Psoriasis: maintenance of an intact monolayer basal cell differentiation compartment in spite of hyperproliferation

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SUMMARY

Frozen sections of punch biopsies from normal epidermis and psoriatic involved and uninvolved epidermis have been examined immunocytochemically using a panel of anti-keratin monoclonal antibodies with various specificities in the skin. Since psoriasis is thought to involve hyperproliferative expansion of the basal compartment from one to about three cell layers in thickness, the samples were screened with antibodies to intermediate filament determinants associated with basal cells, suprabasal cells and hyperproliferating keratinocyte-derived cell lines, respectively. The basal-suprabasal division was observed to be intact, with only one layer of basal cells demarcated by the specific antibodies used under all circumstances. This suggests that (a) psoriatic ‘basal cell hyperproliferation’ may not specifically involve the basal cell compartment containing the stem cells, but rather a population of amplifying transit cells which are predominantly suprabasal, and that (b) while keratinocyte differentiation begins as the cells lose contact with the basal lamina, the first stages at least of differentiation are not dependent on the loss of the capacity to divide.

The epidermis is a compound stratified squamous epithelium in which cells undergo progressive terminal differentiation as they migrate from the basal proliferative zone, through the stratum spinosum to the stratum granulosum and out to the stratum corneum. As epithelial cells, the keratinocytes of the epidermis contain intermediate filaments (tonofilaments) composed exclusively of keratin filament proteins (Franke et al., 1979), and their exact filament composition at any one time reflects their position in the differentiation sequence (Fuchs & Green, 1980). Polyclonal and monoclonal keratin antibodies have been described which label discrete compartments in the epidermis (Viac et al., 1980; Banks-Schlegel, Schlegel & Pinkus,

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1981; Woodcock-Mitchell et al., 1982), in addition to the immunological distinctions which can be made between the epidermis itself and the skin appendages such as sweat glands (Debus, Weber & Osborn, 1982), or between keratinocytes and keratinocyte-derived cell lines (Lane, 1982; Taylor-Papadimitriou et al., 1982). The fine specificity of monoclonal antibodies is therefore particularly suited to the immunological dissection of a complex epithelial tissue such as skin. In this study we have used such antibodies to examine the epidermis in psoriasis, traditionally regarded as a skin disorder involving basal cell hyperproliferation.

METHODS

Epidermal biopsies
Twenty-one paired samples were taken from untreated psoriatic patients using 5 mm punch biopsies. In addition to the paired psoriasis samples a further five involved and nine uninvolved psoriasis biopsies were examined. Uninvolved skin was taken from the inner upper arm and involved skin from the nearest plaque to the uninvolved site, usually extensor upper arm or elbow. Control samples, also 5-mm punch biopsies, were taken from the inner upper arm of normal volunteers with no evidence of psoriasis (12 samples). Both these types of skin samples were snap-frozen with liquid nitrogen and stored at −70°C until the frozen sections were cut.

Keratinocyte culture
Keratinocytes were cultured as described previously (Taylor-Papadimitriou et al., 1982) from neonatal foreskin, redundant normal adult skin from mastectomy specimens, and paired 5 mm punch biopsies of involved psoriatic plaque and uninvolved skin from psoriatic patients. Cells were serially trypsinized at 37°C and cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum, hydrocortisone (5 μg/ml) and cholera toxin (50 ng/ml), in the presence of X-irradiated 3T3 feeder cells.

Monoclonal antibodies
The antibodies used in this study are all described in detail elsewhere. Their principal specificities are as follows. Monoclonal antibody LP34 recognizes an antigenic determinant common to a wide range of keratin polypeptides throughout human epidermis and in most simple epithelia. LE61 and LE65 (Lane, 1982) are specific for simple epithelia, recognizing different determinants on keratin 18 (see classification of keratins by Moll et al., 1982); they do not react with normal keratinocytes in situ, although transformed keratinocytic lines often do express the LE61/LE65 determinants (Lane, 1982; Taylor-Papadimitriou et al., 1982; Rupniak et al., 1982). A third group of intermediate markers listed in Table 1 (LP2K, LP3K) lie between LE61 and LP34 in their range of reactivity, reacting with simple epithelial cells as well as with certain specific subpopulations of keratinocytes (Lane et al., 1984). PAb421 reacts with the nuclear protein p53 (Harlow et al., 1981) but also recognizes keratins in the basal layer of epidermis (see Crawford, 1983); PAb601, raised against SV40-T antigen (Gooding et al., 1983) also recognizes intermediate filaments (L. Gooding, personal communication) of keratin found in epidermal basal cells and in some cultured cells. The nuclear specificity of PAb601 and PAb421 can be disregarded for the purpose of this study, since the filamentous pattern of the keratin staining by these antibodies is easily distinguishable from the nuclear localization of p53 and T antigen. K92 (raised against human hair keratins) and AJ4 (raised against leukaemic cells) also recognize epidermal keratins (K.A. Pulford, unpublished data) in the suprabasal (K92) and the basal (AJ4) compartments. RKSE60 (Ramaekers et al., 1983) is directed against suprabasal
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* Not monoclonal, smaller sample numbers, n = 6. ND = not done.
keratin filaments in normal epidermis. In addition, a polyclonal rabbit antiserum to involucrin (Rice & Green, 1979) was used on some of the samples.

