Cell Kinetics in Skin Disorders with Disturbed Keratinization

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A relatively simple immunohistochemical method was developed and used on cryostat sections. The monoclonal antibody Ki67 was used as marker for actively cycling cells and Pab601 for germinative cells. Counts were expressed as Ki67- or Pab601-positive cells/mm. In order to improve our understanding of the pathogenetic mechanisms in skin disorders with disturbed keratinization we have measured cell kinetic values in dyskeratosis follicularis, pemphigus benigna familiaris chronica, autosomal dominant ichthyosis vulgaris, X-linked recessive ichthyosis, atopic dermatitis and psoriasis and compared them with previous values derived with autoradiography using tritiated thymidine. The results showed that microscopical acanthosis is related to an increase of the germinative population, while the increased epidermal turnover is associated with increased numbers of cycling cells. The cell kinetic changes seem to be all secondary except in psoriatis where a dysregulation in the epidermal growth may cause the epidermal changes. This simple method allows quick evaluation of drug efficacy which might be useful in atopic dermatitis and psoriasis. *Key words: Epidermis; Growth fraction; Skin disease; MoAb Ki67; MoAb Pab601.*

A knowledge of the cell kinetic values in skin disorders with disturbed epidermal growth is essential to the understanding of pathogenic mechanisms and may help to find and evaluate drugs beneficial for these disorders. Since recent reports indicate relatively constant cell cycle times in different experimental settings for keratinocytes(1–5), we focused on two other cell kinetic values: the pool of actively cycling cells and the germinative cell pool (cycling and quiescent (G_0) cells). To measure these the monoclonal antibody Ki67 was used as a marker for the nuclei of actively cycling cells(6,7) and Pab601 as a cytoplasmic marker for the germinative cell pool. On sections Pab601 binds to the basal cells and to part of the suprabasal cells. In flow cytometric studies almost all cells of an epidermal cell suspension in the S-, G_2- and M-phase are Pab601-positive. This indicates its specificity for germinative cells(8). For application of these antibodies a quick immunohistochemical method on cryostat sections was developed and optimized. We applied the technique to normal epidermis and to 6 skin disorders with disturbed epidermal growth.

MATERIAL AND METHODS

Shave biopsies were taken from 5 healthy persons, 2 patients with dyskeratosis follicularis, 2 with pemphigus benigna chronica familiaris, 2 with autosomal dominant ichthyosis vulgaris, 5 with X-linked recessive ichthyosis, 5 with atopic dermatitis and 11 with psoriasis. In the

psoriasis group 5 patients were treated. One patient used methotrexate, 2 etretinate and 2 topical corticosteroids.

Cryostat sections (thickness 8 μm) were made from the biopsies of normal skin of the back of volunteers and lesional skin of patients. They were immediately fixed in acetone/ether (vol 60/40%) for 10–20 min, air-dried and stored at −20°C for no more than one week. After storage, sections were air-dried for a further 5 min and rehydrated for 3 min in phosphate buffered saline (PBS) with 0.05% Tween 80. The immunohistochemical staining procedure was started by an incubation with the mouseantihuman monoclonal antibody for 30 min in a solution of either 1:20 Ki67:PBS pure (Dakopatts, Denmark) or 1:2.5 Pab601:PBS (kindly provided by I. Leigh, London, England), followed by a 3 × 10 min wash in PBS. The second antibody was 1:25 rat-antimouse-peroxidase:PBS containing 5% human AB-serum (Dakopatts, Denmark), again followed by a 3 × 10 min wash in PBS. After 2 min incubation in acetate buffer, sections were incubated in the substrate solution containing 50 ml Na-acetate buffer (pH 4.85), with 2.5 ml dimethylsulfoxide, 20 ml H_2O_2 30% and 10 mg aminoethylcarbazole resulting in a red-brownish precipitate. After a rinse in demineralized water nuclei were stained with hematoxylin according to mayer. Positive cells were counted over a total length of 5 mm epidermis in cryostat sections and expressed as positive cells per mm.

RESULTS

The results are shown in Table I. The values in normal skin for Pab601-positive cells/mm were 312 ± 17 and for Ki67-positive nuclei/mm 13 ± 2. The growth fraction defined by the Ki67/Pab601-ratio is 4%.

The values of the 2 patients with dyskeratosis follicularis (Darier's disease) and autosomal dominant ichthyosis vulgaris were in the normal range. The 2 patients with pemphigus benignus chronica familiaris had normal values for Pab601-positive cells, but the value of the Ki67-positive nuclei/mm was increased 4-fold, leading to a growth fraction of about 16%. In the 5 patients with X-linked recessive ichthyosis Pab601-positive cells/mm were in the normal range, but the mean value of Ki67-positive nuclei/mm was more than doubled, resulting in a mean growth fraction of 9%. This value is explained by a single high count of Ki67-positive cells in one subject.

In atopic dermatitis and in psoriasis (treated as well as untreated lesions) the Pab601-positive cells/mm were about doubled. Ki67-positive nuclei/mm were 7-fold increased in atopic dermatitis, 10-fold increased in treated psoriatic epidermis and 26-fold increased in untreated psoriasis epidermis, yielding growth fractions of 17, 23 and 57 percent respectively. The growth fraction in treated psoriasis was significantly higher than in untreated psoriasis ($p = 0,0137$; Wilcoxon). In psoriatic epidermis an increased number of Ki67-positive cells was observed throughout the whole lesional epidermis, whereas in atopic epidermis this was most pronounced in the areas underlying a parakeratotic stratum corneum.

Cytoplasmic binding of Ki67 was observed in the basal cells. The intensity of this binding in a certain area showed an inverse relation to the number of Ki67-positive nuclei.

