

Increased DNA synthesis of uninvolved psoriatic epidermis is maintained *in vitro*

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SUMMARY

Clinically uninvolved psoriatic epidermis shows increased DNA synthesis *in vivo*. We have studied the DNA synthesis of cultured keratinocytes from uninvolved psoriatic skin. Trypsinized epidermal cells were plated on plastic dishes pre-coated with bovine collagen type I. In initial studies, normal human serum was found to be superior to fetal bovine in supporting the growth of human epidermal keratinocytes. Furthermore, keratinocyte cultures established in the presence of normal human serum produced large keratin proteins (68,000 daltons) indicating that the terminal steps in cell differentiation can occur *in vitro*. In subsequent experiments keratinocyte cultures were grown in medium supplemented with 10% normal human serum. Confluent cultures of keratinocytes from uninvolved psoriatic epidermis had an increased DNA synthesis determined both as the incorporation of [³H]thymidine and as the autoradiographic labelling index. The DNA synthesis of both normal and psoriatic keratinocyte cultures increased in response to incubation in medium with 10% psoriatic serum. The ability of keratinocytes from uninvolved psoriatic epidermis to maintain an increased DNA synthesis suggests the presence of an inherent defect within the population of epidermal keratinocytes in psoriasis. Such a culture system can be used as an *in vitro* model for the study of psoriasis.

Both inflammation and abnormal epidermal growth are essential components of psoriatic skin lesions. Recently, much emphasis has been put on the inflammation occurring in psoriasis, but epidermal hyperproliferation remains the single best marker of psoriasis. An increased labelling index is seen in involved and, to a lesser degree, in uninvolved psoriatic epidermal keratinocytes (Hell & Hodgson, 1966; Goodwin, Hamilton & Fry, 1973; Grove, Auderton & Smith, 1976; Marks, 1978; Rowe, Dixon & Forsythe, 1978). The concept of increased epidermal DNA synthesis in uninvolved psoriatic epidermis is further supported by transplantation studies of uninvolved psoriatic skin to congenitally athymic mice (Krueger, Chambers & Shelby, 1981). This property of uninvolved psoriatic epidermis makes it an attractive model to study the aberrant epidermal growth in psoriasis. The failure of culture systems to maintain the increased

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proliferation of keratinocytes from uninvolved psoriatic skin (Liu & Parsons, 1983; West, Kenicer & Faed, 1983) has, however, limited the use of keratinocyte cultures as an *in vitro* model for the study of psoriasis. Another limitation has been that cultured keratinocytes only produce small keratin proteins (40,000–58,000 daltons) indicating that the final stages of keratin synthesis do not take place *in vitro* (Fuchs & Green, 1978; Sun & Green, 1978).

Using a modified culture system the aim of the present study was to determine the proliferation of keratinocytes from normal and uninvolved psoriatic epidermis.

METHODS

Skin specimens

Informed consent was obtained from adult patients with untreated psoriasis (skin involvement less than 20%) and from an age-matched group of healthy volunteers without a history of skin disease. Keratome biopsies were taken from buttock skin at least 30 cm from psoriatic lesions.

Keratinocyte cultures

To establish keratinocyte cultures we used a modification of the procedure reported by Liu & Karasek (1978). A keratome biopsy was incubated with 0.25% trypsin in PBS containing 5 mM glucose (pH 7.0) for 30–40 min at 37°C. After aspiration of trypsin, minimal essential medium (MEM) with 10% fetal bovine serum (FBS), 50 IU/ml penicillin and 50 µg/ml streptomycin was added. Then epidermis was separated from dermis, and the epidermal cells released into the medium by gently scraping and agitating both the epidermal and the dermal compartment of the biopsy. Epidermal cell suspensions (0.7×10^6 trypan blue-excluding cells per ml) were plated on 16 mm culture dishes pre-coated with a collagen type I gel (Vitrogen, Flow Laboratories). Cells were incubated at 37°C in 100% humidity in a 95% air/5% CO₂ environment. After 24 h the plating medium was replaced with McCoy's 5A medium supplemented with 50 IU/ml penicillin, 50 µg/ml streptomycin, 4×10^{-4} M L-serine and 10% of either FBS or normal human serum (NHS). NHS was obtained from healthy adult volunteers taking no medication. The medium was changed twice a week, and the cultures became confluent after 1–3 weeks. The success rate of growing keratinocytes was 80–90%.

