Results

Dermoepidermal separation and cell isolation

Experiments were carried out to establish the optimal analytical procedures to achieve a selective separation of the dermis and epidermis and a maximum number of intact epidermal cells. After thermolysin incubation for 1–2 h at 4°C of punch and dermatome biopsies taken from both normal and psoriatic skin no reliable dermoepidermal separation was possible (fluorescence microscopic control). A 2-h thermolysin incubation at 37°C did not provide sufficient separation in a punch biopsy from psoriatic skin. In contrast, the following conditions proved to result in optimal cell suspensions: an overnight (16–20-h) incubation with thermolysin (0.5 mg/ml) at 4°C, followed by a 30-min trypsin incubation (0.25 mg/ml) at 37°C. Figure 1 illustrates the typical histological appearance of dermoepidermal separation in punch biopsies from normal skin and from lesional psoriatic skin. It can be seen that the separa-



Fig. 1a–c Dermoepidermal separation obtained after incubation of punch biopsies from (a) normal skin and (b) psoriatic skin with thermolysin (0.5 mg/ml) overnight (16 h) at 4°C. c After removal of the psoriatic epidermis from a thermolysin-treated biopsy the remaining dermis is complete (a \times 400, b \times 200)

tion selectively involved the dermoepidermal junction without dissociation of the individual keratinocytes. The complete basal cell layer was present in the epidermal compartment without any dermal contamination.

Table 2 summarizes the flow cytometric analysis (double-labelling) of the epidermal sheets prepared from nor-

Table 2 Flow cytometric analysis of epidermal cell suspensions prepared from normal and lesional psoriatic skin using trypsin (*t*) and thermolysin–trypsin (*tt*) separation methods.

Biopsy procedure	Skin	Separation method	Vimentin-positive cells (%)	Keratin 10-positive keratinocytes (%)	Keratin 5-positive keratinocytes (%)	Naked nuclei (%) ^a
Dermatome	Normal	<i>t</i>	5.9	65.6	28.0	0.5
Dermatome	Psoriasis	<i>t</i>	9.1	26.3	54.7	9.9
Dermatome	Normal	<i>tt</i> (2 h)	5.9	90.5	3.0	0.6
Punch	Normal	<i>tt</i> (16 h)	7.1	48.1	38.4	6.4
Punch	Psoriasis	<i>tt</i> (16 h)	6.3	29.2	60.8	3.7

^a% naked nuclei = 100 – (% keratin 5-positive cells + % keratin 10-positive cells + % vimentin-positive cells)

