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Initial Hyperproliferation and Incomplete Terminal Differentiation of Cultured Human Keratinocytes from Lesional and Uninvolved Psoriatic Skin

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Human epidermal keratinocytes (KCs) were isolated from lesional and from uninvolved skin of 8 patients with chronic plaque-like psoriasis and from the normal skin of 8 healthy volunteers. Primary KC cultures, grown on 3T3 cell feeder layers, were examined over a period of 4 weeks and their plating efficiency, colony growth area, DNA synthesis and ultrastructural cell differentiation were evaluated. Psoriatic KCs formed colonies one day earlier than non-psoriatic controls and proliferated faster during the first 2 weeks, as assessed by the mean colony growth area and ^{3}H-thymidine incorporation. After 4 weeks, however, no significant differences were observed between the in vitro proliferation parameters of normal and psoriatic KCs. At the ultrastructural level, cultures of lesional psoriatic KCs consisted of more cell layers with adherent transitional cells and incomplete formation of cornified envelopes, even after 4 weeks, while KCs from uninvolved psoriatic skin were characterized by a transient delay of in vitro maturation. These results indicate that the characteristic hyperproliferation of psoriatic KCs may only be maintained over a short period in primary culture, whereas defective terminal differentiation of lesional psoriatic KCs was maintained throughout the culture period. ***Key words: Keratinocyte cultures; Electron microscopy; Colony growth; Thymidine incorporation.***

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Keratinocyte (KC) cultures may be a useful tool to study epidermal cell behaviour in psoriasis, isolated from the various dermal and systemic influences which are effective in vivo. In 1985, Kragballe et al. (1) showed that uninvolved psoriatic epidermis is characterized by increased DNA synthesis in vitro, confirming earlier reports that had demonstrated in vitro hyperproliferation of psoriatic KCs (2-4). In most of these studies, however, only KCs from the uninvolved psoriatic skin were cultured, and long-term follow-up was not performed. Furthermore, some of these results were obtained by explant cultures, and ultrastructural assessment of the cell differentiation after long-term cultivation was lacking. Interestingly enough, other groups failed to detect differences between psoriatic and normal KCs in vitro (5–7).

In order to further characterize the growth of psoriatic KCs in vitro, we examined the plating efficiency, colony growth area and [^{3}H]-thymidine incorporation and the ultrastructural differentiation pattern of primary KC cultures from lesional and uninvolved psoriatic skin and from non-psoriatic controls over a period of 4 weeks, using a well established culture model (8). By these techniques we obtained evidence that defective terminal differentiation is a major feature in lesional psoriatic KCs, maintained over the entire culture period. In contrast, hyperproliferation of psoriatic KCs was only observed during the first 2 weeks in culture and was normalized thereafter.

MATERIAL AND METHODS

Skin samples

Punch biopsy samples (6 mm) were obtained from the central areas of involved psoriatic skin (IPS) and from the uninvolved skin (UPS) of 8 patients with chronic plaque-like psoriasis (age: 40–64 years). These patients had received no systemic treatment for at least 6 weeks, and no topical treatment for at least 3 weeks. For controls, normal skin (NS) was obtained from the lower back of 8 healthy volunteers (age: 35–68 years).

Epidermal keratinocyte cultures

KC cultures were prepared ad modum Rheinwald & Green (8), using Dulbecco's MEM supplemented with antibiotics, 10% normal human AB serum, 0.4 µg/ml hydrocortisone, 1

Table I. *Efficiency of colony formation of cultured keratinocytes from normal skin (NS), involved psoriatic skin (IPS) and uninvolved skin (UPS); results are expressed as medians and 95% confidence limits; N = 8*

Keratinocytes	Number of colonies (per 1×10^4 plated cells)
NS	2.25 (1.50–3.22)
IPS	2.63 (1.75–3.78)
UPS	2.83 (1.75–4.00)

nM cholera toxin, and 10 ng/ml epidermal growth factor. The KCs were plated on mitomycin-treated 3T3 mouse fibroblast feeder layers (2×10^4 cells/cm^2) inside of 24-well tissue culture clusters (Costar, Cambridge, Mass., USA) with a seeding density of 1×10^4 viable cells/cm^2, and incubated at 37°C in an atmosphere of 95% air, 5% CO_2, and the medium was renewed every second day. After 14 days, remaining 3T3 cells were removed by treatment with 0.02% EDTA in PBS for 10 min, and new mitomycin-treated 3T3 cells were added. All chemicals and media were purchased from Biochrom KG, West Berlin, Germany.

