

Psoriatic Hair Follicle Cells

III. Characterization of Aberrant Morphology in Differentiating Cultures

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Psoriatic and control human hair follicle keratinocytes were cultured on bovine eye lens capsules in Epicult dishes for a period of 5-6 weeks and examined using light microscopy. The following morphological differences between cultures were observed: 1. The lower cell layers contained predominantly flattened cells in psoriatic cultures instead of roundish in control cultures. 2. The differentiation pattern was irregular in psoriatic cultures instead of regular in control cultures. 3. The differentiated zone of psoriatic cultures was more compact and thicker in comparison to normal cultures. These differences might allow discrimination between normal and psoriatic cultures. *Key words: Psoriasis; Keratinocyte differentiation in vitro.* (Received August 27, 1986.)

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The human hair follicle is increasingly being appreciated as a source of keratinocytes for in vitro models concerning studies on differentiation, transformation and carcinogenesis (1-3). For dermatologists in particular, the question whether keratinocytes from patients with skin disorders differentiate abnormally in vitro, is intriguing.

In vivo, the human hair follicle plays an important role in the regeneration of the epidermis after wound healing: keratinocytes migrate from the external root sheath of hair follicles to cover the denuded dermis (4, 5). In epidermal wound healing differences have been described between control skin and psoriatic skin: in involved psoriatic skin epidermal repair is markedly higher than in uninvolved psoriatic or normal skin (6). Also in tissue culture differences in early outgrowth and migration between normal and psoriatic keratinocytes have been observed (7). Moreover, after several weeks in culture several morphological differences were found to occur, suggestive of a higher rate of differentiation in psoriatic cells (8, 9). Our studies as well as the findings of others (10) indicate that the differences in the rate of differentiation observed may be due to a faster proliferation of psoriatic keratinocytes versus normal keratinocytes, resulting in the earlier appearance of several markers of differentiation in psoriatic as compared to normal cells.

However, even in older cultures, where most parameters of differentiation were comparable, some of the morphological differences do persist and are even readily observed in light microscopical preparations. The aim of the present study is to further characterize these persisting morphological differences.

MATERIALS AND METHODS

Culturing human scalp hair follicle cells

Hair follicles were obtained from healthy individuals and patients with psoriasis, who had no treatment in the previous month. Keratinocyte cultures were obtained from scalp hair follicles as described previously (11) using the bovine eye lens capsule as a growth substrate (12). Anagen human hair follicles were plucked from the scalp, immersed in culture medium with gentamycin and placed on the preincubated capsules. Cultivation was performed using a culture medium consisting of: Minimal Essential Medium (Eagle) with Earle's salts and 25 mM Hepes (Gibco, Paisley, Scotland), supplemented with 15% foetal calf serum (Gibco), 0.4 µg/ml hydrocortisone (Sigma, St. Louis, USA), 4 µg/ml insulin (Organon, Oss, The Netherlands), 10^{-9} M cholera toxin (Sigma), 10 ng/ml epidermal growth factor (Collaborative Research, Waltham, USA) and 50 µg/ml gentamycin (Sigma).

The Epicults were placed in a humidified incubator without CO₂ at 37°C for an initial period of 3–5 days and subsequently, after first outgrowth had occurred, transferred to an incubator with an atmosphere of 5% CO₂. Thereafter the medium was changed twice per week.

Light microscopy

After a few washings with phosphate buffered saline (PBS) to remove the medium, control cultures or psoriatic cultures (aged 5–6 weeks), were fixed in 2.5% glutaraldehyde in PBS for 1–2 h at room temperature and postfixed in 1% OsO₄ in 0.1 M phosphate buffer pH 7.3 for 1–2 h at room temperature. After dehydration in ascending concentrations of ethanol, the cultures were embedded in EPON. Semithin sections for light microscopy were cut using a Reichert ultramicrotome equipped with glass knives and stained with toluidine blue.

RESULTS

Human hair follicle keratinocytes cultured on the eye lens capsule for a period of 5–6 weeks were studied microscopically with respect to morphological differences which still persisted (8). The age of the controls ranged between 24 and 47 years (mean 35.2 ± 5.8 years, $n=8$), that of the psoriatic patients between 25 and 77 years (mean 48.8 ± 15.4 years, $n=6$).

