Distribution Pattern of Psoriatic Keratoblasts: Computer-Assisted Image-Analysis for Combined Evaluation of DNA Synthesis and Expression of 67 kD Keratin Polypeptides in the Epidermis of Stable Plaques of Psoriasis

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Psoriatic epidermis is characterized by increased DNA synthesis and disturbed differentiation. Even though these processes are closely associated, most investigations do not give insight into temporal/spatial relationships between both events. We previously developed a double labeling method for the simultaneous demonstration of the germinative and differentiated epidermal compartments in normal human skin by using tritium-labeled thymidine ([^{3}H] Thd) incorporation and immunoperoxidase staining of 67 kD keratin polypeptides.

In this paper we report the results of combined evaluation of these compartments in stable plaques of psoriasis. Scanning of skin sections with an automatic image analyzer allows objective quantification of areas of total epidermis, 67 kD$^+$ differentiated epidermis and numbers of [^{3}H] Thdr+ nuclei. Our data indicate that the 67 kD$^-$ undifferentiated psoriatic epidermis is expanded. Increased numbers of [^{3}H] Thd$^+$ basal and suprabasal psoriatic keratinocytes are present and most of them (97.9%) pertain to the 67 kD$^-$ compartment. Keratin identification in scales taken from the same sites showed a variable but distinct decrease of 67 kD keratin polypeptides. Hence, the hyperplastic epidermis of stable plaques of psoriasis is characterized by the presence of increased numbers of [^{3}H] Thd$^+$ cells, which primarily belong to the undifferentiated (67 kD$^-$) basal and suprabasal compartments, especially in the lowermost parts of the elongated interpapillary rete ridges. These changes are associated with a relative decrease of synthesis of 67 kD polypeptides and the presence in the scales of keratins that confer a characteristic hyperproliferative epidermal keratin pattern to the psoriatic plaque. *J Invest Dermatol 90:382–386, 1988*

The affected skin lesions of psoriasis are characterized by a number of changes in the epidermis. These modifications are associated with increased DNA synthesis and disturbed differentiation. Increased numbers of DNA-synthesizing epidermal cells have been reported by almost every investigator interested in the study of epidermal proliferation in psoriatic lesions. This is in agreement with the early reports of increased numbers of mitotic figures in the psoriatic epidermis. Mitoses are present in the three lowermost cell layers of lesions, but in a single basal layer in uninvolved skin of the same patients [1]. When [^{3}H] Thd-labeling indices from various sources are compared, a wide variation exists. The difficulty resides in identifying which cells are actually in the germinative compartment. Indeed, in a recent study using [^{3}H] Thd labeling of normal and psoriatic epidermis in vivo, the germinative labeling index was calculated as the number of labeled basal cells/100 basal cells × 100 and the total labeling index as the labeled cells (basal and suprabasal) per 100 basal cells for each specimen [2]. This implies that neither index is representative of the expanding proliferative cell population. Although the basal cell population only represents a fraction of the proliferating cells [3], the total labeling index, as defined here, overestimates the size of the labeling index by including exclusively S phase cells amidst a population of suprabasal cycling and noncycling cells. This complexity is further illustrated by a statement by Goodwin et al [4]: "Definition of the germinative cell population in psoriasis is difficult and somewhat arbitrary." All these obstacles associated with the potential heterogeneity of basal keratinocytes [5] will not be overcome unless specific markers are developed that are able to distinguish between the various subpopulations in basal and adjacent cell layers pertaining to the growth fraction and differentiating cell populations.

On the other hand, the differentiation is disturbed, as evidenced by abnormal quantitative and qualitative keratin synthesis. These patterns are characterized by expression of keratin gene products that are not present in normal skin and by concommitant reduction of synthesis of high molecular weight keratin polypeptides [6–9]. A useful topographic marker of epidermal cell differentiation is the expression of 67 kD keratin polypeptides.