Colony formation

The efficiency of colony formation was assessed ad modum Rheinwald & Green (8). After 1 week of culture, the number of colonies was determined in triplicate cultures by phase-contrast microscopy and correlated to the number of initially seeded cells. All values were expressed as number of colonies per 1×10^4 seeded cells.

Colony growth

After 1 week and 2 weeks in culture, the mean surface area of KC colonies was determined in triplicate experiments, using a computerized image analysis system (MOP-Videoplan, Contron).

[^{3}H]-Thymidine incorporation

After 2 and 4 weeks, triplicate KC cultures grown in 24-well tissue culture clusters were incubated for 4 h at 37°C with 1 μCi/ml [^{3}H]-thymidine (50–70 Ci/mMol; Amersham-Buchler, Braunschweig, FR Germany). The incorporation of [^{3}H]-thymidine was stopped by medium aspiration followed by two washes with cold PBS and addition of cold 6% trichloroacetic acid. The extraction of DNA and determination of radioactivity were performed as described elsewhere (9). All data were expressed as cpm/well.

Electron microscopy

After 2 and 4 weeks, KC cultures grown on plastic coverslips were processed for electron microscopy as previously described (10). Thin sections were contrasted with lead citrate and uranyl acetate and were examined in a Zeiss 10 CR electron microscope.

RESULTS

Colony formation

Two days after seeding, KC colonies were found in all cultures from IPS and UPS, whereas initial colony formation was observed in control cultures from NS only after 3 days.

By light microscopy, no qualitative morphological alterations were observed in KC cultures from psoriatic skin, as compared with normal skin. The efficiency of colony formation, assessed after 7 days as number of KC colonies per 1×10^4 seeded cells, did not differ significantly between KC cultures from IPS and UPS, compared with that from NS (Table I).

Colony growth area

Quantification of colony growth areas was performed after 1 and 2 weeks by computer-assisted determination of mean surface areas. The results are listed in Table II and show that KC cultures from psoriatic skin had grown to larger colonies than control cultures from NS. The differences were more pronounced after 1 week than after 2 weeks, and were statistically significant. Only slight differences were observed between KC cultures from IPS and UPS.

[^{3}H]-Thymidine incorporation

Statistical analysis revealed a significantly increased [^{3}H]-thymidine incorporation in 2-week-old KC cultures from psoriatic skin, compared with non-psoriatic controls (Fig. 1 A). A comparable growth pattern was found in keratinocyte cultures from both IPS and UPS. After 4 weeks, no differences in [^{3}H]-

Table II. *Colony growth area of cultured keratinocytes from normal skin (NS), involved psoriatic skin (IPS) and uninvolved skin (UPS); Results are expressed as medians and 95% confidence limits; N = 8*

KCs	Area (mm^2)	
	After 1 week	After 2 weeks
NS	1.46 (0.82–1.76)	7.69 (5.78–11.84)
IPS	1.98 (1.64–2.90)*	10.84 (7.59–13.92)***
UPS	2.37 (1.54–2.72)**	10.56 (7.59–13.92)****

* IPS vs. NS: $p<0.01$ (Wilcoxon's test for unpaired samples).
** UPS vs. NS: $p<0.025$
*** IPS vs. NS: $p<0.05$
**** UPS vs. NS: $p<0.05$