The 41 cultures (22 from normal individuals, 19 from psoriatic patients) were examined by light microscopy and pictures were taken along the whole length of the culture, taking into account that differentiation was different close to the explant (the hair follicle) and should therefore not be studied in this region. The above mentioned pictures enabled an impression of the state of differentiation of the culture as a whole. From this series of pictures three major criteria appeared important for the description of differences observed between cultures of healthy and psoriatic origin:

1. The lower cell layers contain predominantly roundish cells in control cultures but flattened cells in psoriatic cultures.
2. The morphological aspect of the culture is either regular in control cultures or irregular in psoriatic cultures.
3. In psoriatic cultures the differentiated zone is very compact and relatively thick in comparison to the lower cell layers.

On the basis of these general observations it was attempted to describe the differentiation pattern of normal hair follicle cells in culture and to define the aberrations encountered in cultures of psoriatic origin. Schematic representations of both differentiation patterns were drawn. Descriptions are given below and the characteristic phenomena observed are illustrated by pictures (it should be noted that variation in the staining intensity between cultures may occur as a result of toluidine blue staining).

In a keratinocyte culture, derived from hair follicles of a normal individual, different structures can be observed and classified in 4 major types.

Layer I: Cells in the lower layers are mostly roundish (*a* in Fig. 1), but sometimes they are flattened (elongated, *b* in Fig. 1).

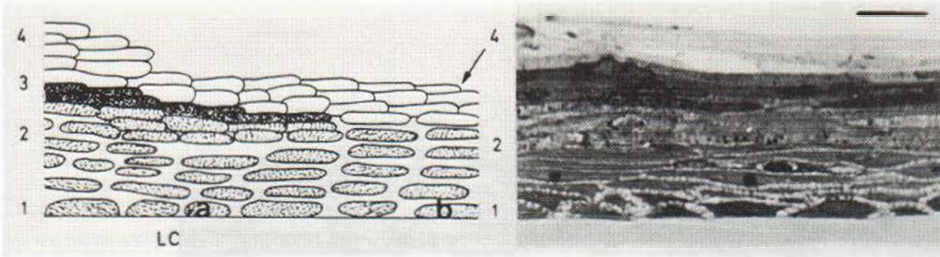


Fig. 1. Semi-thin section of a stratified colony of normal hair follicle keratinocytes in culture: on the left, a schematic representation and, on the right a photograph. The numbers indicate the different cell layers. LC = lens capsule (bar=25 μ m).

Layer 2: The cells (first differentiation zone) are flattened, but nuclei are still present. Relatively large intercellular spaces can be observed as in the lower cell layers.

Layer 3: Above these two cell layers, a differentiated cell layer occurs in cultures older than 4–5 weeks, that may vary in thickness between different cultures. This layer tends to be compact, meaning that intercellular spaces disappear completely. In this layer nuclei are sometimes present and nucleoli can be easily recognized.

Layer 4: On top of the cultures, corneocytes can be observed (these cells are very slightly coloured by the toluidine blue staining procedure). In several cultures clusters of these cells appear to be shed off into the culture medium.

When compared to the normal cultures described above, cultures derived from psoriatic individuals exhibit aberrations.

- As a main rule, psoriatic cultures are irregular in structure. Cellular differentiation is not taking place in an ordered fashion over the entire length of the culture: Fig. 2 gives, on the left, a schematic representation of the morphological pattern observed in psoriatic cultures and, on the right, a typical example of psoriatic hair follicle culture.
- The cells of layers 1 and especially 2 are elongating excessively, thereby enlarging their surface enormously. This gives rise to very long parallel intercellular spaces between the thin and elongated cells (Figs. 2^{III} and 3 A).
- In psoriatic cultures, corneocytes are sometimes encountered in the middle of the compact structure. They appear to be relatively large in size (Figs. 2^{II} and 3 B). The