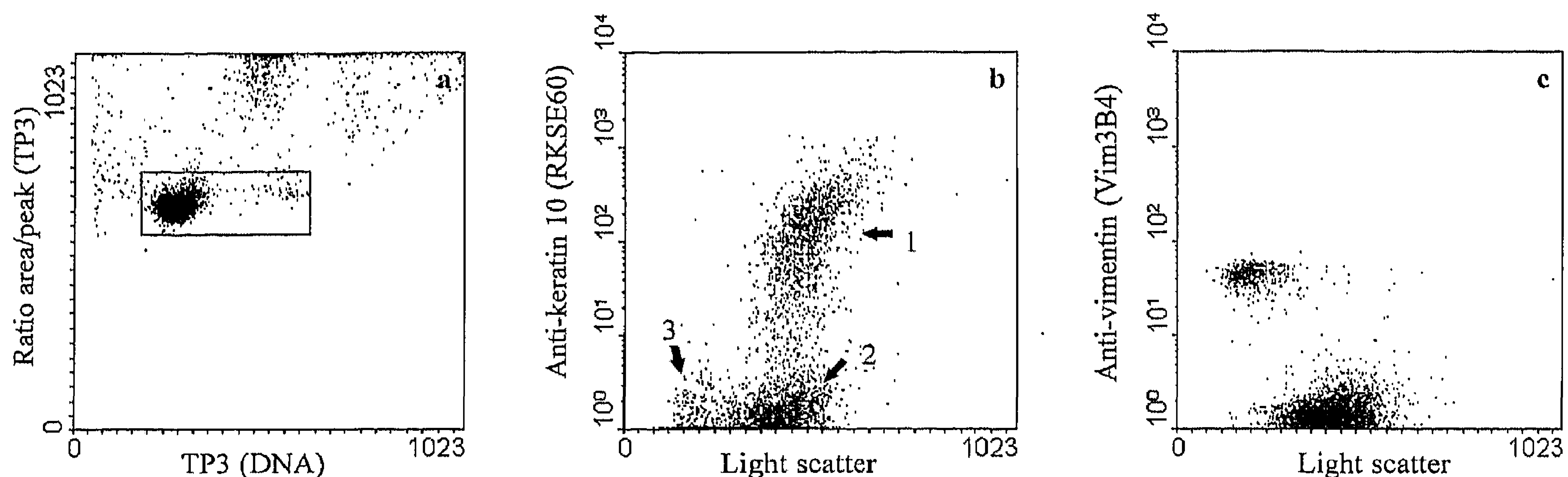


Fig. 2 a–c Discrimination of different epidermal subpopulations with triple-labelling flow cytometry. **a** TP3 (DNA content, red signal) versus the area/peak ratio of the red signal; **b** light scatter versus log orange fluorescence (keratin 10; for explanation of subpopulations 1, 2 and 3, see text); **c** Light scatter versus log green fluorescence (vimentin)

mal and lesional psoriatic skin using trypsin versus thermolysin–trypsin separation methods. It can be seen that the number of vimentin-positive cells and the number of naked nuclei was substantially elevated in the preparation of psoriatic skin using the trypsin separation method. After 2 h thermolysin incubation at 4°C (dermatome biopsy, normal skin) a significant loss of keratin 5-positive keratinocytes, i.e. basal cells, was found. However, using the optimized thermolysin–trypsin method the number of keratin 5-positive keratinocytes tended to be greater than with the trypsin method.

Quantification of the epidermal cell characteristics of normal skin

Representative flow cytometric dot plots obtained by the triple-labelling procedure are given in Fig. 2. Inside the gate (Fig. 2a) all cells with diploid to tetraploid DNA content are present. Cell debris (less DNA content) and clumps (higher area/peak ratio of the TP3 signal) are excluded from the analysis. Antikeratin 10 staining (RKSE60, Fig. 2b) resulted in discrimination of keratin 10-positive cells (1), i.e. suprabasal keratinocytes, from keratin 10-negative cells (2), i.e. basal keratinocytes. Fig. 2b also shows a subpopulation of keratin 10-negative cells with a smaller size (light scatter) (3). However, no clear separation of this subpopulation was achieved. With respect to antivimentin staining (Vim3B4), two distinct epidermal subpopulations were discriminated: a vimentin-positive population with small cell size and a vimentin-negative population with larger cell size (Fig. 2c). The first population corresponds to the cells from subpopula-

Table 3 Flow cytometric comparison of the trypsin (*t*) and the thermolysin–trypsin (*tt*) cell isolation procedures on normal skin (mean ± SEM)

Biopsy procedure	Separation method	Basal cells in S + G ₂ M phase (%)	Intact cells (%) ^a	Coefficient of variation (G ₁ -peak)	Vimentin-positive cells (%)	Keratin 10-positive keratinocytes (%)
Dermatome (<i>n</i> = 10)	<i>t</i>	8.8 ± 0.8	69 ± 2.8	8.0 ± 0.3	7.5 ± 1.1	61.5 ± 4.5
Punch (<i>n</i> = 7)	<i>tt</i>	5.5 ± 0.9	88 ± 2.6	6.6 ± 0.5	7.2 ± 0.6	57.8 ± 3.4

