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INVESTIGATION ON LANGERHANS CELLS IN PATHOLOGICAL HUMAN EPIDERMIS

Paolo Lisi

From the Department of Dermatology, University of Perugia, Italy

Abstract. The ATPase activity, and the number, distribution and morphology of Langerhans cells (L.c.) in human epidermis were studied using the Wachstein–Meisel method suitably modified. In normal epidermis the average number of the L.c. was $696 \pm 70.5/mm^2$. In pathological epidermis the L.c. were greatly increased in number in recent lichen planus, tbc verrucosa, leishmaniasis and mycosis fungoides. As a rule in acanthotic skin diseases the L.c. were decreased in number, while they were increased in skin diseases with reduced epidermal thickness. In squamous cell carcinoma the L.c. were diminished in number and had some morphologic anomalies, while in basal cell carcinoma they were increased and had normal morphology. Furthermore, in the epidermis of nodular lesions due to delayed hypersensitivity, the L.c. were statistically increased in number at the 8th day, while they were normal in number at the 15th day. These findings seem to be consistent with the theories of the reticulohistiocytic derivation of L.c. and of the role of these cells in regulating the differentiation and the mitotic activity of keratinocytes.

The cells first described by Langerhans (8) in 1868 were until recent years classified according to morphological criteria as either intra-epidermal nervous cells or melanocyte-derived cells. Electron microscope studies on Langerhans cells (L.c.) in vitiliginous epidermis were begun by Birbeck et al. (2) in 1961: their findings did not provide any evidence in favour of either of two theories, but revealed the presence of granules typical of these cells. Over the past few years identical granules have been found in the macrophages of infantile-type "histiocytosis X" cutaneous lesions (15, 5, 6); this has given rise to the hypothesis that L.c. are of mesodermal origin and may function as phagocytes.

Kuwahara (7) has proposed that the L.c. may be involved in early antibody production, and the recent findings of Silberberg (14) in contact allergic reactions seem to indicate the same.

For some years we have been studying L.c. under the light microscope using the Wachstein–Meisel method with certain modifications. The light microscope was preferred to the electron microscope because it gives an overall view of the epidermis. We have examined the ATPase activity, the number, distribution and morphology of L.c. in normal human epidermis (9) and in various pathological skin conditions (1, 10, 11).

This paper summarizes the above-mentioned data and describes further findings in pathological epidermis and in nodular lesions due to delayed hypersensitivity.

MATERIALS AND METHODS

From 15 volunteers of both sexes aged from 27 to 71 years specimens of normal epidermis were taken by rotary punch biopsy without anaesthesia. One biopsy was done on 10 of the volunteers on one of the following skin areas: extensor surface of the arm (1 case), back (6 cases), and front (1 case) and back (2 cases) surfaces of the leg. Five of the volunteers had 3 biopsy specimens each taken at the same time from the flexor surface of the forearm, and the back and front surfaces of the leg.

Pathological epidermis was examined for 76 patients affected with:

	No. of cases
actinic degeneration	3
atrophic scar	4
scleroderma	3
ichthyosis vulgaris	1
Darier's disease	2
acanthosis nigricans	1
chronic eczema	3
chronic psoriasis	8
lichen planus	12
lichenification	5
lichen sclerosus et atrophicus	3
prurigo nodularis	1
tbc verrucosa	1

Table I. *The average number of L.c. per mm² of normal human epidermis in various areas*

Area	No. subjects	Average number/mm²
Arm	1	685
Forearm	5	633±94.4
Back	11	724±49.0
Leg (front surface)	6	701±82.7
Leg (back surface)	2	692

	No. of cases
leishmaniasis	2
dermatitis herpetiformis	1
condyloma accuminatum	4
keratoacanthoma	4
epithelial nevus	1
seborrheic keratosis (keratotic type)	4
cornu cutaneum	1
basal cell carcinoma	7
squamous cell carcinoma	3
intermediary cell carcinoma	2
mycosis fungoides	3

Finally, biopsy specimens were examined from 12 volunteers injected intradermally with tuberculin, trichophytin and antipyogenic vaccine. The injections were given in the flexor surface of the forearm and the specimens of epidermis from the resulting nodular lesions were taken by rotary punch biopsy without anaesthesia after 8 days in 8 cases and after 15 days in 4.

Biopsy specimens with surface dimensions of 4–6 mm were fixed in 5% neutral formalin at 0–4°C for from 20 to 30 minutes and 14 μ sections were cut on a cryostat. The Wachstein–Meisel method modified for our purpose and, as a control, the Laidlaw dopa-stain technique were used on the sections.

To examine the ATPase activity, the sections were dipped in double-distilled water and transferred with a glass rod to the previously prepared and filtered incubation medium (10 ml of 0.125% ATP disodium salt (pH 7.2), 10 ml of 0.2 M tris-maleate buffer (pH 7.2), 2.5 ml of 0.1 M magnesium sulphate, 1.5 ml of 2% lead nitrate, and 1.0 ml of double-distilled water). After incubation for 2 hours at 37°C, the sections were transferred to three baths of double-distilled water for a total of 1–2 minutes and then to a solution of 0.5–1% ammonium sulphide for 1 hour. The sections were then laid on coverglasses and left to dry at room temperature; they were mounted with glycerine jelly or Canada balsam.

The ATPase activity was observed and the number of L.c. per square millimetre of epidermis, their distribution, shape and size were noted. Cell counts were made using the lucid chamber system and leaving the horny layer out of the final measurement; this was considered advisable because of the varying thickness af the horny layer and its likely loss during the course of successive passages.