**Immunofluorescence**

Cultured keratinocytes on plastic dishes were fixed using methanol/aceton (1:1). Frozen 6-µm sections of skin biopsies were stained unfixed. Monoclonal antibodies were used as undiluted culture supernatant and incubated on all samples for 45 min at room temperature; specimens were washed three times with phosphate buffered saline (PBS), up to 15 min total, and incubated with the second antibody (fluorescein-labelled rabbit anti-mouse IgG, Miles-Yeda, diluted 1:40 in PBS) for 30 min at room temperature. Samples were again washed in PBS, and then after rinsing in distilled water the preparations were mounted in Gelvatol 20–30 (Monsanto Petrochemicals) and examined by epifluorescence with a Zeiss Photomicroscope III.

**Immunoperoxidase**

Sections were stained and washed as for immunofluorescence, but the second antibody was peroxidase-conjugated rabbit anti-mouse IgG (DAKOatts, diluted 1:50 in PBS with 10% fetal calf serum). Two subsequent washes were carried out in PBS with 0.02%, Tween 20 and

![Figure 1](image-url)

**Figure 1.** Suprabasal keratinocyte markers. Frozen sections of epidermis from psoriatic subjects. (a) Immunofluorescence and (b) phase contrast image of uninvolved skin with edge of sweat gland duct (bottom of picture), stained with RKSE60. Control skin samples were stained similarly. (c) Immunofluorescence and (d) phase contrast image of section of involved psoriatic plaque stained with RKSE60. K92 gave the same result. Arrows indicate basal lamina. Scale bars = 25 µm.
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1% bovine serum albumin. Enzyme localization was visualized by incubation in diaminobenzidine tetrahydrochloride at 2 mg/ml (in PBS with 0.05% stock hydrogen peroxide) for 5–10 min, until the colour developed; sections were then washed again and counterstained with haematoxylin and eosin.

RESULTS

The results of the histochemical analysis are given in Table 1. All the basal cell markers stained a single monolayer of basal cells on the basal lamina and the suprabasal markers gave a complementary distribution. Simultaneous incubation with a basal and a suprabasal marker antibody together stained all the cells in the epidermis as far as we could tell, i.e. there was no zone of cells that remained unstained by both categories.

Keratin common antigen

Although the monoclonal antibody LP34 was raised against psoriatic plaque scale (stratum corneum), its reactivity range is extremely broad, including epithelia from full thickness epidermis to colon epithelium (E.B. Lane et al., unpublished data). Rare negative cells are occasionally seen in the basal layer of the epidermis which are interpreted as non-keratinocytes, but otherwise the whole of the the epidermis is always stained by LP34 in frozen sections of normal control, psoriatic involved and psoriatic uninvolved skin. However, the keratin antigens in the basal layer appear to be less stable than the suprabasal antigens, and staining with all the antibodies was gradually reduced with increasing time after biopsy. After methanol, acetone or aldehyde fixation, LP34 staining in the basal layer was diminished, due either to such instability or to antigen masking.

