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AN ULTRASTRUCTURAL STUDY OF LANGERHANS CELLS IN THE DEPIGMENTED SKIN IN VITILIGO

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The role of Langerhans cells in the pathogenesis of vitiligo is as yet unresolved. Ultrastructurally, these cells are identified by the presence of Birbeck granules, but at times this morphological marker is missed during sectioning. Identification of Langerhans cells was done by monoclonal antibodies and these were quantitated in the depigmented and the pigmented skin of seven vitiligo patients. Observations are in favour of autoimmunity being an aetiological factor for vitiligo.

Key words : Langerhans cells, Vitiligo.

The number of Langerhans cells (Lcs) in skin range from 400-1000 cells/mm^2 in man.[1] The regional variations in man reflect changes in the epidermal thickness. Increase in the number of Lcs in vitiligo was observed by many.[2-4] Others have noted a shift towards the basal cell layer.[5]

Indirect immunofluorescence technique has revealed that Lcs are the only cells in the epidermis that expressed the Ia antigen. Rowden et al[5] and Stingl et al[6] demonstrated receptors for Ig and C_3 on Lcs. This suggested that these cells perform functions similar to those of the monocyte, macrophage-histiocyte system and are thus immunocompetent cells. In our previous report a number of indeterminate cells were observed. It was essential to establish the identity of these cells. Hence the present study.

Materials and Methods

Seven patients (5 males and 2 females), aged 11 to 63 years who had vitiligo for less than one year and had not taken any form of treatment were selected. As a control group 1, five skin biopsies (3 males and 2 females) aged 18 to 53 years were taken from the corresponding sites of normal healthy individuals. Out of the 7 vitiligo patients, additional 2 skin biopsies were taken from the pigmented skin of these patients as a second control. This exercise has been done to appreciate the state of Lcs in the otherwise pigmented skin of vitiligo.

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Skin biopsy procedure

The biopsy was taken from the centre of a depigmented patch by injecting 0.5 ml lignocaine and snap frozen in a mixture of dry ice and methanol bath for 5 minutes.

Five micron thick sections were cut at -20°C and dipped in cold acetone for 1-2 minutes for clarification. These were treated with human AB serum and washed in phosphate buffer saline (PBS) and incubated in a moist chamber with monoclonal antibody OKT_6 (1 : 50 dilution) for 45 minutes at room temperature. The sections were washed in PBS and treated with (1 : 30 dilution) of FITC rabbit anti-mouse antibodies for 30-45 minutes. These were then mounted in glycerol medium and observed under Nikon FX 35 Microscope with Nikon mercury lamp power supply (HBO-100 W/2) and with UV filter. Fluorescing dendritic cells were evaluated in vertical sections in

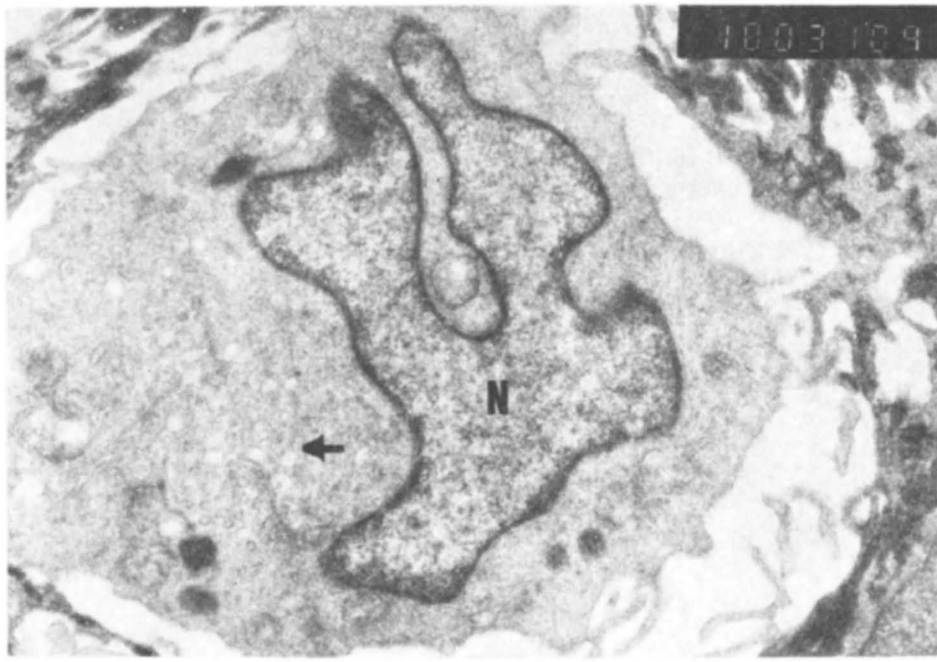

Fig. 7A A Langerhans Cell. Note the Conuoluted nucleus (N) and Birbeck granules (— Mag. x 10,000.