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Abbreviations:
[^{3}H] Thd: tritium-labeled thymidine
kD: kiloDalton
NepHGE: nonequilibrium pH gradient electrophoresis
PBS: phosphate-buffered saline
PMSF: phenylmethylsulfonyl fluoride
SC: stratum corneum
SDS: sodium dodecyl sulfate
PAGE: polyacrylamide slab gel electrophoresis

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Expression of 67 kD occurs almost exclusively in suprabasal cells of normal epidermis and reduced synthesis has been shown in psoriatic epidermal cells (52% instead of 77% labeled epidermal cells [10]). The possibility of combining the immunolabeling of 67 kD with autoradiography after [^{3}H] Thd labeling of S phase keratinocytes [11] prompted us to further characterize the proliferating cell pool in psoriasis. In this report we confirm decreased synthesis of 67 kD polypeptides in psoriatic epidermis and expansion of the 67 kD$^-$ compartment, which involves both basal and suprabasal cells. Interestingly, increased numbers of [^{3}H] Thd-labeled cells were located in the 67 kD$^-$ compartment, which, in psoriasis, extends largely above the basal cell layer especially in the lowermost part of interpapillary rete ridges.

MATERIALS AND METHODS

Skin Specimens Skin specimens were taken from the outer edges of stable psoriatic plaques. Skin lesions were located on the arms and thighs of four patients (aged more than 45 yr) who had not been treated during the last month. All biopsy specimens were taken between 9 and 12 AM in order to avoid possible diurnal fluctuations of DNA synthesis. After oral consent was obtained, the biopsies were performed under local anesthesia (2% lignocaine without adrenalin) exactly 45 min after intradermal injection of 0.1 ml of [^{3}H] Thd (10 μCi, specific activity 10 Ci/mmol: Centre d' Energie Nucléaire, Mol, Belgium). The 4-mm punch biopsy specimens were immediately fixed in Bouin's fluid. For comparative purposes, we made serial sections in control specimens (four subjects, mean age 81 yr) available from a previously published study [11].

Antiserum Antiserum against the 67 kD keratin polypeptide was raised by immunizing Hartley guinea pigs (400 g). We therefore used 67 kD polypeptides that were extracted from normal human corneocytes as described in detail by Viac et al [12]. Briefly, horny layers were obtained by scraping foot callus of healthy subjects. After keratin extraction [6] and separation of fibrous proteins by 12% polyacrylamide slab gel electrophoresis (PAGE), the bands containing 67 kD keratin polypeptides were used for immunization. Specifity of this 67 kD antiserum was controlled by light and electron microscopic studies [13]. The same batch was used for all the skin preparations.

Immunoperoxidase Staining Throughout this study we used a 1:20 dilution of 67 kD antiserum. Serial sections were left in contact with this antiserum for 30 min. Subsequently, they were washed for 30 min in PBS and then treated for 30 min with peroxidase-labeled rabbit antiguinea pig Ig (1:50 dilution; Nordic). After two successive washes (30 min in PBS and 10 min in Tris HCl buffer; pH 7.6), the presence of peroxidase was revealed by a 15-min contact with Graham Karnowsky medium. Slides were then rinsed in Trish HCl (pH 7.6), PBS and finally processed for light microscopy autoradiography. Preliminary experiments established that labeling of 67 kD polypeptides in psoriatic epidermis was not affected by fixation and autoradiographic procedures.

Quantitative Analysis of Keratin Polypeptides in Psoriatic Scales One-dimensional gel electrophoresis was performed in 8.5% polyacrylamide slab gels using SDS in the discontinuous system described by Laemmli [14]. The analysis was made on scales taken at the same time and from the same site as skin specimens in all subjects. Symmetrically located lesions were also sampled at the same time and a second time 4 wk later in two cases. Scales were immediately frozen after sampling and stored at −80°C until transfer to the laboratory for further analysis. They were homoge-

