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CELL PROLIFERATION IN LICHEN PLANUS OF THE BUCCAL MUCOSA

R. Maidhof, O. P. Hornstein, G. Bauer, A. Schönberger and G. Bäurle

Department of Dermatology, University of Erlangen-Nürnberg, Federal Republic Germany

Abstract. The proliferative activity of buccal mucosa epithelium in 16 patients suffering from oral lichen planus was studied by using [^{3}H]-thymidine labelling technique in vitro and histometric methods. Autoradiographic sections of two groups of lesions (12 with atrophic and 4 with acanthotic epithelium) were compared with the buccal mucosa of 19 healthy controls investigated in the same way. Determinations comprised separate and combined labelling indices of the basal and suprabasal progenitor compartment (LI_{bas}, LI_{sbas}, LI_{total}) in relation to basal cells as well as to surface of the epithelium. The following results were obtained. The values of LI_{total} per 100 basal cells were increased in both groups of lesions, whereby the relation of LI_{bas} : LI_{sbas} shifted markedly to LI_{bas}, in particular in the atrophic lesions. When relating the total of labelled nuclei to surface length, however, an increase could be confirmed only in acanthotic lesions, whereas most atrophic lesions showed a decrease. This indicates an impaired capacity of the atrophic epithelium to maintain regenerative steady state. This imbalance could also be confirmed by counting the total of basal cells per surface length, which were significantly lowered in atrophic lesions as compared with acanthotic ones as well as normal mucosa. From the results it can be concluded that the renewal activity of the epithelium in atrophic lesions of lichen planus mucosae becomes virtually deficient, though determination of LI_{total} referred to basal cells simulates a slight increase. Thus, for detecting intrinsic imbalances in the proliferative equilibrium of squamous epithelium, correlation of progenitor compartment labelling to external surface as the site of continuous cell loss is required.

Key words: Lichen planus; Buccal mucosa; In vitro labelling technique; Decreased proliferation in atrophic lesions

In skin lesions of lichen planus (LP) there exists a striking discrepancy between cytolytic degeneration of basal keratinocytes, induced by an underlying infiltrate predominantly of T cells (2, 5) and enhancement of the epithelium proliferation as observed autoradiographically by Marks et al. (9) and Ebner et al. (4). On the other hand, in LP lesions of the buccal mucosa a slight decrease in the total labelling activity in comparison with normal mucosa epithelium was found by Walker & Dolby (12). It has to be realized, however, that the proliferative rate of the healthy mucosa epithelium markedly exceeds that of the skin epidermis (cf. to 8), though a renewal of damaged basal cells from skin appendages such as eccrine ducts as observed in epidermal lesions (4, 9) cannot take place in the oral mucosa.

The question arises whether results obtained from cutaneous lesions and by using different autoradiographic methods (e.g., ^{3}H-labelling in vivo, as did Marks et al.) are biologically comparable to those gained from oral mucosa, being an epithelium of very high turnover capacity. To elucidate the reason for the discrepant results derived from mucosal vis-à-vis epidermal lesions of LP, re-examination should be restricted to lesions of either epidermis or buccal mucosa by applying adequate histoautoradiographic measures.

The present study was designed to answer the open question whether or not the proliferative activity of LP lesions in buccal mucosa is increased in line with epidermal lesions (4) or slightly decreased as suggested by data previously published (12). Thus we not only determined separately the indices of the labelled basal and suprabasal progenitor cells in relation to basal cells, as Walker and Dolby did (12) but also referred some measures of the proliferative activity to the mucosa surface length, as recently described (8).