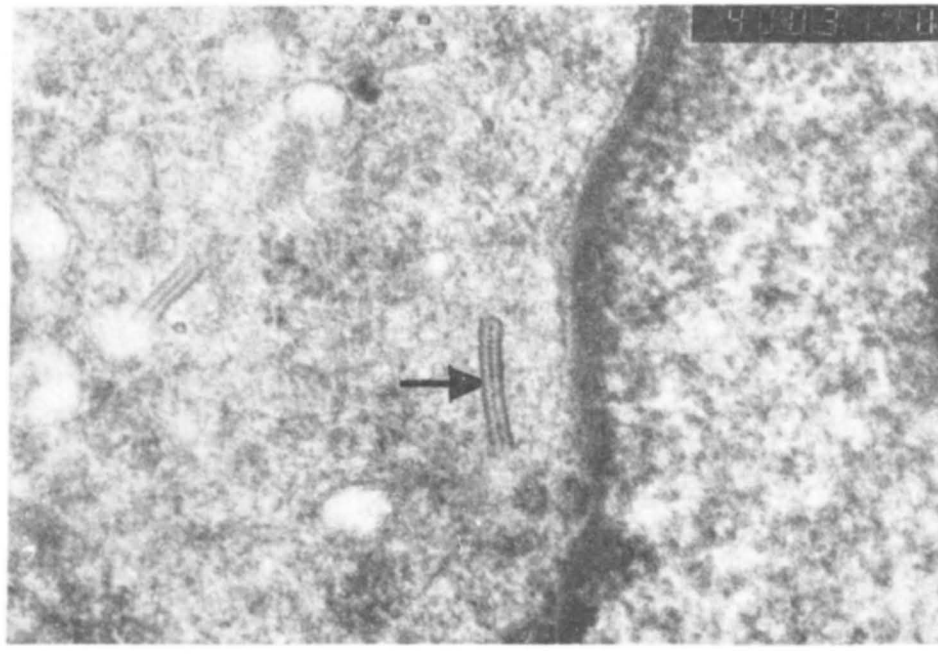

Fig. 7B Higher Mag. of Birbeck granule x 40,000

regard to their intra-epidermal distribution. Cell counts were made by a caliberated graticule with an objective lens of 40X, 5 consecutive fields were counted for fluorescing Lcs and the population was expressed as mean number of cells per millimeter of epidermis. The patient and control details are presented in table I A, B and C along with the mean values.

The results were statistically analysed. Student paired 't' test was employed P values to assess values of significance. The identification of Lcs ultrastructurally was done by the presence of Birbeck granules which are shown in (Fig. 7A and B).

Results

Ultrastructurally, Langerhans cells with Birbeck granules were identified. (Fig. 7A and B).

Indeterminate cells were identified (Fig. 8) and an increase in their number was noted.

Under fluorescence microscopy, the number of Langerhans cells was significantly high in the depigmented patch, while the count was significantly low in pigmented skin of vitiligo patients.

Comments

Ultrastructurally, degenerated melanocytes and Langerhans cells with Birbeck granules were seen in the depigmented patch. But many cells remained unidentified. Rowden et al[5] termed these indeterminate variety as alpha dendritic cells. They thought these could be precursors or immature Lcs. Surface antigens were also demonstrated on them. But it was not confirmed whether these surface antigens were produced or acquired by the indeterminate cells. Use of monoclonal antibody was essential and in the present study these indeterminate cells were identified as Lcs. It is possible that these could be precursors or immature Lcs (as very few Lcs with Birbeck granules were seen ultrastructurally). The significant observation was that of the low count of Lc in pigmented patch of vitiligo. It seems there is mobilisation of Lcs towards the depigmented patch, these being known as immunocompetent cells.

Table I. The number of Langerhans cells detected by fluorescence microscopy.