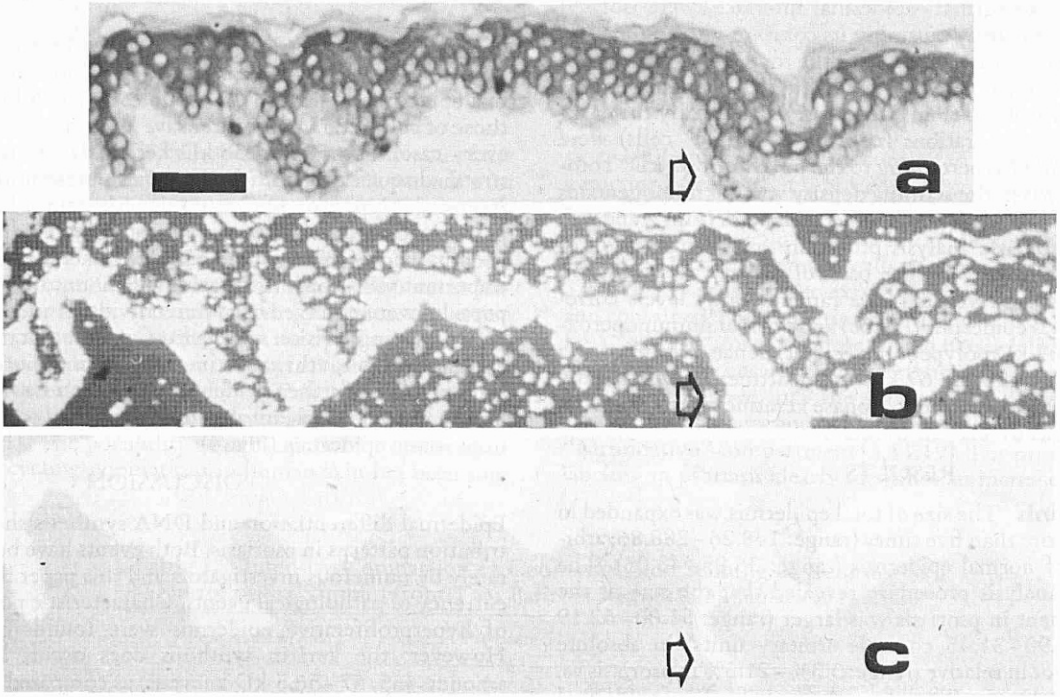


Figure 1. Image analysis of epidermal compartments of skin biopsy (control specimen) by combined labeling of S phase cells (keratoblasts-[^{3}H] Thd$^+$) and of 67 kD keratin polypeptides (keratinocytes and keratoblasts already engaged in cellular differentiation). The image of the skin section (*a*, scale bar = 50 μm) is recorded by a video camera and projected on a high resolution video screen (a slight enlargement ensues when screen is photographed, see *b* and *c*). The epidermis is delineated by using an interactive device. According to the intensity of gray at any given point of the selected area, two discrete compartments can be separated: an unstained area (basal layer, *b*). In this picture, empty areas are visible. They represent 67 kD$^-$ nuclear spaces. These and the stratum corneum (67 kD$^-$ band at the top of *b*) can easily be cleared as nonrelevant signals; a complementary dense 67 kD$^+$ compartment from the first row suprabasal cells to granular layer. One of the pitfalls of automated image selection is illustrated *(arrows)*: a tiny string of 67 kD$^+$ cells *(a)* is recognized as such in *(c)* but is included in the 67 kD$^-$ compartment in *(b)*. This is because cells which have a prominent nucleus and show a limited band of stained cytoplasm are considered at the screen level as nonadjacent dark elements in an otherwise homogeneous 67 kD$^-$ compartment. With these limitations in mind, and by superimposing *a*, *b*, and *c* [^{3}H] Thd cells can easily be alloted to the 67 kD$^+$ or 67 kD$^-$ compartments.

nized in 25 mM Tris HCl (pH 7.4) containing 1 mM phenylmethylsulfonylfluoride (PMSF), 1 mM EDTA, 1% Triton ×100. After centrifugation at 4°C at 10,000 *g* for 10 min, the water insoluble pellet was further extracted by heating at 100°C for 5 min in a solution containing 25 mM Tris HCl (pH 7.4), 10 mM dithiotreitol, 1% SDS, 1 mM EDTA, 1 mM PMSF [15]. Cytoskeletal proteins were electrophoresed and stained by Coomassie blue. The gels were scanned using a laser densitometer (Shimaku Corporation). For two-dimensional gel electrophoresis, samples were first separated by nonequilibrium pH gradient electrophoresis (NepHGE) and then a right angle in the second dimension by SDS PAGE (8.5% polyacrylamide) as described by Bowden et al [16].