DNA synthesis

DNA synthesis via the salvage pathway was determined by [³H]thymidine ([³H]TdR) incorporation into terminally labelled cultures and by autoradiography as previously described by us (Marcelo *et al.*, 1978). For [³H]TdR incorporation cultures were pulsed for 6 h with 1 µCi/ml [³H]TdR (60 Ci/mmol). For autoradiography keratinocytes were grown on plastic coverslips (Lux, Flow Laboratories) and labelled for 16 h with 10 µCi/ml [³H]TdR. The labelling index was determined by counting the number of labelled nuclei of 250 cells in twenty-five fields.

The activity of the *de novo* pathway of DNA synthesis was evaluated by measuring the incorporation of 1 µCi/ml [¹⁴C]orotic acid (46 Ci/mmol) into cultures labelled for 6 h. Since orotic acid is incorporated into both DNA and RNA, the DNA was separated from RNA before counting (Marcelo *et al.*, 1978).

Differentiation of keratinocyte cultures

Extraction of epidermal protein fractions was performed as earlier described by us (Marcelo & Tong, 1983). After a 2 h pulse with 10 µCi/ml [³H]leucine, five extraction buffers were used in

series to separate the cell proteins into six fractions: high salt-soluble protein (S_2), keratohyalin protein (R_2), sodium dodecyl sulphate-soluble proteins (S_3), non-disulphide cross-linked keratin (S_4), disulphide cross-linked keratin (S_5) and envelope protein (R_5). Each protein fraction was then subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis and fluorography. For fluorography the gels were impregnated with EN³HANCE, dried and exposed to X-Omat X-ray film at -70°C for 2 weeks.

Psoriatic serum

Whole blood serum was obtained from untreated psoriatic patients with a skin involvement of 20% to 80%. Ten per cent psoriatic serum in McCoy's 5A medium was added to confluent epidermal keratinocyte cultures. After incubation for 72 h at 37°C the DNA synthesis was measured as described above.

RESULTS

In order to define the importance of the serum source on the growth of human epidermal keratinocytes *in vitro*, the cells were grown in McCoy's 5A medium supplemented with 10% of FBS or NHS (Table 1). Measured as the day of complete confluency of the keratinocytes, NHS was superior to FBS in supporting cell growth.

TABLE 1. Growth of normal human epidermal keratinocytes cultured in McCoy's 5A medium with 10% of fetal bovine serum (FBS) or normal human serum (NHS)

| Serum | Day of confluence |
|-------|-------------------|
| FBS | 13 (7-23) |
| NHS | 9 (5-17)* |

Results are expressed as medians and 95% confidence limits for experiment with 5 different keratinocyte donors. Experiments with NHS from 4 different donors gave similar results.

* $P < 0.01$ (Wilcoxon's test for paired samples).

This capacity of NHS could be due to a mitogenic effect or to an enhanced growth secondary to increased plating efficiency. To evaluate these possibilities, DNA synthesis was measured in confluent keratinocyte cultures. Under these circumstances cells grown in NHS continued to synthesize more DNA than cells in FBS (Table 2). Heat treatment (56°C for 30 min) of FBS and NHS had no effect on the DNA synthesis of keratinocytes (Table 2). Also, no difference was found between isologous and autologous NHS (Table 2). In subsequent experiments human epidermal keratinocyte cultures were established in medium supplemented with untreated, isologous NHS. Under these culture conditions, sodium dodecyl sulphate-polyacrylamide gel electrophoresis of the non-disulphide cross-linked keratin fraction (S_4) of normal human