MATERIAL AND LABELLING TECHNIQUE

Investigations were performed on 16 patients (5 males, 11 females, mean age 49 years) suffering from histologically verified LP mucosae. Punch biopsies (4 mm ∅) were taken from involved buccal mucosa strictly during the morning in order to keep stable the conditions of diurnal variation in proliferative activity. For local anaesthesia 1 % solution of Mepivacain (Scandicain®) without epinephrine was

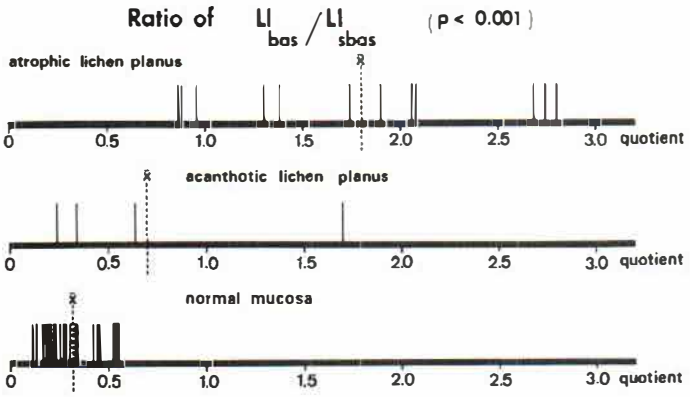

Fig. 1. Quotient of the labelling indices of basal and suprabasal progenitor cells.

used. Immediately after biopsy each specimen was split, one portion cut perpendicular to surface into small pieces and incubated in Minimum Essential Medium (Flow Lab. Ltd.), to which 5 μCi [^{3}H]thymidine/ml (spec. act. 6 Ci/mmol, Schwarz Bio-Research Inc., Orangeburg, N.Y.) had been added, at 37°C and 1.4 atm. oxygen partial pressure for 60 min. Autoradiographs were prepared from 5 μm thick paraffin sections. Exposure time of stripping film (AR 10 Kodak) at 4°C was 4 weeks. Only nuclei with ≥4 silver grains were regarded as labelled.

The other portion of each biopsy specimen was taken for usual histologic examination.

MEASUREMENTS

In each specimen at least 1 000 basal cells were evaluated and the following data were determined:

1. Index of labelled basal nuclei (LI_{bas}).
2. Index of labelled suprabasal nuclei (LI_{sbas}).
3. Index of all labelled nuclei (LI_{total}).

The data of indices 1–3 are related to 100 basal cells, respectively.

4. Number of basal cells related to surface length (1 000 μm).
5. Total of labelled nuclei (LN_{total}) related to surface length (1 000 μm).
6. Length of basement membrane related to surface length (1 000 μm).
7. Ratio of LI_{bas} : LI_{sbas}.

Details of the methods used have been published elsewhere (8). All biopsy specimens were histologically categorized as LP of either acanthotic or atrophic type.

For statistical evaluations, the variance analysis was applied, when the data were normally distributed (Fig. 2). The H-test of Kruskal & Wallis (11) was employed, if this condition was not given (Figs. 1 and 3). Procedure: All data of one variable are sorted in increasing order and ranked with numbers from 1 to n. Nevertheless, for demonstration purposes, all data are plotted as original values.

Table I. *Cell kinetic data of atrophic lichen planus buccalis*

LI_{bas}=labelled basal nuclei/100 basal cells; LI_{sbas}=labelled suprabasal nucl./100 basal cells; LI_{total}=total of labelled nucl./100 basal cells; BC/SL=number of basal cells/1 000 μm surface length; $LN_{total}/SL_{1000\,\mu m}$=total of labelled nucl./1 000 μm surface length; $BM/SL_{1000\,\mu m}$=relation of basement membrane length to surface length

