Epidermal Langerhans' cells in Behçet's disease

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SUMMARY Langerhans' cells were studied in the epidermis of two patients with active Behçet's disease and compared with those in two normal controls. Ultrastructural morphology and the percentage of Langerhans' cells found were similar in patients' (1.88%) and the control epidermis (1.79%). The density of Langerhans' cells in adjacent sites of the same epidermis was not homogeneous, being in the range of 0.8–2.8% in Behçet's disease and 0.6–4% in the controls. In the controls, Langerhans' cells were distributed unevenly. Some were located near the basal layer of the epidermis while the rest were in the mid and upper layers. In Behçet's disease most Langerhans' cells were in the mid-epidermis, but some were immediately beneath the stratum granulosum. In the Behçet's disease epidermis the area occupied by Langerhans' cells was increased by about 25% and the number of granules found increased by about 44%. It is suggested that in Behçet's disease the Langerhans' cells are in a more active state.

The aetiology and pathogenesis of Behçet's disease are not fully understood, but there are reports suggesting a cell mediated immune mechanism.^{1,2} Recently Saito *et al*³ found an increased number of epidermal Langerhans' cells after the prick test in Behçet's disease. We wished to clarify differences, if they exist, between Langerhans' cells in normal epidermis and in epidermis of patients with Behçet's disease by a more detailed ultrastructural study.

Material and methods

Biopsies were taken under local anaesthesia from skin showing erythema nodosum of the lower leg in two patients with active Behçet's disease. The patients were an 18 year old man and a 14 year old girl, with a duration of disease of four and nine years respectively. The diagnosis in these patients was based on accepted clinical criteria.^{4,5} In both patients the presenting symptoms were: severe recurrent oral and genital ulcers and erythema nodosum. The male patient had recurrent uveitis. Normal skin from two healthy volunteer men served as controls.

The tissue specimens were fixed for 2.5 h with 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3, washed overnight with the same buffer but containing 7.5% sucrose, then postfixed for 1 h in phosphate buffered 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon 812 mix-

ture.⁶ Thin sections from five to seven blocks of tissue from each biopsy were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Jeol 100 B electron microscope at 80 kV.

The number of epidermal cells and Langerhans' cells was determined by counting the profiles of the cells, including those not containing a nucleus, in a single thin section about 60 µm thick prepared at random from every block of tissue. Mononuclear blood cells occasionally found in the epidermis and cells from the stratum granulosum and stratum corneum were excluded. The areas of Langerhans' cell profiles were measured on electron micrographs with a G Coradi AG Swiss made model 7160 Planimeter. Langerhans' cell granules were counted at a magnification of ×15 000 on intact Langerhans' cell profiles. Student's *t* test for unrelated samples was used to determine the significance of the difference between mean values from patients and controls. The correlation between Langerhans' cell section area and number of Langerhans' cell granules was tested by Pearson's correlation coefficient.

Results

The basic ultrastructural morphology of Langerhans' cell profiles in Behçet's disease epidermis was similar to that in the controls. Their shape was irregular with or without one or more larger cytoplasmic dendrites and some smaller pro-

Fig. 1 Langerhans' cell (LHC) in Behçet's disease epidermis showing the characteristic granules (R), rough endoplasmic reticulum (arrow), mitochondria (M), Golgi bodies (G), and a lysosome (L). K: adjacent keratinocyte. Original magnification $\times 10\,000$.

cesses. They contained abundant, often swollen mitochondria, well developed Golgi bodies, and rough endoplasmic reticulum, occasionally one or more lysosomes, a usually deeply indented nucleus with one or two nucleoli, and the characteristic rod/racket shaped cytoplasmic granules (Fig. 1). These features are in agreement with those previously described.⁷ A well developed rough endoplasmic reticulum was more obvious in Langerhans' cells from patients' skin than in controls. A centriole was seen in Langerhans' cells as often in patients as in controls. Some of the Langerhans' cells in patients' skin contained a lipid droplet. This was not seen in the controls.

A total of 3085 Behçet's disease epidermal cell profiles were counted: 58 were Langerhans' cells (1.88%). In the normal skin 2064 profiles were

counted: 37 were Langerhans' cells (1.79%). Differences were observed in the density of these cells in the random thin sections derived from the same tissue in the controls as well as in Behçet's disease, ranging from 0.6% to 4.2% and from 0.8% to 2.8% respectively. The number of Langerhans' cells and their non-homogeneous density found in the population of epidermal cells was similar in Behçet's disease and in the controls.

The distribution of Langerhans' cells throughout the epidermis showed that in controls 27% of the profiles were located near the basal cell layer, 65% of them were in the mid-epidermis, and about 8% were in the upper epidermis. This contrasted with Behçet's disease, where most of Langerhans' cell profiles (89%) were in the mid-epidermis and 11% in the upper region just beneath the stratum granulosum; no Langerhans' cells were found near the basal cell layer. Occasionally, close contact between lymphocytes and Langerhans' cells was observed. The frequency of such contact was similar in patients and in controls, affecting 1.7% and 2.7% of the Langerhans' cells respectively. Langerhans' cells in the dermis were only seldom encountered.

Five per cent of Langerhans' cells in Behçet's disease epidermis and 8% of Langerhans' cells in the control epidermis showed signs of structural damage. The intercellular space between them and the surrounding keratinocytes was enlarged and they appeared shrunken with loss of cytoplasmic contents.

In an attempt to clarify differences between Langerhans' cells in Behçet's disease and in the controls, a more detailed analysis of the cytoplasmic granules was carried out. In order to minimise inclusion of tangential sections, Langerhans' cell sections were chosen at random from among those with a diameter of over $5\,\mu\text{m}$ and with an area over $10\,\mu\text{m}^2$. Langerhans' cell profile area was measured and the Langerhans' cell granules in these profiles were counted (Table).