Suprabasal markers

K92 and RKSE60 both stain suprabasal cells in normal epidermis, with only a single basal cell layer remaining unstained; in the samples of psoriatic lesions, both antibodies still stained all the suprabasal cells (Fig. 1). Immunofluorescence preparations showed a reduction of K92 and

![Figure 1. Reduction of suprabasal staining in involved psoriatic lesions. Sections of involved (a) and uninvolved (b) psoriatic epidermis stained by immunofluorescence with K92. Suprabasal cells only are stained; (a) and (b) were photographed at the same exposure. Similar results were obtained with RKSE60, but were not so obvious since that antibody is stronger. Scale bar = 25 μm.](image-url)
FIGURE 3. Basal keratinocyte markers. AJ4 staining sections of uninvolved skin, grazing hair follicle [(a) fluorescence, (b) phase contrast] and involved psoriatic epidermis [(c) fluorescence and (d) phase contrast]. The extent of the staining of uninvolved skin was indistinguishable from that of normal control skin; as in psoriatic plaque, a single continuous basal layer only is stained. PAb601 and PAb421 gave the same results. Scale bars = 38 μm.

FIGURE 4. Qualitative difference in staining patterns between suprabasal and basal keratinocytes. While the staining with the suprabasal markers K92 (a) or RKSE60 was not resolvable into filaments, higher power micrographs do show filamentous staining in basal cells by AJ4 (b), also by PAb421 and PAb601. This is thought to be due to the changes in keratin organization that are taking place as differentiation commences. Arrows indicate basal lamina. Scale bar = 15 μm.
RKSE60 suprabasal staining intensity in involved psoriatic epidermis (Fig. 2). Saturation staining with immunoperoxidase also left only a single basal cell layer unstained. Occasional stained cells (usually fewer than 1%) were seen which still appeared to be in contact with the basal lamina, both in normal and psoriatic skin; these were assumed to be in transit between the basal and the epibasal layers. Involucrin expression in the involved psoriasis samples also showed no significant difference from control normal skin, possibly even appearing at a slightly deeper level in the epidermis (Fig. 3) than might be expected (Banks-Schlegel & Green, 1981).

**Basal cell markers**

Antibodies AJ4 (Fig. 4), PAb601 and PAb421 all show staining of a single basal layer in normal epidermis. In the basal cells, filamentous staining patterns can be seen with these antibodies by immunofluorescence of thin frozen sections (Fig. 5), in contrast to suprabasal keratin staining, which is rarely resolvable into filaments. Immunofluorescence with AJ4 gave variable results in psoriatic material, but saturation staining with immunoperoxidase showed a single basal layer of positive cells in involved (and uninvolved) psoriatic epidermis. (Staining with AJ4 was observed to decrease with time on storage of the supernatant at 4°C). Immunoperoxidase staining with PAb601 or PAb421 showed a single positive basal cell layer staining in all specimens.

In passing, it is curious to note that all three monoclonal antibodies used here as basal cell markers were identified primarily as antibodies to other proteins (see Methods), and their cross-reactivity to basal layer keratins was discovered afterwards. Whether the molecular similarity implied by cross-reacting monoclonal antibodies, between keratins and these (and other) growth regulation related proteins is of any biological significance, remains to be seen.
Keratin simple epithelium antigens
There was no expression of simple epithelial cytokeratin antigens detectable by LE61 or LE65 in any epidermal keratinocytes in situ. Similarly, there was no staining by LP2K or LP3K in psoriatic or normal epidermis neither in basal nor suprabasal keratinocytes (excluding hair follicles, which were not examined in these experiments). Parts of the sub-epidermal glands were positively stained. Rare positive cells were seen along the basal lamina of the epidermis, which are believed to be non-keratinocytes, probably Merkel cells (Lane & Klymkowsky, 1982).

Technical factors influencing interpretation of results
One potential problem in interpretation of the data on skin sections is the ambiguity arising from changes in the angle between the plane of the basal lamina and the plane of section (Penneys et al., 1970). When serial sections cannot be compared, this problem can be reduced by observing the cell shape in the basal layer. In areas where the plane of the basal lamina is at less than 90° to the plane of the section, multiple layers of round cell profiles can apparently be stained with basal markers (or not stained with the suprabasal markers), but where the section runs through the longitudinal axis of the columnar basal cells, the basal compartment is clearly restricted to a single cell layer. An example of this can be seen around the rete ridge in Fig. 3c. Due to the convoluted nature of the psoriatic basal lamina, we cannot prove that there is not a single 'basal cell' (as defined by our antibodies) that is truly out of contact with the basal lamina, but most of the basal compartment is clearly only one cell thick. The immunohistochemical staining cannot be reconciled with the image of three cell layers in the basal compartment that is usually assumed for psoriasis.