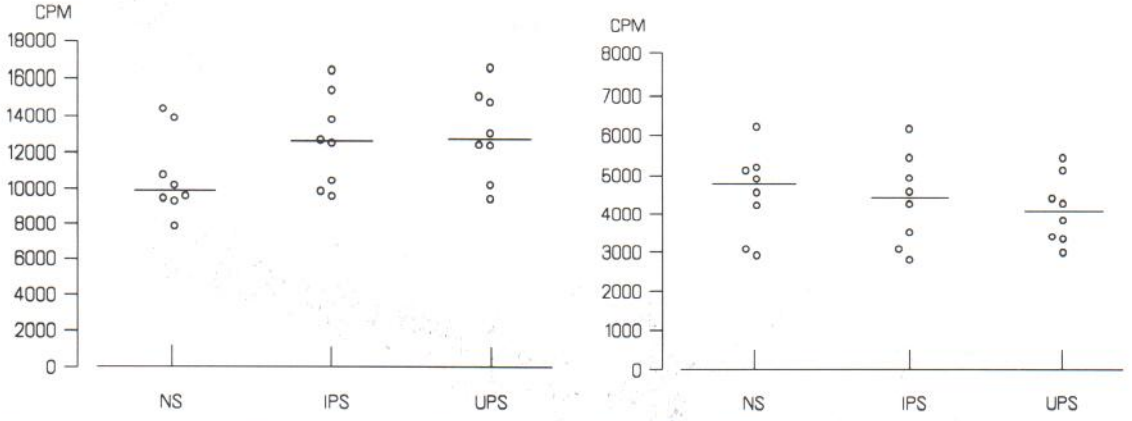

Fig. 1. Incorporation of [^{3}H]-thymidine into KC cultures from NS, IPS and UPS after a 4 h incubation with 1 μCi/ml [^{3}H]-thymidine. Horizontal bars indicate the median values. (A) 2-week-old cultures. UPS vs. NS: $p<0.05$ (Wilcoxon's test for unpaired samples). (B) 4-week-old KC cultures. No statistically significant differences were observed. All values are expressed as cpm/well.

thymidine incorporation were found between the three groups (Fig. 1 B).

Electron microscopy

After 2 weeks, control cultures from NS were composed of several cell layers showing prominent features of KC differentiation. The flat intermediate cells contained regular bundles of tonofilaments, and the uppermost KCs exhibited an electron-dense 12-nm layer in the inner part of their plasma membranes, representing early stages of cornified envelope formation (Fig. 2 A). Cell organelles were virtually absent in these corneocyte-like cells. In contrast, cultures from IPS consistently displayed more cell layers of KCs with irregular bundles of tonofilaments (Fig. 2 B). The upper cell layers were composed of adherent transitional cells with a thin, frequently incomplete cornified envelope, small bundles of tonofilaments and remnants of cell organelles. Cultures from UPS showed similar, yet less pronounced alterations.

After 4 weeks, cultures from NS still maintained a higher degree of differentiation than cultures from IPS. The upper cell layers of NS cultures showed

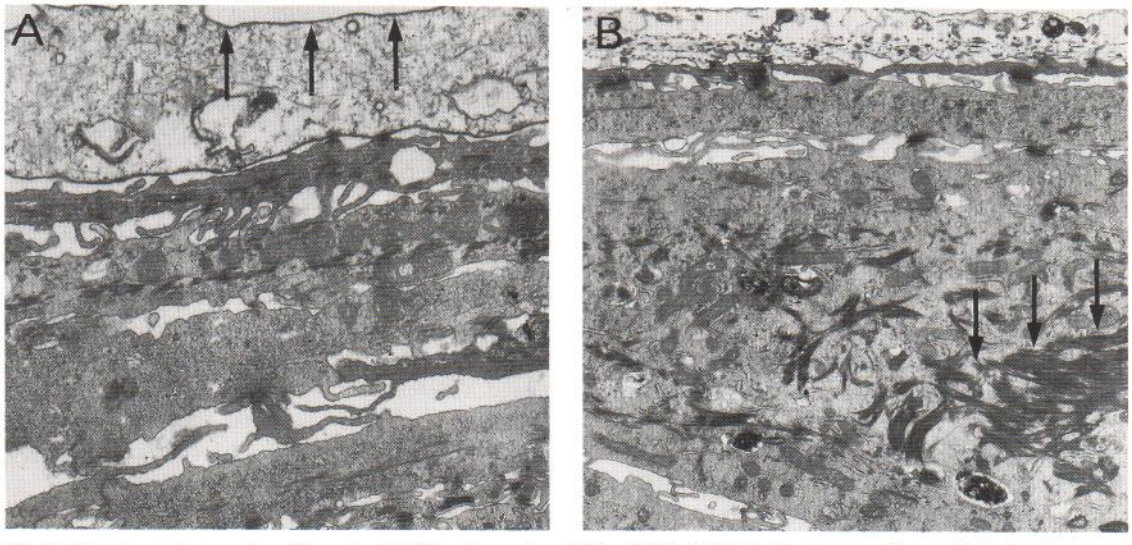

Fig. 2. Electron micrographs of 2-week-old KC cultures from NS and IPS. (A) NS: Flat intermediate cells and early stages of cornified envelope formation (*arrows*) in the uppermost cell, ×17,415. (B) IPS: Irregular bundles of tonofilaments in the intermediate cells (arrows) and transitional cell at the culture surface ×12,750.