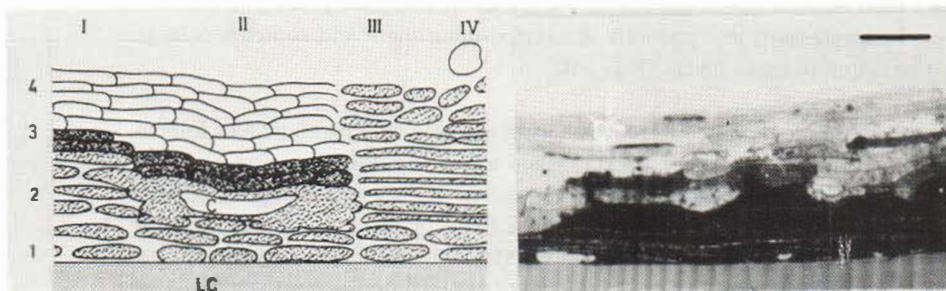


Fig. 2. Semi-thin section of a colony of psoriatic hair follicle keratinocytes in culture: on the left, a schematic representation indicating the different aspects that may occur in different parts of the culture (roman numerals); arabic numerals indicate the different cell layers. C = inclusion of corneocyte; on the right a typical example of psoriatic hair follicle culture (bar=25 μ m).

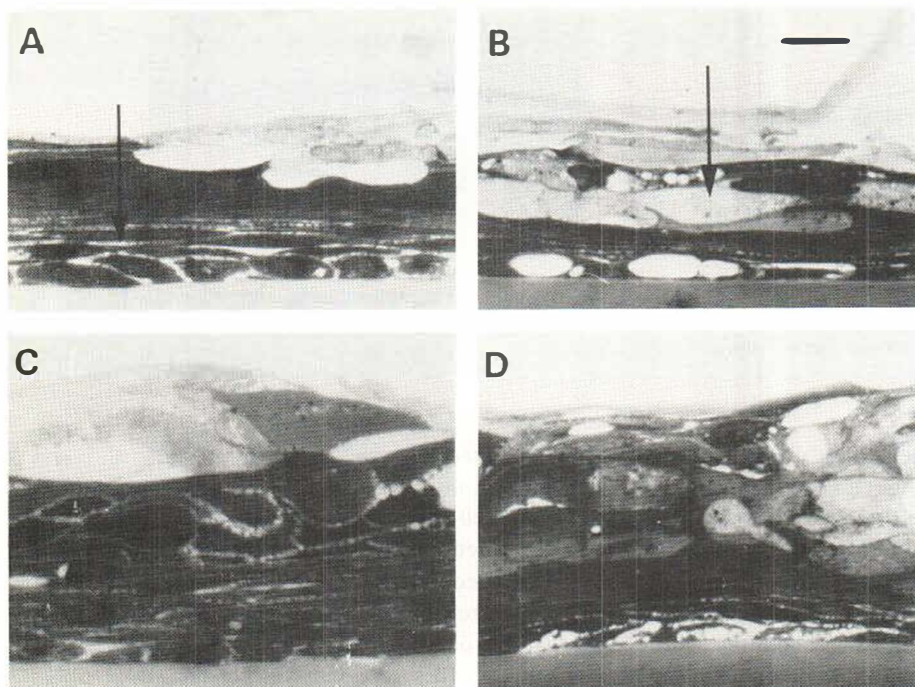


Fig. 3. Illustration of the different aspects that may occur in different parts of psoriatic hair follicle cultures: (A) Irregularity in structure and presence of very long and parallel intercellular spaces (arrow). (B) Presence of corneocytes in the middle of less differentiated cells (arrow). (C) Apparition of a new layer of large basal-type cells above layers 1 and 2. (D) Existence of a very thick compact zone above Layers 1 and 2 (bar=25 μ m).

presence of less differentiated cells above and all around corneocytes is very typical of psoriatic cultures.

- In some cases, a new layer of large basal-type cells exists above layers 1 and 2, where excessive flattening has occurred. These cells can be easily distinguished due to the fact that intercellular spaces are clearly visible. In these cases corneocytes (if present) do not form a compact layer (Figs. 2^{IV} and 3C).
- If a compact zone is formed (Figs. 2 II and 3D), it tends to be thicker than in normal cultures (more than 70–75% of the total culture thickness). The underlying layers (layers 1 and 2) give the impression that pressure is exerted upon them and sometimes large vacuoles can be found in these layers (Fig. 3B).