The area of Langerhans' cell profiles increased by about 25% and the number of Langerhans' cell granules was significantly increased by about 44% in the patients' epidermis compared with that of con-

Changes in number of Langerhans' cell granules in the Langerhans' cell sections from normal and Behçet's disease epidermis

	No of LHC sections examined	Long diameter of LHC sections (μm) $\pm$ SE	Small diameter of LHC sections (μm) $\pm$ SE	Area of LHC sections (μm^2) $\pm$ SE	No of LCG in LHC sections $\pm$ SE
BD epidermis	28	11.90 $\pm$ 0.86	7.14 $\pm$ 0.49	53.21 $\pm$ 4.02	16.79 $\pm$ 2.18
Control epidermis	31	10.86 $\pm$ 0.74	5.62 $\pm$ 0.33	42.44 $\pm$ 3.76	11.68 $\pm$ 1.51

LHC = Langerhans' cells.
BD = Behçet's disease.
LCG = Langerhans' cell granules.

Fig. 2 Part of a Behçet's disease Langerhans' cell. Note the groups of parallel closely apposed rod shaped granules (R). Arrows point to places of bending and possible future splitting of the elongating granule. Original magnification $\times 32\,000$.

trols ($p < 0.05$). There was no correlation between the area of Langerhans' cells and the number of Langerhans' cell granules found in them as shown by a low correlation coefficient (r for controls = 0.36; r for Behçet's disease = 0.18).

Usually the Langerhans' cell granules were found throughout the cytoplasm as single rackets or rods or in groups of two to six parallel closely apposed rods (Fig. 2). This tendency of the granules to form groups was more obvious in Behçet's disease.

Much information has accumulated in the published work about Langerhans' cells including: their location in the body;^{8,9} their origin from the bone marrow;¹⁰ their possible functions, especially in immunological reactions¹¹ serving as traps and processors for external contact antigens;¹² their macrophage like characteristics such as antigen presentation;^{13,14} Fc – IgG and C₃ receptor sites;¹⁵ express Ia antigens¹⁶ and ability in vitro to transfer antigen in lymphocyte stimulation.¹⁴ Langerhans' cells have been implicated in the pathogenesis of *Mycosis fungoides*^{17,18} and they play an important role in the development of contact hypersensitivity.¹⁹

Saito and colleagues⁹ suggested that Langerhans' cells may have causative importance in the skin lesions in Behçet's disease. We support this idea by the results obtained in our study. The results of our investigation, however, differ in certain respects from those of Saito *et al.* An increased number of Langerhans' cells in the epidermis was not evident in our patients with Behçet's disease. We found a similar low percentage and non-homogeneous density of Langerhans' cells in the population of epidermal cells in Behçet's disease and in the controls. Our

findings are in agreement with other reports concerning the small (2–4%) subpopulation of Langerhans' cells in human epidermis.^{15,20} The difference between our results and those of Saito *et al.*⁹ may be due to different methods of cell counting. There are difficulties in making a precise quantitative evaluation of such irregularly shaped dendritic cells by direct electron microscopy. Because of their scarce number in the epidermal population we counted all the Langerhans' cell profiles including small ones not containing the nucleus and part of which could be profiles of dendrites. The frequency of these small cells was similar in epidermis from patients and controls.

The non-homogeneous density of the Langerhans' cells was not surprising. These cells have the ability to move in the epidermis and in some conditions²⁰ many Langerhans' cells can appear in a particular site within a short time. The distribution of Langerhans' cells seen by us in the control epidermis was in agreement with the other studies.^{21,22} In Behçet's disease none of the Langerhans' cells was seen in or near the basal cell layer. Most were in the mid-epidermis and some of them were in the upper layer. Saito *et al.*⁹ reported also that Langerhans' cells were rarely seen in the basal cell layer. Their presence in the upper layers could be related perhaps to a more active state of these cells in Behçet's disease.

Breathnach²³ distinguished two types of Langerhans' cells in normal human epidermis. Type I contained many Langerhans' cell granules and type II, which had fewer Langerhans' cell granules, were less dendritic and occurred more frequently in the basal cell layer. We tried in this study to differentiate the Langerhans' cells into these two types. We have not succeeded in this because in most cells the characteristics of type I and type II Langerhans' cells were mixed. It is noteworthy, however, that in the Langerhans' cells of the controls the number of Langerhans' cell granules was lower and did not correlate with the location of the cells in the epidermis. The count of Langerhans' cell granules in Behçet's disease was significantly higher than in the controls. The increase in Langerhans' cell granules may be an expression of the active state of Langerhans' cells. The increase in Langerhans' cell granules found in Langerhans' cells in eyelid epitheliomata has also been related to an active state of Langerhans' cells.²⁴

Some structural features such as direct attachment between Langerhans' cell granules and the plasma membrane and continuity between Langerhans' cell granules and Golgi vesicles have been suggested as possible sources of origin for Langerhans' cell granules.^{25–27} In our study these structures were rarely seen and then only in Behçet's disease. We

have, however, seen a tendency, especially in Behçet's disease, for granules to form parallel groups (Fig. 2). Another mode of Langerhans' cell granule formation which may explain such grouping is elongation with bending and splitting producing closely apposed stacks of granules.

Our observations indicate that in Behçet's disease Langerhans' cells are situated in the mid and upper parts of the epidermis. They are in an active state and are bigger, with prominent, well developed rough endoplasmic reticulum and significantly more granules than in controls. It is suggested that the active state of Langerhans' cells may be part of the complex pathogenesis of this chronic inflammatory disease, which needs further clarification.

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