Age, sex	LI_{bas}	LI_{sbas}	LI_{total}	BC/ $SL_{1000\,\mu m}$	LN_{total}/ $SL_{1000\,\mu m}$	BM/ $SL_{1000\,\mu m}$
24 ♂	11.5	12.8	24.3	182.8	44.4	1.36
29 ♀	23.9	11.5	25.3	270.1	95.3	2.29
45 ♂	28.6	10.4	39.0	171.6	67.6	
46 ♂	9.9	11.2	21.1	266.7	56.3	2.10
48 ♀	18.0	18.3	36.4	179.2	65.2	
52 ♀	10.7	4.0	14.7	210.3	30.9	1.36
54 ♀	25.4	14.6	39.9	138.2	55.1	1.57
55 ♀	20.9	11.0	32.0			
57 ♀	15.6	11.2	26.8	131.5	35.2	1.17
62 ♀	25.3	12.3	37.6	196.1	73.7	1.84
65 ♂	9.9	3.5	13.4	99.6	13.3	1.06
67 ♀	28.4	21.5	50.0	156.7	78.4	1.18
M	19.0	11.9	30.9	182.1	55.9	1.54
S.D.	7.3	5.0	11.0	52.9	23.7	0.49

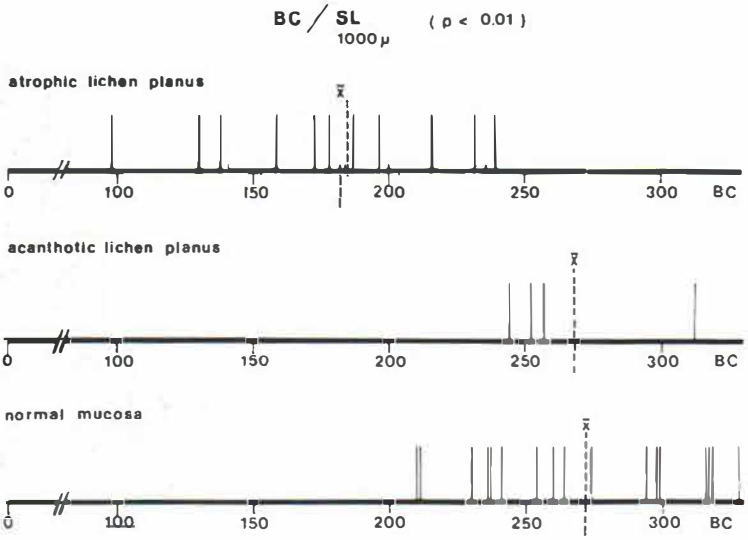

Fig. 2. Number of basal cells per surface length

RESULTS

The average of LI_{total} in 12 atrophic lesions of LP amounted to 30.9±11.0 whereas in 4 acanthotic lesions, the mean value was 35.1±11.7. Autoradiographic details of each case are set out in Tables I and II. All the data were compared with those of healthy buccal mucosa (Table III), which in a previous study was found not to be subject to any age-dependency of proliferative activity in adult life (8).

The increase in LI_{total} is not statistically significant in either atrophic or acanthotic lesions. When studying separately LI_{bas} and LI_{sbas}, however, the differences between the groups of atrophic LP, acanthotic LP and normal mucosa were highly significant ($p<0.001$). The mean ratio of LI_{bas} : LI_{sbas} exceeds that of normal mucosa (0.3) very strikingly. In acanthotic lesions it reaches 0.74 ($p<0.001$) and in atrophic ones, even reaches 1.79 ($p<0.001$) as shown in Fig. 1.

In a few cases, the data of LI_{total} showed a drop. In the specimens of 12 patients with atrophic LP, values were found to be elevated in 7, lowered in 3, and normal in 2. Regarding acanthotic LP, 3 cases showed a rise of LI_{total} and one a normal value.