On the basis of the above mentioned differences between normal and psoriatic hair follicle keratinocyte cultures, a questionnaire was established to see if a discrimination could be made between normal and psoriatic cultures (Table I). Two independent observers screened 99 cultures derived from 15 control individuals (7 males and 8 females) and 9 psoriatic patients (5 males and 4 females). The ages of the controls ranged between 24 and 60 years (mean 37.3 ± 11.9), that of the psoriatic patients ranged between 26 and 67 years (mean 52.7 ± 13.0). Cultures were classified as psoriatic when 4 or more questions were answered positively. After screening, these results were listed per individual. The most frequently occurring classification was taken as the final diagnosis. The results for both observers were comparable and gave the right classification for more than 70% of the

individuals. The results were analysed statistically using the chi-square test and differences between both groups were found to be statistically significant for both observers ($p<0.01$).

DISCUSSION

In a previous study (8) we observed that cultures of psoriatic and normal origin differentiate to a different extent during the first three weeks in culture. However, cultures aged 5–6 weeks appear comparable with regard to several markers of differentiation such as membrane coating granules, keratohyalin granules, basal lamina/cellular debris and the presence of corneocyte-like cells. In vivo, an early psoriatic lesion is characterized histologically by the following parameters (13):

- 1. Sparse mixed inflammatory-cell infiltrate of lymphocytes, histiocytes, and neutrophils; extravasated erythrocytes are common.
- 2. Dilated slightly tortuous capillaries in the papillae.
- 3. Slight edema of the papillary dermis.
- 4. Slight psoriasiform epidermal hyperplasia.
- 5. Small collections of neutrophils within the epidermis, especially within the spinous and granular layers (spongiform pustules), beneath the cornified layer (subcorneal pustules), and within mounds of parakeratosis (pustular scale-crusts).
- 6. Granular layer largely preserved.
- 7. Orthokeratosis above regions of intact granular layer and between pustular scale-crusts.

In our culture system, neither containing dermis nor cells from the circulating system, only differences related to epidermal structure can be expected such as those mentioned above

Table I.

	Control/no	Psoriatic/yes
1. Is the structure of the culture irregular at the major part of the culture?		
2. Has a differentiated layer been formed only at some sites of the culture?		
3. Has a differentiated layer been formed over the entire length of the culture with a compact structure and does this layer show at several parts of the culture:		
a. 70% or more of the total thickness of the culture?		
b. much variation in thickness of the culture?		
c. the impression that pressure is exerted on the lower cell layers?		
4. Do the lower cell layers contain predominantly flattened cells?		
5. Are there corneocytes that are completely surrounded by less differentiated cells?		
6. Are there corneocytes present at the top of the culture which are relatively structureless?		
7. Are there basal-type cells present in the upper part of the culture or on top of the so-called extensively flattened cells?		
Total		

under 4, 6 and 7. Moreover, epidermal hyperplasia cannot be psoriasiform since the growth substrate is flat and immobilized. Nevertheless, increased cellular mitosis is suggested by recent findings (10) and appears to be further confirmed by our own findings on initial outgrowth of psoriatic hair follicle cells in culture (7) as well as by the increased number of layers found in cultures of psoriatic origin (8).

With regard to the presence of a granular layer, our cultures appear comparable to an early psoriatic lesion rather than to a long-standing plaque. This is a result one should expect considering the age of our cultures. Although its morphological structure is irregular, orthokeratosis exists at many sites in almost every culture, again a phenomenon identical to that observed in early lesions.

The differences in morphology described in the present paper provide further evidence that the psoriatic keratinocyte behaves abnormally in vitro, as well as in vivo, even in the absence of a psoriatic dermis. However, the present results also show that morphological observations alone, although giving rise to a statistically significant discrimination between both groups, are too time consuming and insufficiently clear cut as to allow the testing of drugs meant to normalize the epidermal differentiation.

In vitro differences might become easier to distinguish if differentiation of the cultures would proceed towards the formation of a mature stratum corneum. A recently developed new culture device might serve this purpose (14). Moreover it would allow in vitro recombination with dermal components and cells from the circulating system. A prerequisite for this approach however is an adaptation that renders the system less time consuming. The use of a disposable system would be an important step towards this goal. Meanwhile the actual results indicate that the present system might be useful to study the biochemical parameters known to be affected in psoriatic lesions. It would help to acquire more insights into the sequence of events leading in vivo to a psoriatic plaque. Our system could allow, better than studies on affected whole skin, to distinguish primary biochemical shifts from secondary ones.

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