Functionally Different Langerhans' Cells in Human Epidermis

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Experiments comparing the L-dopa histofluorescence method for the visualization of epidermal Langerhans' cells and immunocytochemical labelling with monoclonal anti-T6 antibodies have demonstrated the existence of two functionally different human Langerhans' cells: those that take up L-dopa by a mediated transport and those that lack the capacity to take up L-dopa. ***Key words: L-dopa; Amino acid transport; Immunocytochemistry; Fluorescence histochemistry.***

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Langerhans' cells (LC) can be visualized selectively in normal epidermis because on incubation in vitro they can take up and retain L-dopa (1) which can be transformed to highly fluorescent molecules by the histofluorescence technique of Falck and Hillarp (2, 3). The method was recently applied in our laboratory to a large number of biopsies (12–14; forearm skin) taken from each of five volunteers in the period from August 1987 through January 1989. It was found that the L-dopa method visualized reproducibly a numerically stable population of LC in each individual. However, there were very marked inter-individual variations in the number of L-dopa-containing LC, which aroused suspicion as to whether the L-dopa method visualizes all LC and prompted us to compare the number of dopa-fluorescent LC with T6-positive LC. This comparison led to the revelation of two functionally different populations of LC, those which take up L-dopa and those which seem to lack this capacity.

MATERIAL AND METHODS

Two punch biopsies (3mm) from the volar forearm were taken close to each other without anesthesia. The subjects were again the 5 intensively studied volunteers mentioned in the Introduction and 2 additional volunteers (No 6 and 7 in Table 1). One biopsy was processed for L-dopa histofluorescence and the other was immediately frozen and processed for immunocytochemisty.

Dopa histochemistry was performed according to the method of Sjöborg et al. (4) as modified by Warfvinge et al. (5). The specimens were incubated in 10 mM L-dopa dissolved in Krebs-Ringer phosphate buffer (1 h, 37°C) and washed in the same buffer (1 h, 37°C). Immunohistochemistry was done as described recently (6). A monoclonal antibody, anti-T6 (DAKO-T6 from Dakopatts) was used, and the antigen-antibody complex was visualized with an avidin-biotin-immunoperoxidase (VECTASTAIN^{R} ABC KIT from Vector Lab.)

From 15 to 20 sections (10 μm) were obtained from each specimen. Visualized dendritic cells were counted in 5 non-consecutive sections from each specimen. The mean cell densities were expressed per 0.9 linear mm of epidermal surface.

RESULTS

The results are summarized in Table 1. The number of fluorescent LC did not deviate from that found previously in the same subjects (5). The LC exhibited a strong, green formaldehyde-induced fluorescence which could easily be distinguished from the yellowish fluorescence displayed by the melanocytes. The latter fluorescence is also formaldehyde-induced but derives from cysteinyldopa (for ref. see 4).

DISCUSSION

The volar forearm skin of five of the volunteers had been intensively studied in earlier experiments (see

Table I. *Number of dopa-fluorescent LC and T6+ LC in volar forearm skin.*

Volunt	Fluorescent cells	T6+ cells
1	5.8	14.6
2	13.4	14
3	15.4	13
4	4.6	13.8
5	9	12.4
6	2.6	15.4
7	17.6	16.6

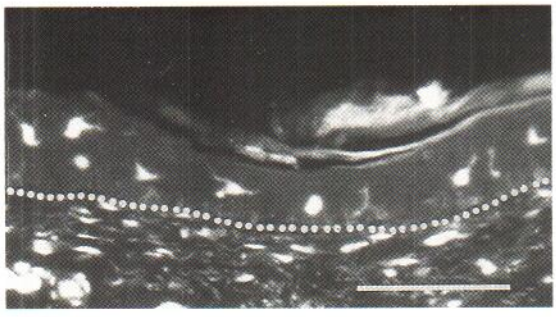

Fig. 1 Flourescence microscopic picture of normal epidermis obtained from individual No 2. The LC display intense flourescence. Dotted line indicates the epidermal-dermal junction.

above). Each of them had the same number of fluorescent LC in the present experiments and, accordingly, the large inter-individual variations remained the same.

No such inter-individual variability could be shown when anti-T6 antibodies were used for visualization (Table I). Thus, there appear to exist two different types of LC, one which can take up L-dopa (dopa(+) LC) and one which cannot (dopa(−) LC). This finding raises two possibilities. Either LC have two functional states with regard to L-dopa uptake or there are two functionally dissimilar populations of LC, one which has the capacity to transport L-dopa across the plasma membrane and the other which lacks this capacity.

Axelsson et al. (7) found features of the L-dopa transport mechanism that were compatible with the concept of exchange diffusion, that is, a carrier-mediated counter-transport, where the influx of L-dopa is linked with the outflow of an intracellular substance. The identity of this postulated substance is unknown. If the exchange diffusion hypothesis is correct, and if there exist two functionally separate types of LC, one implication is that the dopa(−) LC lack the amino acid pump or the capacity to synthesize and/or store the unknown partner of L-dopa.

Several problems remain to be solved if the significance of the L-dopa uptake mechanism and the existence of dopa(+) LC and dopa(−) LC are to be understood. For example, it is not certain whether L-dopa is the natural substrate for the amino acid pump. There is evidence supporting the possibility that L-dopa only fits into a pump normally transporting some other unknown amino acid(s) and it has been suggested that L-dopa and its related catecholamines only act as markers of an amino acid/amine system operating in the LC (7). Also, it is puzzling that some individuals seem to lack dopa(−) LC, whereas these cells do occur, sometimes in high numbers, in other individuals. Preliminary studies in our laboratory indicate that the proportion of dopa(−) LC to dopa(+) LC shows great regional variations in the same individual. Thus, dopa(+) LC can be few in volar forearm skin but numerous in dorsal forearm skin. These different proportions of dopa(−) and dopa(+) cells need more detailed study.

Note added in proof: It was recently suggested by Schuler et al. (In: Epidermal Langerhans Cells. G. Schuler, editor. CRC Press, Inc., Boca Raton, 1991) that the L-dopa uptake reported earlier had been interpreted as reflecting a postulated major function of LC to take up small molecular compounds of all sorts (the "reticuloepithelial trap" concept). It is, however, obvious that an indiscriminate connective uptake of amino acids in mammalian cells is not conceivable. In fact, as is clear from the present communication, the LC transport L-dopa by a specialized amino acid pump.

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