Concerning the data in relation to surface length,

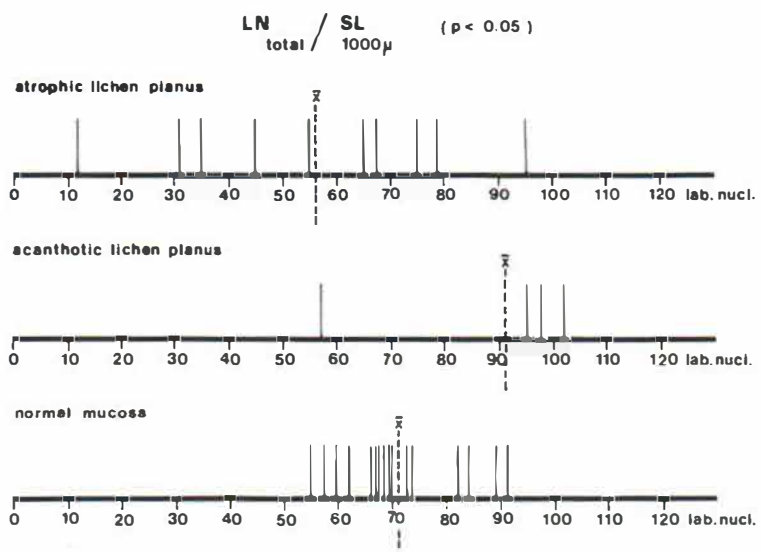

Fig. 3. Total of labelled nuclei per surface length unit.

Table II. *Cell kinetic data of acanthotic lichen planus buccalis*
Explanation see Table I

Age, sex	LI_{bas}	LI_{sbas}	LI_{total}	BC/ $SL_{1000\,\mu m}$	LN_{total}/ $SL_{1000\,\mu m}$	BM/ $SL_{1000\,\mu m}$
28 ♀	28.0	16.6	44.6	251.4	112.1	1.96
37 ♀	4.7	13.4	18.1	313.2	56.7	2.02
58 ♂	7.3	30.3	37.6	256.1	96.3	1.97
64 ♀	16.1	24.0	40.1	244.4	98.0	1.83
M	14.0	21.0	35.1	266.2	90.8	1.95
S.D.	10.5	7.6	11.7	31.7	23.8	0.08

the number of basal cells (Fig. 2) was found to be markedly reduced in atrophic lesions ($p<0.01$), as was the mean length of the basement membrane (Table I). The mean value of LN_{total} was also lowered but the wide range of data did not reveal any statistical significance. In acantholytic lesions, however, the rate of basal cells per surface unit was normal (Fig. 2), whereas both LN_{total} and length of basement membrane were significantly increased (Table II, Fig. 3).

DISCUSSION

In the majority of buccal mucosa lesions of acanthotic as well as atrophic LP, we found a rise in the total [^{3}H]thymidine labelling index (LI_{total}) in the damaged epithelium in comparison with normal buccal mucosa, whereas the number of basal cells per surface length proved to be reduced. This result corresponds to some autoradiographic data obtained from epidermal lesions of LP by Ebner et al. (4) who also were able to demonstrate a marked though different increase in the mean LI in LP of either atrophic or acanthotic type. On the other hand, the present study does not confirm the results of investigations on buccal LP reported by Walker and Dolby (12) as far as LI_{total} are concerned. Contrary to a reduction in mean LI_{total} shown by these authors in acanthotic (33.8±6.0) and particularly in atrophic mucosa lesions (23.6±10.0), as compared with a very high level in normal mucosa (39.9±3.3), we found the same parameters to be increased in both atrophic and acanthotic lesions (30.9±11, and 35.1±11.7, resp.) in comparison with normal buccal mucosa (26.0±2.6).

Interestingly, Walker & Dolby (12) noted a 'redistribution' of labelled cells within the epithelium, inasmuch as the LI of basal cells exceeded that of normal controls and the labelling in the suprabasal layer was decreased. A similar 'shift' within the proliferative pool can be confirmed by the present investigation, which fits in well with the results of a previous study on benign oral leukoplakias other than LP (7). In our opinion, the changing arrangement of proliferative cells in mucosa lesions of either LP or benign leukoplakia is adaptive in nature, being due to a tendency to premature keratinization of squamous cells close to the underlying progenitor compartment.