^a% intact cells = % particles with DNA content (2c–4c) without clumps or debris

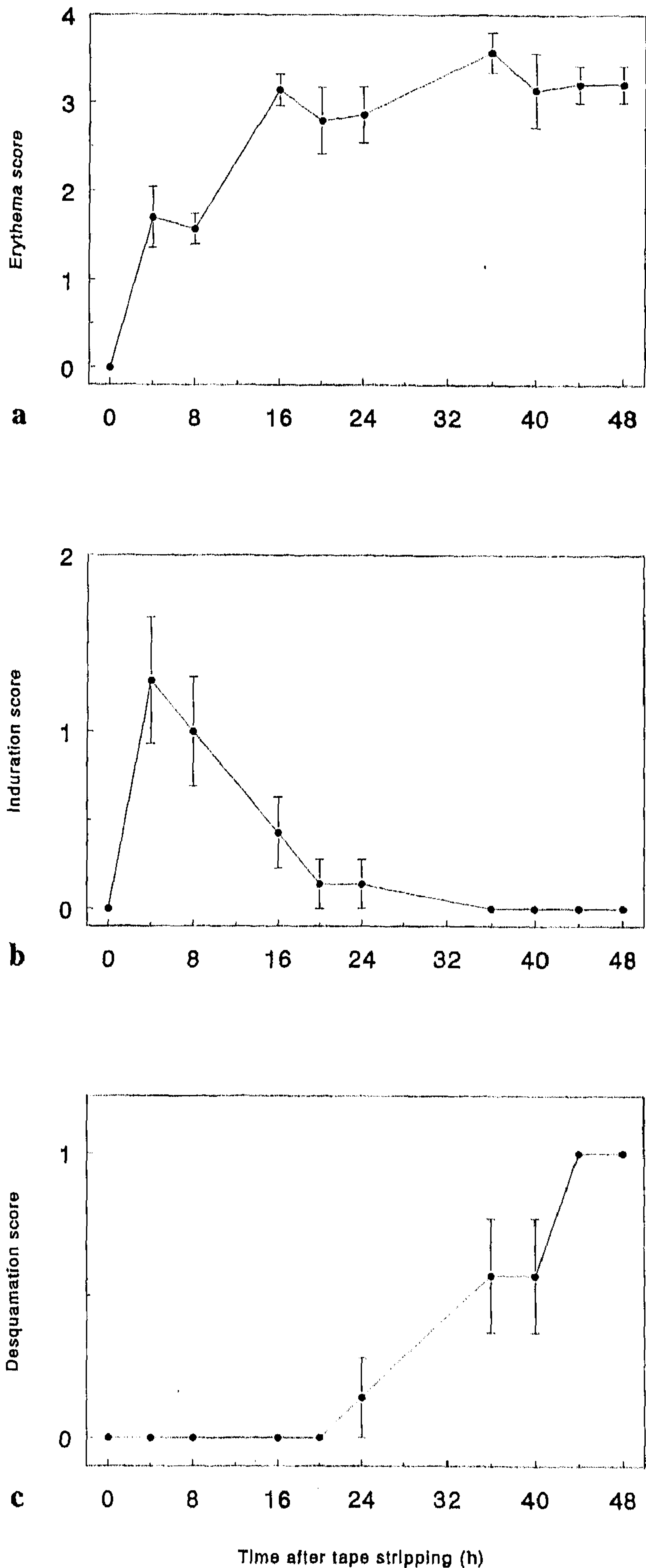


Fig. 3a-c Clinical scores after Sellotape stripping (mean \pm SEM; 0 = not present, 1 = slight, 2 = moderate, 3 = severe and 4 = very severe). a Erythema; b induration; c desquamation

tion 3 in Fig. 2b. The second population represents all keratinocytes. Cell cycle analysis was performed on histograms representing the germinative layer of the epidermis after exclusion of vimentin- and keratin 10-positive cells by software gating. The flow cytometric quantification of the different epidermal subpopulations using the trypsin separation protocol on dermatome biopsies versus the thermolysin-trypsin protocol on punch biopsies is summarized in Table 3. It can be seen that the population separated with thermolysin-trypsin was characterized by a decreased percentage of basal cells in S and G₂M phases ($P = 0.012$, *t*-test assuming equal variances, two-tailed), and increased percentage of intact cells ($P < 0.01$), a decreased coefficient of variation of the G₁ peaks ($P = 0.016$), and a slightly decreased percentage of vimentin-positive cells ($P = 0.79$) and keratin 10-positive cells ($P = 0.56$).