Morphometric Analysis For each specimen we determined the following characteristics:

- length of the skin specimen at the surface (stratum corneum, SC)
- number of [^{3}H] Thd labeled 67 kD$^-$ cells (i.e, cells that were below the stratum granulosum or parakeratotic SC down to the dermal–epidermal interface and not stained with the anti-67 kD antiserum)
- number of [^{3}H] Tdh labeled 67 kD$^+$ cells.

All data were then expressed in arbitrary units relative to 1 mm length of SC. Representative segments of psoriatic skin as well as control specimens and a 2-mm rule (one interval = 0.01 mm; Ernst Leitz Gmbh, Wetzlar) were photographed at a standard magnification. Slides were scanned under a high quality video camera and data were recorded, after contrast enhancement, with an IBAS II image analyzer (Kontron, D). Two running procedures were sequentially adopted: first semi-automatic selection of the epidermis followed by automatic recording of the intaepidermal 67 kD$^+$ and 67 kD$^-$ compartments.

Epidermal segments, from granular layer (or stratum corneum in parakeratotic areas) to dermal–epidermal interface, were isolated manually from the image by outlining its contour with an interacting device. After automatic recording of the total epidermal surface in normal skin specimens, the 67 kD$^+$ and 67 kD$^-$ compartments were automatically isolated (Fig 1), measured, and finally the darkest elements of the preparations (i.e., the [^{3}H] Thd$^+$ cells) were identified and recorded as pertaining to the 67 kD$^+$ or 67 kD$^-$ compartment. As in psoriasis the staining density was not homogeneous but increased from deep to superficial keratinocytes (Figs 2 and 3*a*); we adopted an automatic analysis procedure for quantification of the 67 kD$^+$ compartment. On the basis of density of staining in control skin specimens, we defined a range of gray levels corresponding to the total epidermis (Fig 3*b*) and normal immunoperoxidase detection of 67 kD polypeptides (Fig 3*c*) hence discriminating between the 67 kD$^+$ and the 67 kD$^-$ compartments. Once again, the darkest elements correspond to S phase keratinocytes, which can easily be classified as 67 kD$^+$ or 67 kD$^-$.

RESULTS

Psoriatic Epidermis The size of total epidermis was expanded in psoriasis usually more than five times (range: 148.26–288.86, arbitrary units) that of normal epidermis (range: 36.95–43.03). The automatic image analysis procedure revealed that the size of the 67 kD$^+$ compartment in psoriasis was larger (range: 51.00–62.19 in psoriasis vs. 7.90–31.45 controls arbitary units) in absolute values, but by far not in relative (range: 3.3%–21.5% in psoriasis vs. 18%–83% controls) terms. Therefore, the main factor of epidermal expansion is in the 67 kD$^-$ compartment (range: 146.75–226.67 in psoriasis vs. 6.2–35.1 controls, absolute arbitrary units; range: 78.5%–99.04% in psoriasis vs. 17.1%–81.6% controls relative to total area of epidermis). Interestingly, the vast majority of [^{3}H] Thd labeled cells, either basal or suprabasal, belong to the 67 kD$^-$ compartment and they are preferentially distributed in the lowermost segments of the interpapillary rete ridges.

