# Preliminary Observation on Langerhans' Cells in Leprosy<sup>1</sup>

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Since the target role of Langerhans' cells (LC) in contact allergic reactions was noted by Silberg (<sup>8</sup>), further advances in the immunological techniques of characterizing such cells have been made (<sup>6, 9</sup>), and LC are well confirmed as special epidermal macrophages (<sup>7</sup>). But very little is known about the condition of LC in leprosy, even about the number and shape. Accordingly, it was decided to observe the state of LC in different types of leprosy, and the investigation of their number and morphology was our first trial.

## MATERIALS AND METHODS

Skin specimens were obtained by biopsy from eight patients with active leprosy. The patients were classified according to the Ridley-Jopling classification on clinical and histological grounds as: TT, 3 cases; BT, 4 cases; BL, 1 case. None of the patients had previously been treated with any antileprosy drugs.

In seven cases of TT and BT leprosy, biopsies were taken from the well demarcated marginal area of the lesions, about  $3 \times 1.5$  cm in size. One half of each biopsy contained the leprosy lesion. The other half was the adjacent, normal-appearing skin used as the control. In the BL leprosy case, besides the biopsy of the lesion, a control specimen was obtained from the same region on the contralateral side showing no obvious lesion. The biopsies were divided into two pieces: a small piece was provided for routine histological examination (hematoxylin and eosin and Harada's acid-fast stain), and the rest was processed for the demonstration of LC using an enzyme histochemical technique for ATPase as described by Juhlin and Shelley (<sup>4</sup>).

Quantitative cell counts were made as described by Brown, *et al.* (<sup>2</sup>) on epidermal sheets by means of a reticule fitted into the eyepiece of a microscope with an objective lens of  $\times 16$ . The size of the field outlined by the reticule was calibrated; the cells in each of ten randomly chosen fields were counted, and cell populations were expressed as the mean number of cells per square millimeter of epidermis. The morphology of the LC was also examined with these epidermal sheets microscopically.

## RESULTS

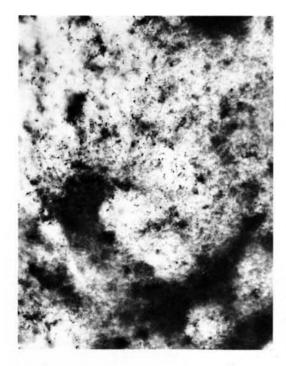
Individual values obtained from the eight patients examined are shown in The Table. The number of ATPase-positive dendritic LC in normal appearing skin varied from 266/mm<sup>2</sup> to 548/mm<sup>2</sup> (mean 376). In involved areas, the number varied from 103/ mm<sup>2</sup> to 256/mm<sup>2</sup> (mean 184). These differences were highly significant statistically. In addition, the dendritic processes of LC in these epidermal sheets from involved areas appeared to be reduced in number, shortened, or diminished (Figs. 1 and 2).

### DISCUSSION

In 1956, Richter (5) used Ferreira-Marques' method for the demonstration of LC in the epidermis overlying leprous lesions and found that they were damaged early in leprosy. They were not only reduced in number but were also typically degenerated. These observations were based solely upon light microscopic examination of skin sections impregnated with gold chloride. Six years later, Breathnach, et al. (1) investigated nine patients with leprosy, using similar materials both with Gaion's technique of impregnation with gold chloride and electron microscopy. With the possible exception of one tuberculoid case, they found that the number of LC was not greatly re-

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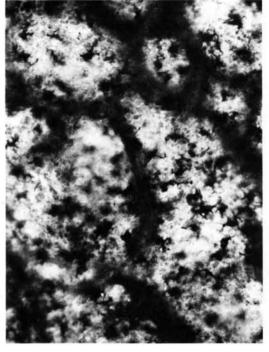


FIG. 1. Langerhans' cells were destroyed in the annular erythematous lesion of a 48-year-old female patient with TT leprosy of six months' duration. The lesion was located on the lateral surface of the left thigh ( $\times$ 300).