It should be noted that the rate of basal cells per unit of surface length was markedly reduced in atrophic lesions in contrast to the normal value in acanthotic ones, both compared with buccal mucosa of healthy controls (Table III). To our knowledge, in studies on the histokinetics of buccal LP, neither the number of basal cells nor the total of labelled nuclei has yet been determined in relation to surface length. The importance of referring the total of labelled nuclei in oral mucosa not only to basal cells but in particular to external surface has already been emphasized (1, 7, 8), since the latter method yields a better measure of the renewal rate than the former one in tissues such as mucous

Table III. *Cell kinetic data of the buccal mucosa of 16 healthy adults*
Mean values, for explanation, see Table I

	LI_{bas}	LI_{sbas}	LI_{total}	BC/ $SL_{1000\,\mu m}$	LN_{total}/ $SL_{1000\,\mu m}$	BM/ $SL_{1000\,\mu m}$
M	6.2	19.8	26.0	270.7	70.9	1.75
S.D.	2.0	2.6	2.6	38.6	10.4	0.16

membranes, where the high turnover rate can be calculated from the size of the proliferating pool and the duration of the cell cycle.

The reduction of basal cells in lesions of atrophic LP corresponded closely to a flattening of rete ridges due to an underlying infiltration of mononuclear cells. Despite a 'liquefying' degeneration of many basal cells with reduction in number, the LI of the progenitor epithelium related to basal cells proved to be raised in lesions of acanthotic and, not so prominently, of atrophic type (Tables I and II). For maintaining the proliferative homeostasis of the mucosa, the cell renewal can either be increased, or desquamation of superficial cells be reduced. There is much evidence from recent work (4, 9, 10) that the increase in mitotic activity is recruited from cells of either dermal eccrine ducts or perilesional epithelium. The latter mode of renewal applies to LP lesions in the oral mucosa. In addition, cell loss from the mucosal surface can be reduced by orthokeratinization (7) being a typical feature of many leukoplakic and also of LP lesions. This view can be supported by a few of our cases in which both atrophy and hyperorthokeratosis were found to be very pronounced.

From a purely histological point of view, it seems rather surprising that the cytolytic effects of the inflammatory cells do not hamper the steady state of epithelial cell proliferation so long. The mechanism of basal cell destruction, however, is not likely to influence primarily the DNA replication, but may rather act via an immunological disorder affecting the cytomembrane or other constituents of basal cells (2, 3, 5). The inflammatory process, if directed against cellular antigens, may lead to a progressive decay of basal cells with an increasing imbalance of epithelial growth regulation, terminating in pronounced atrophy of the epithelium and often followed by erosion. In advanced stages of atrophy (e.g., the penultimate case in Table I), the data of LI_{total} as well as of $LN/SL_{1000\,\mu m}$ are markedly lowered, indicating a markedly reduced rate of proliferation.

From the present results it can be concluded that the damage to the proliferative pool in acanthotic lesions of buccal LP will be, at least temporarily, compensated by an enhancement of cellular renewal. This becomes obvious especially when the total of labelled nuclei is referred to surface length, since in acanthotic lesions the mean value of $LN/SL_{1000\,\mu m}$ (90.1 ± 23.5) exceeds that of normal buccal mucosa (70.9 ± 10.4). In atrophic lesions of LP, on the other hand, a minor rate of epithelial renewal still remains, which is reflected by a moderate decline in the rate of $LN/SL_{1000\,\mu m}$ in spite of a drastic reduction in the total of basal cells.

With regard to former studies on the proliferative state of the epithelium in LP (4, 9, 12), some contradictory results can be explained by an inadequacy of the reference structure with which the autoradiographic data have been connected. To gain deeper insight into the actual mitotic activity of the epithelium in LP and other epidermal disorders, the index of labelled progenitor cells should be applied to the *external* surface of the epithelium, since an exfoliative cell loss accounting for the regenerative nature of squamous epithelium takes place there (1, 8). If the LI of this epithelium is only referred to the progenitor compartment, it will fail to eclucidate the real extent of any alteration of the renewal capacity as far as the replacement of lost superficial cells is concerned.

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Prof. Dr O. P. Hornstein
Dermatol. Univ.-Klinik
Hartmannstr. 14
D-8520 Erlangen
Fed. Rep. Germany