Quantitative Analysis of Keratin Polypeptides in Psoriatic Squames The keratin patterns observed in psoriatic scales were consistent with earlier findings [6–9]. The 65–67 kD and 56.5 kD keratins, markers for advanced stages of keratinocyte differentiation, were markedly diminished when compared to keratins of normal epidermis. A 46–48 kD doublet and a 56 kD band, similar to those of cultured hyperproliferative keratinocytes, were detected in every case. The 50 and 58 kD keratins that are expressed in all stratified squamous epithelia, were also present in all samples. The intensity comparison of Coomassie blue stained keratins in six samples of two subjects (three samples per subject) is presented in Table I. A marked decrease of the 65/67 kD keratins was observed and important variations in the relative amount of these keratin polypeptides were observed between individuals and for a single subject between a single lesion sampled at two different times or symmetrical lesions taken at the same time. Two-dimensional gel electrophoresis provided further evidence that an acidic 48 kD and a basic 56 kD keratin which are absent from normal scales were expressed in psoriatic epidermis (Fig. 4).

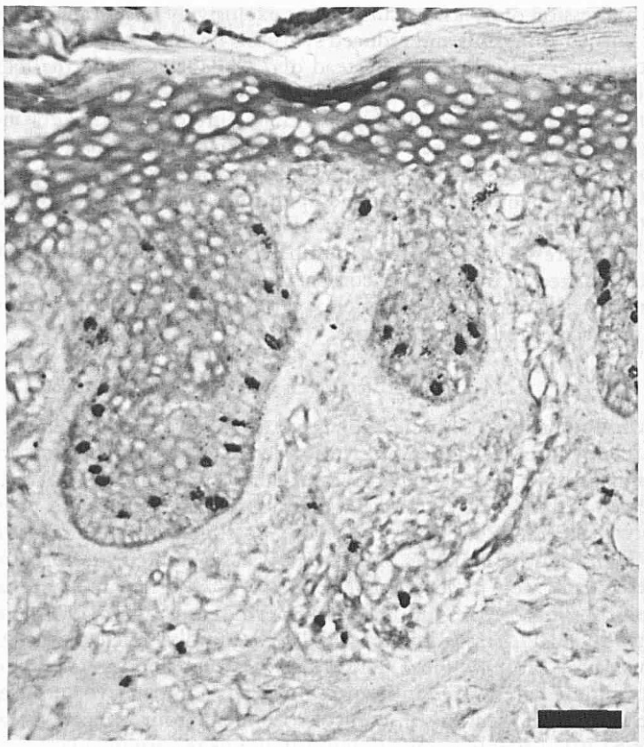
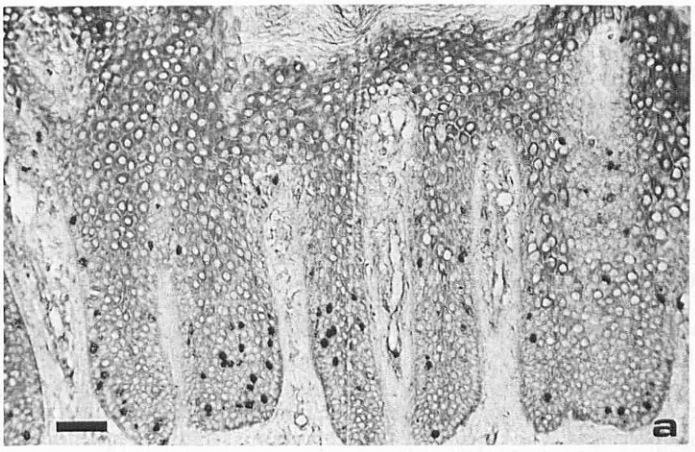
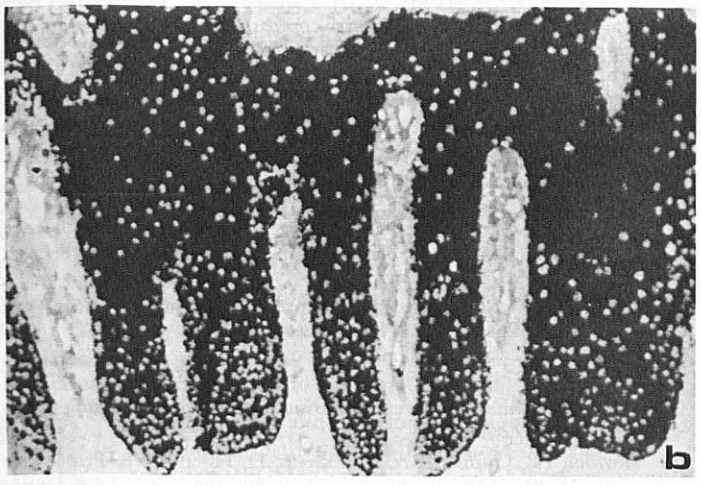