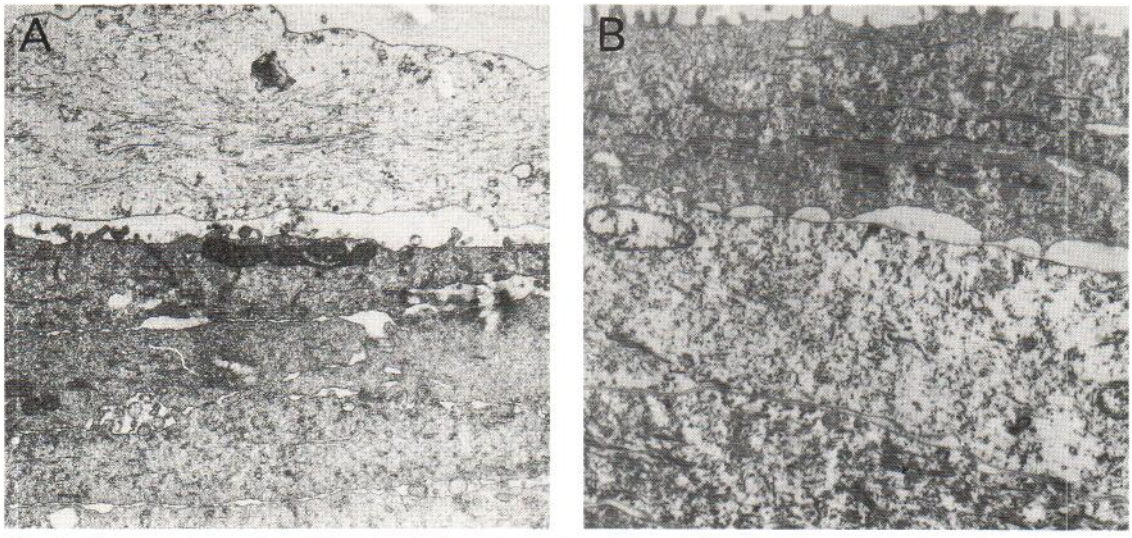

Fig. 3. Electron micrographs of 4-week-old KC cultures from NS and IPS. (A) NS: Regular formation of cornified envelopes in the upper cell layers. ×11,275. (B) IPS: Transitional cells with sparsely distributed cell organelles in the upper layers. ×11,480.

regular cornified envelopes (Fig. 3 A), whereas KCs from IPS remained immature, the upper layers consisting of transitional cells with irregular cornified envelopes and sparsely distributed cell organelles (Fig. 3 B). In contrast, 4-week-old cultures from UPS did not differ markedly from NS cultures.

DISCUSSION

The present investigation provides evidence that isolated psoriatic KCs are characterized by transient hyperproliferation in primary cell culture. We found an earlier onset of colony formation in KC cultures from psoriatic skin, significantly later colony growth areas after 1 and 2 weeks, and an increased rate of [^{3}H]-thymidine incorporation after 2 weeks. The hyperproliferative growth behaviour, however, was not maintained throughout the culture period, as we could not detect significant differences in thymidine incorporation after 4 weeks. These results suggest that the observed hyperproliferation of psoriatic keratinocytes might be due to the only transient influence of factors enhancing the cell proliferation. These factors might be growth factors or cytokines produced in the psoriatic skin, or altered properties of the psoriatic KCs such as plasma membrane glycoconjugates acting as receptors for growth-promoting substances (11, 12).

Increased in vitro proliferation rates of KCs from IPS have also been reported by other authors, using epidermal explant cultures (2), cultures grafted onto nude mice (3), conventional KC cultures (1, 4), or psoriatic hair follicle KC cultures (13). Other investigators, however, failed to detect hyperproliferation in KCs derived from psoriatic epidermis (6, 7).

Several explanations should be considered to elucidate the conflicting in vitro proliferation data of psoriatic KCs: (a) differences of the disease activity (hot spots in guttate psoriasis, chronic plaque-like psoriasis), the age of the donor, and the site of biopsy may result in different growth activities of KCs in vitro; (b) culture conditions such as growth factors, mitogens, serum additives, feeder layers and medium supplements may exert distinct influences on KCs from IPS and NS. To our knowledge, none of the above-mentioned investigations have used the same standardized experimental conditions.

By electron microscopy, KC cultures from lesional psoriatic skin presented several layers of transitional cells and failed to form complete cornified envelopes such as were consistently found in control KC cultures. Thus, lesional psoriatic keratinocytes maintained a defective differentiation pattern in vitro, mainly concerning the terminal steps of KC maturation, while KCs from UPS were characterized by a delayed onset of terminal differentiation.

These results indicate that the disturbed cell differentiation is a prominent feature of lesional psoriatic keratinocytes in vitro, while the initially observed hyperproliferation may not be maintained

over the entire culture period. Thus, it is conseivable that hyperproliferation and altered differentiation of psoriatic epidermis may take place independently from each other. The cause of this disorder still remains to be explored, but the KC culture model described here may serve as a suitable tool to further identify factors influencing growth and differentiation of psoriatic KCs.

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