RESULTS

L.c. in normal human epidermis

The average measurement of the L.c. was found to be $696 \pm 70.5/mm^2$; their average number in the various areas examined is given in Table I. With regard to the morphology and the distribution of L.c., our study has provided confirmation of previous findings.

There were no significant differences in morphology or ATPase activity in relation to sex, age or cutaneous areas. The L.c. are less numerous in the forearm epidermis ($p < 0.05$).

In the thick epidermis the L.c. were less numerous, and they were located in the upper layers; in the thin epidermis the opposite was the case.

L.c. in pathological human epidermis

To a greater or lesser extent, the ATPase activity of the L.c. was increased in scleroderma, ichthyosis vulgaris, Darier's disease, acanthosis nigricans, chronic eczema, chronic psoriasis, lichen planus, lichen sclerosus et atrophicus, tbc verrucosa, leishmaniasis, keratoacanthoma, seborrheic keratosis and mycosis fungoides; it was decreased in lichenification, prurigo nodularis, squamous cell and intermediary cell carcinoma.

The differences with regard to number, distribution and morphology may be summarized for the L.c. as follows.

a) In skin diseases with acanthosis the L.c. were less numerous ($p < 0.01$) and generally located in the upper half of the stratum Malpighii; they had irregular cell bodies, and the dendritic processes were short and relatively few.

b) In skin diseases with reduced epidermal thickness the L.c. were present in increased numbers ($p < 0.05$).

c) In recent lichen planus, tbc verrucosa and leishmaniasis the L.c. were found in greatly increased numbers ($1.176 \pm 250.5/mm^2$ in lichen planus, $1.005/mm^2$ in tbc verrucosa, $1.070/mm^2$ in leishmaniasis) and had numerous branching and apparently anastomosed dendritic processes.

d) In seborrheic keratosis (keratotic type) the number of L.c. was increased ($900/mm^2$).

e) In keratoacanthoma the L.c. were mostly located in the periphery of the proliferative tissue.

f) In basal cell carcinoma the L.c. averaged $785 \pm 157/mm^2$.

Table II. *The L.c. in nodular lesions due to delayed hypersensitivity*

	No. of cases	ATPase activity	Average number per mm²	Distribution	Morphology
After 8 days	8	Slight increase	899 ± 117 ($p < 0.01$)	Upper half of stratum Malpighii	Normal
After 15 days	4	Slight decrease	640 ± 37.4	Normal	Irregular cell body, short and relatively few dendritic processes

g) In squamous and intermediary cell carcinoma the L.c. averaged 450/mm² and 590/mm² respectively and were mostly in the periphery of neoplastic tissue; they had large, elongated cell bodies and short, relatively few dendritic processes.

h) In mycosis fungoides the L.c. were present in increased numbers (852/mm²) and were located in the upper half of the stratum Malpighii; they had small cell bodies, and short, relatively few dendritic processes.

i) In lichen sclerosus et atrophicus and atrophic scar (conditions with fewer ATPase-positive dermic structures) we observed, at the top of the papillary corium, cells similar in shape to the epidermal L.c. but with greater ATPase activity. Cells of this type were also present in the fluid of a dermatitis herpetiformis dermo-epidermal bulla.

L.c. in nodular lesions due to delayed hypersensitivity

The results of this study are given in Table II.

DISCUSSION

The data resulting from our examination of L.c. in normal human epidermis are largely in agreement with the findings of other investigators using different techniques (4, 3, 13).

Our observations on pathological epidermis have shown that the L.c. are statistically increased in number and have numerous dendritic processes in recent lichen planus ($p < 0.01$), tbc verrucosa and leishmaniasis. The L.c. are to some extent more numerous in mycosis fungoides. There is no clear explanation for these findings, but they support the theories which consider L.c. as reticulohistiocytic cells (or, to use a more current terminology, as mononuclear phagocytic cells); in the above-mentioned skin diseases these cells are present in large numbers.

The increased number of L.c. ($p < 0.01$) in the epidermis of nodular lesions due to delayed hypersensitivity at the 8th day and their normal number at the 15th also tend to confirm this theory. It is significant in this respect that the decrease in the number of L.c. coincides with the diminution of the mostly mononuclear dermal infiltrate in these nodular lesions. Attention is drawn to the morphological changes noted in the L.c. at the 15th day, changes for which, at present, we are not able to offer an explanation.

Moreover, skin diseases in which the epidermis thickens show a decrease in the number of L.c. ($p < 0.01$), while the reverse process occurs in skin diseases in which the epidermal thickness is reduced ($p < 0.05$). This finding, which requires for confirmation an analysis of the numerical relationship between L.c. and keratinocytes, may be indicative of a chalone-like activity of the L.c. and may be consequently in agreement with Prunieras's (12) theory, which holds that the L.c. are necessary for the maintenance of a physiological equilibrium between the differentiation and the mitotic activity of the keratinocytes. In fact, in the material we have studied, the L.c. are present in decreased number in Darier's disease, in chronic eczema, chronic psoriasis, lichenification and prurigo nodularis, all diseases characterized by increased mitotic activity and parakeratosis. The reverse is true in scleroderma and lichen sclerosus et atrophicus, diseases in which mitotic activity is limited.

Our observations on carcinoma material have not provided further supporting evidence for this hypothesis.

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P. Lisi, M.D.
Department of Dermatology
University of Perugia
06100 Perugia
Italy