TABLE 2. DNA synthesis of confluent cultures of normal human keratinocytes grown in medium with 10% fetal bovine serum (FBS) or normal human serum (NHS)

| Serum | <i>n</i> | Heat (56°C for 30 min) | DNA (µg/16 mm well) | [³ H]TdR incorporation (c.p.m./µg DNA) |
|------------------|----------|---------------------------|------------------------|---|
| FBS | 7 | o | 7.9 (5.2-10.4) | 1085 (720-1507) |
| FBS | 5 | + | 8.1 (4.9-11.8) | 995 (690-1510) |
| NHS (isologous) | 7 | o | 8.5 (5.2-11.7) | 1931 (1223-2955)* |
| NHS (isologous) | 5 | + | 8.4 (5.5-12.1) | 1830 (1115-2861)* |
| NHS (autologous) | 6 | o | 8.0 (5.3-10.8) | 1842 (1222-2743)* |

Results are expressed as medians and 95% confidence limits. Experiment with NHS from three different donors gave similar results.

* NHS vs. FBS: $P < 0.01$ (Wilcoxon's test for unpaired samples).

94 kd →

67 kd →

43 kd →

30 kd →

20 kd →

14.4 kd →

FIGURE 1. Separation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis of polypeptides in the S₄ fraction (non-disulphide cross-linked keratins) extracted from day 14 human keratinocyte cultures. Gels were stained with 0.25% Coomassie Brilliant Blue. Authentic standards are shown on the left.

keratinocytes resolved bands with molecular weights ranging from 40,000 daltons to 68,000 daltons (Fig. 1).

Table 3 compares the DNA content and the DNA synthesis of confluent epidermal keratinocyte cultures obtained from normal human skin or uninvolved psoriatic skin. Keratinocytes from uninvolved psoriatic skin showed an increase of DNA synthesis determined as the incorporation of [^3H]TdR into DNA. There was, however, an overlap of data when DNA synthesis of cultures from normal and uninvolved psoriatic epidermis was compared (Fig. 2). The [^3H]TdR used in both these assays is a precursor of the salvage pathway of DNA synthesis. The incorporation of [^{14}C]orotic acid, a precursor of the *de novo* pathway, was equally low in cultures of keratinocytes from normal and uninvolved psoriatic skin (Table 3). The acid-soluble

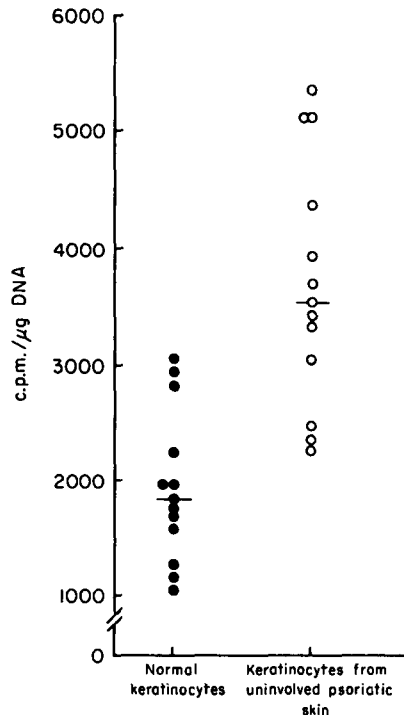


FIGURE 2. Incorporation of [^3H]thymidine into DNA of cultured keratinocytes from normal (●) and uninvolved psoriatic skin (○). The horizontal bars indicate the median values. Psoriasis vs. normal: $P < 0.01$ (Wilcoxon's test for unpaired samples).

pool of [^3H]TdR was normal in keratinocyte cultures from psoriatics, indicating a normal cellular transport of [^3H]TdR (data not shown). To confirm that the [^3H]TdR data represented actual increase in proliferative rate rather than simply an altered uptake of [^3H]TdR, cells were prepared for autoradiography (Table 3). Measured as the labelling index psoriatic keratinocytes also showed an increased DNA synthesis.