Figure 2. Stable plaque of psoriasis. Combined labeling of S phase cells and of 67 kD keratin polypeptides. There is a clear-cut distinction between [^{3}H] Thd labeled cells *(black dots)* which belong to the 67 kD$^-$ compartment and the absence of [^{3}H] Thd labeling in the 67 kD$^+$ area (scale bar = 50 μm)

DISCUSSION

Epidermal differentiation and DNA synthesis show abnormal distribution patterns in psoriasis. Both events have been reported separately by numerous investigators and this paper illustrates the concurrency of pathological events. Characteristic polypeptide profiles of hyperproliferative epidermis were found in psoriatic scales. However, the keratin synthesis does occur, albeit in reduced amounts (65/67–56.5 kD keratins) as compared with normal subjects. We can emphasize that the failure to make the correct amount of these keratin polypeptides is not due to a defect of a specific subset of abnormal keratinocytes within the psoriatic plaque. Our morphologic study indicates instead a partial defect of all the epidermal cells, with gradually increasing but delayed expression of the 67 kD polypeptide in the lesion. Indeed, the final size of the 67 kD$^+$ compartment is only moderately increased as compared to the tremendous increase of the total epidermal volume in psoriatic epidermis. Conversely, the expansion of the 67 kD$^-$ compartment could be due to a partial deficiency of the proliferative cell in undergoing the cellular commitment to terminal differentiation. At the same time,





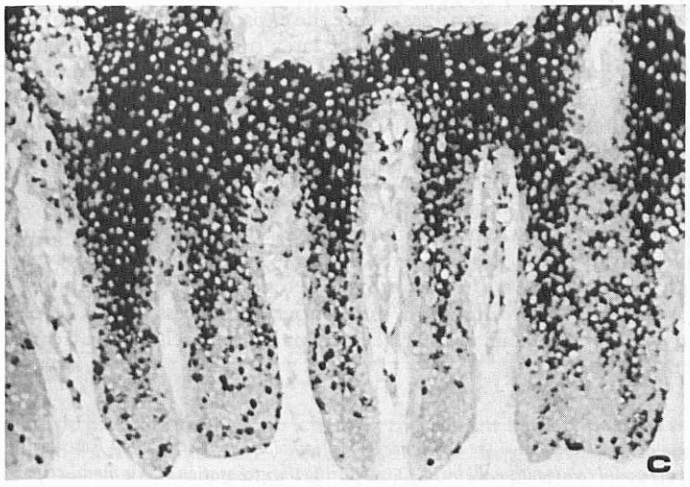


Figure 3. Image analysis of epidermal compartments in psoriasis by combined labeling of S phase cells and of 67 kD keratin polypeptides. *a* Immunohistochemical detection of 67 kD keratin polypeptides shows gradual expression of the marker of keratinocyte differentiation while cells in S phase are 67 kD$^-$ (scale bar = 50 μm). *b* Same as *a*: by selecting the appropriate gray levels in control biopsy specimens, a homogeneous epidermal compartment can be automatically preselected. Elimination of nonepidermal and nonrelevant structures is possible by utilizing an interactive device. *c* Same as *a* and *b*: more specific targetting of the 67 kD$^+$ areas is obtained by selecting a more specific range of gray levels. This preselection is made on control specimens. This picture clearly shows that S phase cells are 67 kD$^-$.