Quantification of the epidermal cell characteristics of normal skin following tape stripping

In preliminary experiments flow cytometric analysis was performed on cell suspensions prepared from dermatome biopsies taken at different intervals after tape stripping using the standard trypsin separation method. Following trypsinization, a reliable separation of dermis from epidermis turned out to be virtually impossible in these thin specimens with a partially to totally removed stratum corneum and hardly any dermis present. This was confirmed by the flow cytometric analysis which gave very inconsistent results especially with respect to the numbers of vimentin- and keratin 10-positive cells (unpublished data). In contrast, following incubation of punch biopsies taken at different intervals after tape stripping with thermolysin-trypsin the dermoepidermal separation was reliable, and pure epidermal single-cell suspensions could be prepared.

None of the seven healthy volunteers complained as a result of the tape stripping study. The clinical scores at different time intervals after tape stripping are shown in Fig. 3. Maximum erythema was observed 36 h after tape stripping. The triple-labelling flow cytometric analysis of the dynamics of the tape stripping model is summarized in Fig. 4. A significant decrease in the relative number of keratin 10-positive keratinocytes was observed at 24, 44 and 48 h after tape stripping ($P < 0.01$, paired *t*-test, two-tailed; Fig. 4a). The relative number of vimentin-positive cells increased after tape stripping with a maximum value at 44 h ($P < 0.08$; Fig. 4b). With respect to epidermal proliferation the number of basal cells in S and G₂M phases increased significantly at 44 and 48 h after tape stripping ($P < 0.01$). Four volunteers showed a peak value after 44 h. The peak value (all volunteers) was 46.7 ± 2.5 (mean \pm SEM). Remarkably, a less pronounced but still substantial increase in proliferative activity was also observed in the suprabasal compartment of the epidermis ($P < 0.01$, at 44 and 48 h). The proliferative activity of the non-keratinocytes in the epidermis was not influenced by tape stripping (Fig. 4c).