Replacement of the NHS with serum from patients with active psoriasis resulted in a stimulation of the DNA synthesis of keratinocyte cultures from both normal and uninvolved psoriatic skin (Table 4). The difference between normal and psoriatic keratinocytes was significant at the same statistical level as before adding psoriatic serum.

TABLE 3. DNA synthesis of confluent cultures of human keratinocytes from normal and uninvolved psoriatic skin

| Keratinocytes | DNA ($\mu\text{g}/16$ mm well) | Labelling index (%) | [^3H]TdR incorporation (c.p.m./ μg DNA) | [^{14}C]orotic acid incorporation (c.p.m./ μg DNA) |
|---------------|------------------------------------|------------------------|--|--|
| Normal | 8.4 (6.2-12.2) | 11 (6-18) | 1890 (1258-2810) | 3 (1-5) |
| Psoriasis | 8.6 (6.4-12.5) | 16 (10-23)* | 3555 (2484-5119)* | 4 (2-5) |

Results are expressed as medians and 95% confidence limits for thirteen experiments.

* $P < 0.01$ (Wilcoxon's test for unpaired samples).

TABLE 4. Effect of psoriatic serum on the DNA synthesis of cultured keratinocytes from normal and uninvolved psoriatic skin

| Keratinocytes | Serum | n | DNA ($\mu\text{g}/16$ mm well) | Labelling index (%) | [^3H]TdR incorporation (c.p.m./ μg DNA) |
|---------------|-----------|----|------------------------------------|------------------------|--|
| Normal | Normal | 11 | 8.2 (6.6-10.4) | 12 (6-19) | 1854 (1410-2675) |
| Normal | Psoriasis | 8 | 8.6 (6.0-10.4) | 17 (11-24)* | 3417 (2280-5222)* |
| Psoriasis | Normal | 8 | 8.9 (6.1-11.2) | 16 (9-23)** | 3900 (2510-5510)** |
| Psoriasis | Psoriasis | 5 | 7.9 (5.5-11.0) | 22 (14-30)*** | 6574 (4730-7911)*** |

DNA synthesis was measured after incubation with 10% normal human serum or 10% psoriatic serum for 72 h at 37°C.

* Psoriatic serum vs. normal serum: $P < 0.01$.

** Psoriatic cells vs. normal cells: $P < 0.01$.

*** Psoriatic serum vs. normal serum: $P < 0.01$.

DISCUSSION

In this study we have demonstrated that it is possible to maintain the increased DNA synthesis of uninvolved psoriatic epidermis in primary cultures of epidermal keratinocytes. The biopsies were obtained from patients with minimal clinical disease and taken at least 30 cm from lesional skin. It is, therefore, unlikely that subclinical disease or residual involvement from a previous clinical episode explains the increased DNA synthesis of epidermal keratinocytes from psoriatics. As in the *in vivo* situation, this increase of keratinocyte DNA synthesis was not accompanied by an increased DNA content of the cultures. Since enhanced DNA synthesis of keratinocyte cultures stimulated by leukotrienes also show no change of their DNA content (Kragballe, Desjarlais & Voorhees, 1984), it is likely that the enhanced keratinocyte DNA synthesis is accompanied by an enhanced shedding of differentiated cells.