FIG. 2. Langerhans' cells in the normal appearing skin of the same patient at the same site (  $\times 300$ ).

duced. The morphology of the majority of the LC in their gold-impregnated material appeared to be normal, but some of them displayed features identical to those of de-

generated cells as defined by Richter. Therefore, they believed that the occurrence of degenerated cells could be attributed to poor impregnation. Under the elec-

THE TABLE. Langerhans' cell counts in involved skin and normal appearing skin in leprosy.

| Case<br>no. | Sex    | Age | Dura-<br>tion<br>(yr) | Classifi-<br>cation | Site         | Clinical appearance   | Langerhans' cells (no./mm <sup>2</sup> ) |                       |
|-------------|--------|-----|-----------------------|---------------------|--------------|-----------------------|--|-----------------------|
|             |        |     |                       |                     |              |                       | Involved<br>skin                         | Normal appearing skin |
| 1           | F      | 49  | 1                     | TT                  | thigh        | annular<br>erythema   | 103                                      | 548                   |
| 2           | М      | 47  | 1                     | BL                  | thigh        | yellowish<br>erythema | 211                                      | 341                   |
| 3           | F      | 38  | 5                     | BT                  | buttock      | erythema              | 191                                      | 342                   |
| 3<br>4<br>5 | F<br>F | 17  | 1                     | BT                  | pretibial    | erythema              | 207                                      | 410                   |
| 5           | F      | 46  | 1                     | ВТ                  | buttock      | erythema<br>reddish   | 172                                      | 362                   |
| 6           | F      | 29  | 3                     | BT                  | thigh        | depigmented<br>macule | 256                                      | 414                   |
| 7           | М      | 27  | 3                     | TT                  | thigh        | erythema              | 167                                      | 324                   |
| 8           | Μ      | 49  | 1                     | TT                  | knee         | erythema              | 167                                      | 266                   |
|             |        |     |                       | М                   | ean ± standa | rd deviation          | $184.3 \pm 44.4^{a}$                     | $375.9 \pm 84.2$      |

<sup>a</sup> Significantly less than normal appearing skin, p < 0.01, Student's t test for paired comparisons.

tron microscope, LC in leprosy appeared to be essentially normal. In 1972, Job, *et al.* (<sup>3</sup>) studied two cases of indeterminate leprosy with electron microscopy and noticed that a number of LC had atrophic changes and that there was an appreciable increase in their number. Most of the eight cases of leprosy in the present study were classified at TT and BT. In these cases, the number of LC was obviously reduced in involved skin compared with the number of LC in uninvolved areas.

The enzyme histochemical method for the demonstration of ATPase is now widely accepted as a simple, reproducible, and probably specific demonstration of LC in several mammalian species. When applied to isolated epidermal sheets, it is an ideal method for quantitatively evaluating the LC population. Although LC in our specimens showed their dendritic processes to be reduced in number, shortened, or diminished, the morphology observed in the epidermal sheets was not satisfactory. Further detailed study with electron microscopy is needed.

#### SUMMARY

Langerhans' cells were enumerated in the skin of eight borderline and tuberculoid leprosy patients by the enzymatic histochemical demonstration of ATPase in these cells in epidermal sheets. The number of Langerhans' cells was significantly reduced in the skin lesions of these patients compared with normal appearing skin from the same patients. The morphological details of these cells were not clear under light microscopy; further study with electron microscopy is desirable.

#### RESUMEN

Se enumeraron las células de Langerhans en la piel de ocho pacientes con lepra intermedia o tuberculoide por la demostración histoquímica de ATPasa en estas células presentes en las capas epidérmicas. El número de células de Langerhans estuvo significativamente reducido en las lesiones dérmicas de estos pacientes en comparación con el observado en la piel no lesionada de los mismos pacientes. Los detalles morfológicos de estas células no fueron claros bajo la microscopía de luz; es pertinente extender estos utilizando microscopía electrónica.

#### RESUME

On a procédé à l'énumération des cellules de Langerhans dans la peau de huit malades atteints de lèpre borderline et tuberculoïde. La méthode utilisée était basée sur la démonstration histochimique enzymatique de l'ATPase dans ces cellules des couches épidermiques. Le nombre de cellules de Langerhans était significativement diminué dans les lésions cutanées chez ces malades, par comparaison avec les résultats obtenus par l'examen de peau normale prélevée chez ces mêmes malades. Les détails morphologiques de ces cellules n'apparaissaient pas clairement à la microscopie optique. Il serait souhaitable de procéder à une étude par microscopie électronique.

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