Table I. *Means of Ki67-positive and Pab601-positive cells per mm with standard deviation and their ratio*

	n	Ki67+/mm SD	Pab601+/mmSD	Ki67/Pab601
Normal skin	5	13± 2	312± 17	4%
Darier's disease	2	18± 7	322± 46	6%
M. Hailey-Hailey	2	54± 42	320± 42	16%
A.D. icht. vulg.	2	13± 1	273± 17	5%
X-linked icht.	5	28± 22	315± 45	9%
Atopic derm.	5	88± 34	531± 69	17%
Psoriasis treated	5	127± 71	560±171	23%
Psoriasis untr.	6	340±113	589±130	57%

DISCUSSION

In normal epidermis as in other squamous cell epithelia a cytoplasmic binding site for Ki67 in the basal layer cells is observed (8). Although the scoring of most nuclei is easy, this cytoplasmic binding, which is strongest in normal unstimulated epidermis (7) may sometimes interfere with the visualization of weakly Ki67-positive nuclei, leading to an underestimation of the Ki67-positive nuclei/mm, especially in the normoproliferative epidermis. Compared to van Erp et al. (3), who used a similar technique, finding 25 ± 2 Ki67-positive nuclei/mm, our present value of 13 ± 2 Ki67-positive nuclei/mm is on the lower side.

The average values in dyskeratosis follicularis were within the normal range. On individual cryostat sections, however, small areas were observed with strong hyperkeratosis, parakeratosis, decreased cytoplasmic Ki67 binding and increased density of Ki67-positive nuclei. The mild acanthosis and hyperkeratosis generally found in this condition are not caused by a large overall increase of cell production. The absense of an increased rate of desquamation indicates epidermal hypertrophy through an increased epidermal turnover time. The acantholysis in pemphigus benigna chronica familiaris, which is much stronger than in dyskeratosis follicularis, leads to blister formation. This might well explain the increased number of Ki67-positive nuclei. In autosomal dominant ichthyosis vulgaris the decreased stratum granulosum formation and the formation of adherent squamae indicate that the metabolic error is expressed in the differentiating keratinocyte. The absence of evident cell kinetic abnormalities matches the absence of acanthosis and indicates that the hyperkeratosis is the result of an increased residence-time of the corneocytes in the stratum corneum. In earlier work Frost et al. (1966) also found normal values for the mitotic counts/cm (9) and the labelling index (10) using tritiated thymidine in ichthyosis vulgaris. Assuming a normal cell cycle these values and the values for Ki67-positive cells/mm should be related. In the X-linked recessive form of ichthyosis the measurements were similar. The clinical picture caused by the adherent hyperkeratotic scales is also often quite similar to the autosomal dominant form.

The macroscopical lichenification and the microscopically prominent acanthosis of the chronic lesions in atopic dermatitis are in line with a germinative population which is nearly doubled. Together with the 4-fold increase of the growth fraction, this implies a 7-fold increase in cell production, presuming a normal cell cycle time. This causes a speeding up of differentiation reflected in the focal parakeratosis, occasionally seen in these lesions microscopically, and the fine macroscopical desquamation, catalyzed by scratching. These findings are roughly in line with the 5-fold increase in the labelling index found by Marks & Wells in 1973 for lichenified eczema (11).

The increase of the germinative cell pool that was measured in psoriasis already pointed out by Weinstein et al. 1985 (12) is much in line with the marked acanthosis. The profuse desquamation generally observed in psoriasis is reflected in the grossly increased growth fraction. This is in line with the 6-fold increase in the labelling found by Weinstein & Frost 1971 (13). The small difference in the germinative populations and the marked difference in the growth fractions between treated and untreated psoriasis indicate that the cells leave the cycle following therapy. The fact that psoriatic lesions often relapse after withdrawal of therapy may thus be the consequence of the still increased germinative cell pool.

The data presented show a relationship between the histological finding of acanthosis and the increase of the germinative population. Furthermore, the Ki67-positive cells/mm (that is the number of actively cycling cells also expressed in the growth fractions) were proportionally increased with increased epidermal turnover: in pemphigus benigna chronica familiaris in the form of intraepidermal blister formation, in atopic dermatitis in the form of fine scaling and in psoriasis in the form of larger scales. Especially the histological finding of parakeratosis was associated with high counts of Ki67-positive cells. This supports the concept that the values of Ki67-positive cells/mm are a measure for the cell production rate and the cell cycle times in the disorders studied here are in the same order of magnitude. It also indicates that parakeratosis may be a symptom of disturbed maturation due to a large increase in epidermal turnover. Marks reported in 1975 a ± 60% increased labelling index in thyrotoxicosis and a ± 55% increased thymidine units in uninvolved psoriatic epidermis (14), indicating a relatively moderately increased epidermal turnover. Since thyrotoxicosis and psoriatic uninvolved skin are not associated with parakeratoses, these moderate increases in epidermal turnover might be considered as too small to induce parakeratoses.

Comparing the values of atopic dermatitis and untreated psoriasis there is a clear difference in the pool of actively cycling cells, despite the very similar values for the germinative cell populations. Whereas in the atopic and pemphigus benigna chronica familiaris lesions the increased growth fraction may be secondary to cell loss at the surface, in psoriasis the grossly increased pool of cycling cells would seem to be the driving force leading to massive parakeratosis, desquamation and a turnover time of about 3.5 days instead of 21 days in normal skin (15). This may suggest a primary disorder in the regulation of the epidermal growth, leading to the transformation of quiescent germinative cells into cycling cells. The advantage of the immunohistochemical method used here is that it is quick and simple compared to the tritiated thymidine technique and that it also measures the germinative pool directly, which is an important cell kinetic value. The method

may serve in psoriasis and in atopic dermatitis to provide quantitative evaluation of therapies.

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