The abnormal proliferative activity of uninvolved psoriatic epidermis observed *in vivo* could be induced by signals from local dermal factors or from systemic factors born via the blood. However, the ability of epidermal keratinocytes to maintain the abnormal DNA synthesis *in vitro* suggests that this abnormality in psoriasis is inherent to the epidermal cells. Incubation with psoriatic serum resulted in a stimulation of the DNA synthesis of normal keratinocyte cultures. It can, however, be excluded that the increased DNA synthesis of cultured psoriatic

keratinocytes is secondary to an *in vivo* exposure to psoriatic serum, because psoriatic keratinocytes were stimulated to the same degree as normal keratinocytes by psoriatic serum. The mitogenic properties of psoriatic serum will be further characterized in a future study.

Previously the *in vitro* behaviour of uninvolved psoriatic epidermis has been studied in both organ cultures and cell cultures. Using whole skin organ explants Harper, Rispler & Urbanek (1978) reported a high labelling index of epidermal cells from uninvolved skin. In contrast to our results, two previous studies of epidermal cell cultures showed a normal proliferation of uninvolved psoriatic keratinocytes determined as the mitotic activity (Liu & Parsons, 1983) and as the surface area per cell colony (West *et al.*, 1983). It is difficult to compare our results directly with those of the other studies because the culture techniques were different. In both of the cited studies epidermal keratinocytes were initially grown on 3T3 feeder cell layers in the presence of FBS and hydrocortisone. Additional supplements were cholera toxin, epidermal growth factor (Liu & Parsons, 1983) and insulin (West *et al.*, 1983). Growth rates were then measured in secondary cultures without feeder layers and without the addition of most of the growth factors, although West *et al.* (1983) added cholera toxin. As demonstrated in the present study, the proliferation of human epidermal keratinocytes is suboptimal in medium with FBS. On the other hand, hydrocortisone, cholera toxin and epidermal growth factor enhance epidermal cell growth (Rheinwald, 1980; Marcelo & Tomich, 1983). In such a culture system the fine regulation of epidermal cell DNA synthesis may be disturbed leading to a change of the intrinsic behaviour of psoriatic keratinocytes. In support of this hypothesis is the lack of 67,000 dalton keratins in keratinocyte cultures established on 3T3 feeder cell layers in the presence of FBS, hydrocortisone and cholera toxin (Fuchs & Green, 1978; Sun & Green, 1978). In contrast, our cell cultures synthesize these large keratins. Keratins of similar size, although not necessarily identical, are found in normal human stratum corneum (Inone, Fukuyama & Epstein, 1976; Sun & Green, 1978). The presence of these keratins in our keratinocyte cultures, therefore, shows that the terminal stages of differentiation can occur *in vitro*. Since this group of keratins was not detected in previous studies of cultured keratinocytes (Sun & Green, 1978), it has been assumed by some investigators that keratinocytes do not undergo terminal differentiation *in vitro* (Rheinwald, 1980; Friedberg, 1983). Our results do suggest that the production of keratins is determined by the culture conditions rather than by a basic difference in the *in vivo* and *in vitro* behaviour of keratinocytes.

The superiority of human serum to FBS in supporting the *in vitro* growth of human cells is not specific for keratinocytes, but has also been demonstrated for monocytes. Human monocytes can be maintained *in vitro* in the presence of human serum (10%), whereas fetal or newborn calf, dog, horse and guinea-pig serum do not support monocyte cultures (Johnson, Mei & Cohn, 1977). For monocytes, heat treatment (56°C for 30 min) of the human serum resulted in a poor cell viability (Johnson *et al.*, 1977), but from our study it appears that human keratinocytes are not dependent on the use of fresh serum.

In conclusion, we have demonstrated that it is possible to establish human epidermal keratinocyte cultures that synthesize the high molecular weight keratins typical of terminal cell differentiation. In such a culture system the epidermal keratinocytes from uninvolved psoriatic skin express increased DNA synthesis. The ability of psoriatic keratinocytes to maintain increased DNA synthesis *in vitro* strongly supports the presence of an inherent defect within the population of epidermal keratinocytes in psoriasis. Using this culture system as an *in vitro* model for the study of psoriasis, the differentiation of psoriatic keratinocytes is presently under investigation.

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