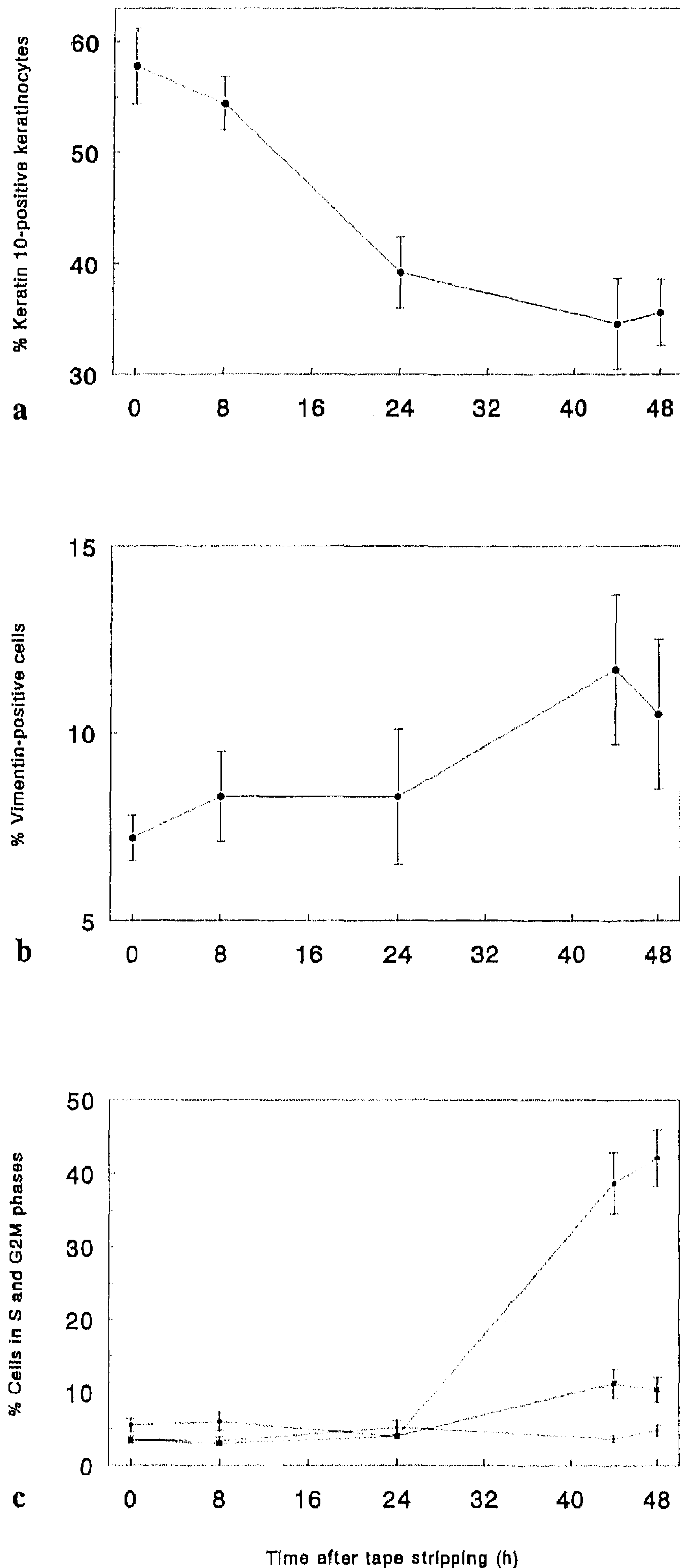


Fig. 4 a–c Flow cytometric analysis of the dynamics of the tape stripping model (mean \pm SEM). **a** Percentage of keratin 10-positive keratinocytes; **b** percentage of vimentin-positive cells; **c** percentage of basal (●) and suprabasal (■) keratinocytes and of vimentin-positive cells (◆) in S and G₂M phases

Discussion

The epidermis and dermis of biopsies taken from normal and hyperproliferative skin can be separated completely following a combined thermolysin–trypsin digestion method. In the present study the optimal conditions for this method were developed. Furthermore, a reproducible and quantitative analytical method was described to measure simultaneously inflammation, differentiation and proliferation in epidermal cell suspensions.

The separation of the dermis from the epidermis has a major influence on the composition of the epidermal cell suspension, in particular in the inflamed skin of psoriasis. Thermolysin is a proteolytic enzyme which cleaves the lamina lucida and is therefore, from a theoretical point of view, an interesting tool to separate the dermis from the epidermis. In contrast to the findings of Walzer et al. [29], our results indicate that a 1–2-h incubation with thermolysin at 4°C does not provide a reliable dermoepidermal separation. This is supported by Willstedt et al. [30] who observed intraepidermal separation in four of five cases after thermolysin incubation for 1 h at 4°C. Even incubation for 2 h at 37°C, as used by Germain et al. [10], does not provide a reliable separation in psoriatic skin. The optimized procedure for punch biopsies included an overnight (16–20-h) incubation with thermolysin at 4°C [25]. Figure 1 shows that a selective dermoepidermal cleavage can be achieved in punch biopsies following the optimized thermolysin procedure. In psoriatic skin, in particular, this method proved to reduce dermal contamination, to preserve the integrity of epidermal cells and to increase the relative number of basal cells. An intriguing observation was the decrease in the percentage of vimentin-positive cells in the biopsies from psoriatic skin. The inflammatory infiltrate cells in psoriatic lesions contribute to an absolute increase in the vimentin-positive cells. However, in psoriatic plaques the number of germinal cells per surface area has been estimated to be increased by a factor of 7 and the number of suprabasal cells by a factor of 3 [3]. This implies that an absolute increase in mesenchymal cells may not represent a relative increase in psoriatic plaques due to the pronounced increase in keratinocyte populations.

In multiparameter flow cytometry, the DNA fluorochrome TP3 has the advantage of minimal spectral overlap with PE. This is in contrast to other DNA dyes, such as 7-amino-actinomycin D or PI, with which measurements are substantially influenced by the electronic compensation for this overlap [15]. With the three-colour flow cytometric approach using TP3, high quality DNA histograms were obtained. Furthermore, discrimination of different epidermal subpopulations allowed a highly selective quantification of the DNA content in basal, suprabasal and mesenchymal cells. Using the trypsin method and the combined thermolysin–trypsin method the subpopulations in epidermal cell suspensions prepared from normal skin were analysed. The percentages of vimentin-positive cells and keratin 10-positive keratino-

cytes (Table 3) are in accordance with previous results [9, 19].