increased numbers of [^{3}H] Thd$^+$ cells, presumably in S phase of the cell cycle, were present and interestingly all (97.9%) but a few of these cells are in the 67 kD$^-$ compartment. Those S-phase cells were preferentially located in the elongated interpapillary rete ridges where the 67 kD$^-$ compartment comprised basal and suprabasal cells. These cells probably represent cycling cells because mitotic figures were also observed in the three lowermost epidermal cell layers [1]. This is in sharp contrast with the apparent restriction of mitotic figures to the basal cell layer of normal involved psoriatic epidermis [1] and the identification of [^{3}H] Thd labeled DNA synthesizing cells in the basal and adjacent supra-basal cells in normal skin biopsies [3,11]. The possibility of participation of differentiating cells into the cycling populations in human skin has been suggested [17] by sorting cells according to their DNA content. Indeed a fraction of cells with DNA content compatible with S and G2/M stages of the cell cycle could be labeled with KL1 monoclonal antibody, which is specific for 56.5 kD acidic keratin polypeptide and is expressed in the suprabasal cells of normal human epidermis [18]. These KL1$^+$ cells did also express bullous pemphigoid (BP) antigen and contained a high fraction of cycling cells as compared with the KL1$^-$ and BP$^+$ (or not differentiated basal) cells. These results suggest that early onset of differentiation can occur in basal cycling cells, which move into the suprabasal cell compartment further supporting the concept of heterogeneity in the basal–suprabasal "germinative" compartment [3,11,19]. The present study of double labeling in psoriasis clearly identifies an increased number of kera-

Table I. Intensity Comparison of Coomassie Blue Staining of Keratins[a] in a Control of Six Samples from Two Patients with Stable Plaques of Psoriasis[b]

		Psoriasis 1			Psoriasis 2		
Keratin (Type Mw)	Control	Site 1 Time 1	Site 2 Time 1	Site 2 Time 2	Site 1 Time 1	Site 2 Time 1	Site 2 Time 2
I:56.5	29.0	—	—	—	—	—	—
I:50	16.5	10.3	14.9	16.3	9.9	11.6	15.9
I:48	—	13.6	13.9	17.8	20.1	17.9	16.5
I:46	—	8.6	9.0	14.1	18.6	17.6	9.6
II:67–65	24.1	10.2	9.1	2.4	0.8	2.0	8.4
II:58	16.6	18.6	21.1	18.4	4.1	12.1	17.9
II:56	—	17.8	19.4	20.1	26.2	20.1	17.9

[a] Expressed in percentage, according to molecular weight.
[b] Paired samples taken simultaneously at symmetrical sites (1 and 2) or at 4-wk intervals at a given site (time 1 and 2).

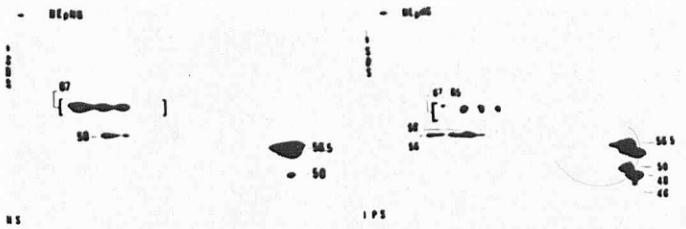


Figure 4. Comparative analysis of total human epidermal keratins by two-dimensional nonequilibrium pH gel electrophoresis (NepHGE) on squames from control skin (NS) and scales from involved psoriatic skin (IPS). Coomassie blue staining, molecular weight is indicated on detected spots.

toblasts belonging to the 67 kD$^-$, or nondifferentiated, epidermal compartment. The expansion of the epidermis seems to be predominantly dependent upon proliferation of keratinocytes in the elongated interpapillary rete ridges where the expression of 67 kD polypeptides is delayed and occurs only later on, after cells migrate outwards (i.e., closer to a level roughly corresponding to the suprapapillary areas of the epidermis). This is very similar to the nonrandom proliferation pattern observed in palmar skin of monkeys [19]. Furthermore, in view of the fact that a single basal cell layer population maintains its normal immunocytochemical characteristics in psoriasis [20], our data support the hypothesis of a transient amplifying suprabasal cell subpopulation with cell division capacity (even though it has lost the basal cell markers) and which is not yet confined in a process of irreversible differentiation. The possibility to modulate the proliferation/differentiation process at this level may well represent an alternative concept in approaching the treatment of psoriasis.