Following fixation of tissue with formalin or methanol–acetone, staining with basal layer markers was reduced. Increasing storage time of samples at temperatures above −70°C, and delay in freezing specimens, also diminished basal layer staining. Antibody stability also varied; AJ4 was the least stable and fresh antibody had to be used every couple of weeks. For visualizing the antibody binding, immunofluorescence clearly gave greater resolution of cellular structure, but immunoperoxidase could be made much more sensitive by incubating the specimen in enzyme substrate until saturation was reached. This also reduced variation in staining intensity.

Keratinocytes in culture
Expression of the basal and suprabasal specific markers was reduced or variable in cultured keratinocytes from all sources. Basal cell markers are variably expressed in culture (AJ4 and PAb601 staining was not detectable by immunofluorescence but PAb421 shows weak tonofilament staining of monolayer keratinocytes) and no significant differences could be found between normal and psoriatic keratinocytes. Suprabasal markers were expressed in stratified colonies of cultured keratinocytes (tonofilaments of suprabasal cells were stained weakly with K92 and more strongly with RKSE60) and also in a squamous carcinoma-derived line, TR131, which differentiates substantially in culture (Rupniak et al., 1982). Simple epithelium markers such as LE61 showed no staining of normal or psoriatic keratinocytes in culture, in spite of the hyperproliferation seen in these situations. While LP2K and LP3K were always negative on keratinocytes in situ, we occasionally found weakly positive colonies in the cultured keratinocytes. These cells may originate from part of the hair follicle (Lane et al., 1984), or they may reflect changes in keratin expression since it is known that such changes can be induced by tissue culture (Fuchs & Green, 1978).
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LP34 staining was consistently strong in cultures of normal keratinocytes, neonatal and adult, as well as in cultured adult psoriatic keratinocytes.

DISCUSSION

Abnormal keratinization is a characteristic feature of the hyperproliferative lesions of involved psoriatic epidermis. Histopathologically, a normal stratum corneum fails to develop in these areas, with retention of cell nuclei in the stratum corneum as parakeratotic scale. Biochemical analyses of keratins from involved psoriatic epidermis have shown a reduction of high molecular weight (67–70 kd) keratins characteristically expressed by suprabasal cells (Thaler et al., 1980; Baden et al., 1978; Skerrow & Hunter, 1978; Hunter & Skerrow, 1981a; Bowden, Wood & Cunliffe, 1983). Alterations of the keratin profile suggesting incomplete keratinization (Thaler et al., 1978) may be due to the greatly decreased keratinocyte transit time through the epidermis, i.e. from 350 h in normal skin to 36–48 h in psoriatic lesions (Weinstein et al., 1983). Abnormal keratin expression has been detected biochemically in a variety of hyperproliferative skin diseases (Baden et al., 1978), suggesting that this is not specific to psoriasis but rather is a secondary effect of pathological hyperplasia. However, the hyperplasia induced by tape-stripping the skin does not show such reduction of high molecular weight keratins (Hunter & Skerrow, 1981b).

Immunological studies using antiserum to 67 kd keratin showed reduction of intensity and irregular staining on psoriatic plaque (Thaler et al., 1978), confirming the biochemical findings. The diminished intensity of staining with suprabasal markers such as K92 and RKSE60 (Fig. 2) also fits with these observations. From some recent immunohistochemical results (Weiss et al., 1983) using a monoclonal antibody AE1 on several hyperproliferative skin disorders including psoriasis it appears that this reduction of high molecular weight keratins might be associated with anomalous exposure of a determinant which is inaccessible to immunochemistry in the suprabasal layers of normal skin.

The epidermal hyperproliferation in psoriasis is generally thought to be hyperproliferation of the basal cell compartment. This interpretation is based on two factors, (a) the demonstrable increase in suprabasal mitotic activity in psoriatic lesions (Van Scott & Ekel, 1963; Weinstein & Van Scott, 1965; Penneys et al., 1970), together with (b) the morphological similarity between cells in this wide proliferative zone and normal basal layer keratinocytes. The standard model for psoriatic epidermis describes a basal proliferative zone three cells thick, as opposed to one cell thick in normal skin (Weinstein et al., 1983). Whether this expansion of the proliferative compartment is due to a reduction in the cell cycle time (i.e., the same number of cells dividing more rapidly) or to an increased recruitment of G0 cells into division (i.e., an increase in the proportion of the epidermal cells dividing), or both, is a matter for discussion (Wright, 1977; Weinstein & Frost, 1968).