Significantly fewer basal cells with hyperdiploid DNA content were present in cell suspensions from normal skin prepared according to the thermolysin procedure (5.5%) compared with the trypsin procedure (8.8%). This implies that after thermolysin–trypsin separation, despite the presence of more basal cells, a lower proliferative activity was measured in the germinative compartment. One explanation for this observation might be the presence of a larger quantity of clumps in the cell suspensions prepared with only trypsin, mimicking cells with a more than diploid DNA content. Indeed, the mean percentage of intact cells was 88% in the cell suspensions isolated according to the thermolysin–trypsin protocol, whereas this percentage was only 69% after trypsin separation. Another explanation might be the lack of proliferative activity of exclusively those basal cells that were preserved with the thermolysin protocol. As we cannot exclude the possibility that the germinative layer contains subpopulations with different proliferating potential, there might exist nonproliferating or very slowly proliferating epidermal cells in the basal layer which were lost in the trypsin separation procedure. These cells might correspond to the stem cells described by Potten and Morris [22]. Future research should resolve this question.

In previous flow cytometric studies the percentage of cells in S and G₂M phases in suspensions prepared from normal skin varied from 2.9% to 7.0% [5, 6, 9, 19]. However, in those studies dermoepidermal separation was obtained with trypsin only, which might have resulted in a relatively high number of clumps and therefore in artificially high values for the percentages cells in S and G₂M phases. The monoclonal antibody Ki-67 detects a nuclear antigen that is only present in proliferating cells. In an immunohistochemical study the growth fraction (number of cycling cells divided by the number of basal cells) of normal epidermis was calculated to be 5.2% [27]. This value approximates the percentage of cells in S and G₂M phases in the basal compartment of 5.5 ± 0.9% (Table 3).

Tape stripping, first described by Pinkus [21], provides a useful method for studying epidermal proliferation, differentiation and inflammation *in vivo* [1, 7, 11, 19]. Using flow cytometry, we reconfirmed that maximal epidermal proliferation can be observed 44–48 h after removal of the stratum corneum. Proliferative activity was mainly located in the basal compartment, but was also present in the suprabasal compartment, and, as expected, was not induced in the non-keratinocytes. After tape stripping we observed the proliferative activity of the basal cells to be increased by a factor of 8.5 compared with unchallenged skin. The decrease in keratin 10 expression (24 h) preceded any measurable change in DNA synthesis. Therefore, it is evident that the onset of keratinocyte differentiation does not imply a loss of proliferative activity as already suggested by Régnier et al. [26] and Bata-Csorgo et al. [2]. Using an enzymological assay Chang et al. quantified epidermal polymorphonuclear leucocytes (PMNs) after tape stripping [8]. Maximal numbers of PMNs were

observed 8 h after stripping. In the present study, however, clinical scores for erythema were maximal 36 h after tape stripping. Interestingly, maximal vimentin expression was observed after 44 h. At this time the percentage of vimentin-positive cells was 11.7%. In contrast to the situation in psoriasis, following tape stripping (without substantial changes in the total number of keratinocytes) the increase in vimentin-positive cells represents a relative increase as well as an absolute increase. These cells comprise Langerhans' cells, melanocytes and infiltrating cells such as monocytes, lymphocytes and PMNs. In inflamed skin the number of infiltrating cells can be approximated from the total number of vimentin-positive cells by subtracting the number of mesenchymal cells in normal epidermis (7.2%). In the tape stripping experiments the maximal number of infiltrating cells was 4.5%.

In conclusion, the combined approach of cell isolation with thermolysin–trypsin and multiparameter flow cytometry was shown to provide the optimal approach to analysing epidermal single-cell suspensions from normal and hyperproliferative skin. Different epidermal subpopulations could be discriminated and reliable cell cycle analysis performed. Furthermore, the characterization of epidermal cell suspensions using triple-labelling flow cytometry provided important information on the regeneration of the epidermis *in vivo* following tape stripping.

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