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REFERENCES

1. Van Scott EJ, Ekel TM: Kinetics of hyperplasia in psoriasis. Arch Dermatol 88:373–380, 1963.
2. Weinstein GD, McCullough JL, Ross P: Cell proliferation in normal epidermis. J Invest Dermatol 82:623–628, 1984.
3. Penneys NS, Fulton JE, Weinstein GD, Frost P: Location of proliferating cells in human epidermis. Arch Dermatol 101:323–327, 1970.
4. Goodwin P, Hamilton S, Fry L: The cell cycle in psoriasis. Br J Dermatol 90:517–524, 1974.
5. Milstone LM, LaVigne JF: Heterogeneity of basal keratinocytes: nonrandom distribution of thymidine-labeled basal cells in confluent cultures is not a technical artifact. J Invest Dermatol 84:504–507, 1985.
6. Baden HP, McGilvray N, Cheng CK, Lee LD, Kubilus J: The keratin polypeptides of psoriatic epidermis. J Invest Dermatol 70:294–297, 1978.
7. Thaler M, Fukuyama K, Epstein WL, Fisher KA: Comparative studies of keratins isolated from psoriasis and atopic dermatitis. J Invest Dermatol 75: 156–158, 1980.
8. Bowden PE, Wood EJ, Cunliffe WJ: Comparison of prekeratin and keratin polypeptides in normal and psoriatic human epidermis. Biochim Biophys Acta 743:172–179, 1983.
9. McGuire J, Osber M, Lightfoot L: Two keratins MW 50,000 and 56,000 are synthesized by psoriatic epidermis. Br J Dermatol 111(suppl 27):27–37, 1984.
10. Staquet MJ, Faure MR, Reano A, Viac J, Thivolet J: Keratin polypeptide profile in psoriatic epidermis normalized by treatment with etretinate (aromatic retinoid Ro10-9359). Arch Dermatol Res 275:124–129, 1983.
11. Van Neste D, Staquet MJ, Viac J, Lachapelle JM, Thivolet J: A new way to evaluate the germinative compartment in human epidermis, using [^{3}H] thymidine incorporation and immunoperoxidase staining of 67 K polypeptide. Br J Dermatol 108:433–439, 1983.
12. Viac J, Staquet MJ, Thivolet J, Goujon C: Experimental production of antibodies against stratum corneum keratin polypeptides. Arch Dermatol Res 267:179–188, 1980.
13. Viac J, Schmitt D, Staquet MJ, Thivolet J, Ortonne JP, Bustamante R: Binding specifity of guinea pig anti-alpha-keratin polypeptide sera on human keratinocytes: comparison of their receptors with those of human epidermal cytoplasmic antibodies. Acta Derm Venereol 60:189–196, 1980.
14. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277:680–685, 1970.
15. Tseng SCG, Jarvinen MJ, Nelson WG, Huang JW, Woodcock-Mitchell J, Sun TT: Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. Cell 30:361–372, 1982.
16. Bowden PE, Quinlan RA, Breitkreutz D, Fusenig NE: Proteolytic modification of acidic and basic keratins during terminal differentiation of mouse and human epidermis. Eur J Biochem 142:29–36, 1984.
17. Régnier M, Vaigot P, Darmon M, Pruniéras M: Onset of epidermal differentiation in rapidly proliferating basal keratinocytes. J Invest Dermatol 87:472–476, 1986.
18. Viac J, Réano A, Brochier J, Staquet MJ, Thivolet J: Reactivity pattern of a monoclonal antikeratin antibody (KL1). J Invest Dermatol 81:351–354, 1983.
19. Lavker RM, Sun TT: Epidermal stem cells. J Invest Dermatol 81(suppl): 121s–127s, 1983.
20. Leigh IM, Pulford KA, Ramaekers FCS, Lane EB: Psoriasis: maintenance of an intact monolayer basal cell differentiation compartment in spite of hyperproliferation. Br J Dermatol 113:53–64, 1985.