In view of this background, it is of interest to find that basal cell associated antigens are still expressed only in a single basal cell layer in psoriatic plaque despite the documented presence of numerous suprabasal mitoses (Penneys et al., 1970; Lavker & Sun, 1983). The complementary finding of expression of suprabasal antigens immediately above this single basal layer (and then extending all the way to the surface) confirms that there is no breakdown of the basal-suprabasal distinction in psoriasis. This observation matches that of van Neste et al. (1983), who observed suprabasal keratins expressed by mitotic cells in normal and experimentally hyperproliferative epidermis. Even the distribution of involucrin (cornified envelope precursor protein; Rice &
Green, 1979) did not show a significant shift between normal and psoriatic epidermis. If anything, it appeared slightly closer to the basal lamina in the psoriatic lesions.

The positive staining of the basal layer keratinocytes appears to be homogeneous. From cell kinetic studies on mouse skin (Potten et al., 1982; Potten, 1974), it has been shown that only a fraction of cells in the basal layer are actually stem cells. Staining of the basal layer keratinocytes showed no patchiness to correlate with the distribution of 'serrated' and 'non-serrated' cells (Lavker & Sun, 1983; a morphological feature suggested to be correlated with stem cell identity), or with the presence of so-called 'dark' cells (Klein-Szanto, 1977). From such homogeneity of staining it has been suggested that the basal-suprabasal distinction depends primarily on contact with the basal lamina (Woodcock-Mitchell et al., 1982). However, we have observed the basal cell antibody binding phenotype (basal marker positive, suprabasal marker negative) in cells several layers removed from the dermo-epidermal junction in a variety of epidermal tumours (Lane et al., 1984) so that this aspect of keratin differentiation is neither solely a position effect nor solely a reflection of a cell staying in the stem cell compartment. The occasional cells in the basal layer which we observed to be positive with the suprabasal markers further serve to confirm that the differentiation stimulus is not entirely a topographical one.

The concept of epidermal cell division as a phenomenon limited to the basal cell layer in normal human skin is also an over-simplification, as was recently pointed out by Lavker & Sun (1983). There is good evidence for a sub-population of dividing keratinocytes among the suprabasal cells of normal epidermis (Penneys et al., 1970; van Neste et al., 1983), and the proportion of mitoses in any epidermis which are suprabasal in their location is correlated with the overall mitotic activity of the epidermis (see discussion in Lavker & Sun, 1983). Like all self-renewing tissues that have been studied, the cell kinetics of the epidermis fit into a two-(or more)-compartment model of proliferation (Potten et al., 1982; Potten, 1981), in which the stem cell (slow cycling, and of unlimited division potential) gives rise to an amplifying population of transit cells (Lajtha, 1979; faster cycling but with a limited number of potential divisions), which are already committed to differentiation. Differentiation of these amplifying transit cells is then completed once they have passed into the post-mitotic compartment. Thus:

stem cells $\rightarrow$ amplifying transit cells $\rightarrow$ post-mitotic cells

Based on morphological data and an extensive literature review, Lavker & Sun (1983) have suggested that the suprabasal mitotic cells may belong to this amplifying transit compartment. The two-compartment theory could thus be used to explain our results satisfactorily, in that the hyperproliferation of psoriasis may not be one of basal cells, and thus not of stem cells since stem cells do not appear to occur outside the basal layer in the epidermis. Instead, non-malignant hyperproliferative conditions, from psoriasis as discussed here to the experimentally induced hyperproliferation assessed with similar differentiation markers by Van Neste et al. (1983), may represent hyperproliferation of a suprabasal and partially differentiated amplifying transit cell compartment.

In this study we have used a group of monoclonal antibodies showing various specificities for keratin filaments (tonofilaments) to look for differences between normal and psoriatic epidermis. While our search for positive markers has proved unsuccessful, this very lack of difference between psoriatic and control skin allows us to draw important conclusions about epidermal differentiation and psoriasis.

1. The hyperproliferation of psoriasis predominantly involves a suprabasal, and partially differentiated, proliferative compartment, and therefore probably not a stem cell compartment.
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2. Keratin differentiation in the epidermis is thus neither solely controlled by a reduction in proliferative potential (in agreement with recent conclusions by Van Neste et al., 1983), nor is it solely controlled by a change in position with respect to the basal lamina. This indicates that other as yet unidentified factor(s) must provide an important stimulus for differentiation.

Our results also suggest that the use of such monoclonal antibodies to distinguish between subpopulations of proliferating cells in the skin may allow us to identify a fundamental difference between benign and malignant hyperplasias. This possibility is now under investigation.

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