A. In the pigmented skin of normal individuals.

	Age	Sex	Site	No. of Lcs in field 1	2	3	4	5	Mean Lcs/mm
1	40	F	Thigh	3	2	1	1	3	2
2	18	M	Forearm	2	0	0	1	0	0.6
3	48	F	Calf	0	2	1	1	1	1
4	53	M	Knee	1	0	2	1	1	1
5	35	M	Knee	2	3	2	1	2	2
				Mean ± SD					1.32 ± 0.28

B. In the pigmented skin of vitiligo patients.

	Age	Sex	Site	No. of Lcs in field 1	2	3	4	5	Mean Lcs/mm
1	55	F	Ankle	0	2	0	1	0	0.6
2	60	M	Ankle	0	0	1	1	1	0.6
				Mean ± SD					0.6 ± 0

C. In the depigmented skin of vitiligo patients.

	Age	Sex	Site	No. of Lcs in field 1	2	3	4	5	Mean Lcs/mm
1	55	F	Ankle	2	1	4	1	2	2
2	60	M	Ankle	4	0	2	3	3	2.4
3	19	F	Knee	0	2	1	1	1	1
4	11	F	Hand	3	1	2	1	1	1.60
5	40	F	Calf	2	1	0	2	0	1
6	55	M	Knee	4	0	3	1	0	1.60
7	63	F	Knee	7	8	3	4	4	5.20
				Mean ± SD					2.54 ± 0.48

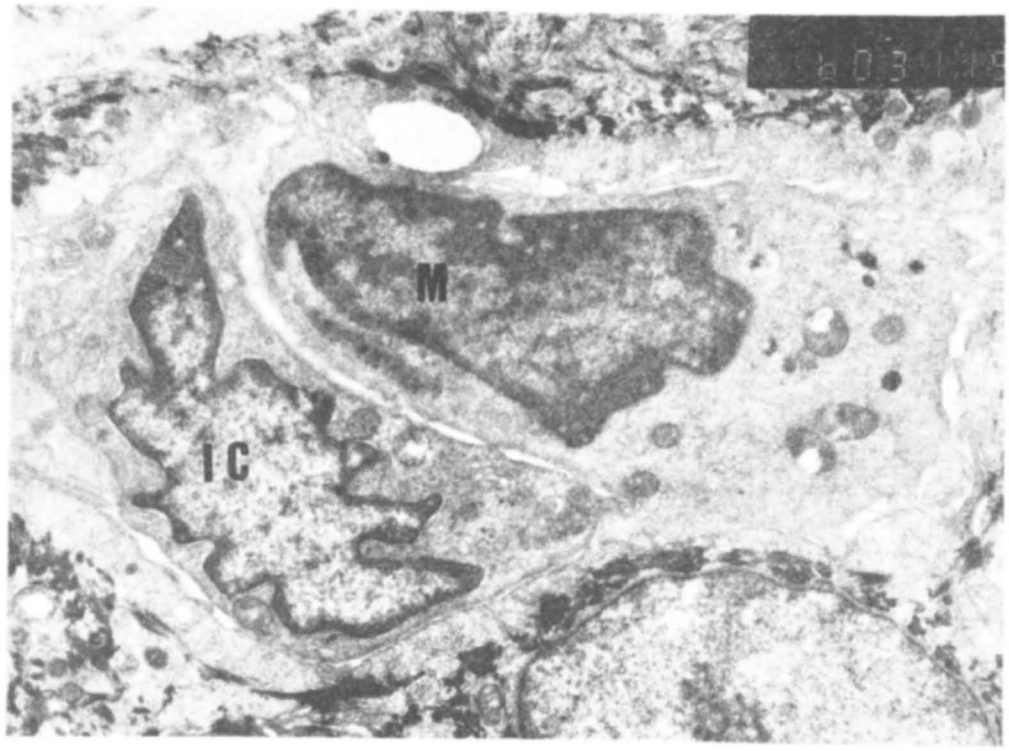

Fig. 8 An indeterminate Cell (IC) near a degenerating melanocyte (M) Note the conuoluted nucleus and absence of melanosomes and Birbeck granules in IC Mag. X 6,000

Stingl et al[6] stated that functionally, Lcs represent the peripheral output of the skin associated lymphoid tissue of the immune system. These probably function as specialized macrophages with a distinct role in maintaining the protective function of the epidermis.

Langerhans cells bind a range of haptens, transport antigens to regional nodes and may also act as target cells for the effector T cell population resulting from lymph node sensitization. Presumably a protective response for binding haptens to the skin is located on the surface of Lc. If the resident Lc population in the epidermis is to be maintained, some form of replacement must occur. The increase in number of fluorescing dendritic cells in the current study suggests that after the migration of Lc in response to haptenic stimulation, a migration of indeterminate cells occurs to restore the status quo. This also explains a significant decrease in the number of Lcs in the pigmented skin of patients.

The following model is based on the hypothesis that hypopigmentation is a morphological expression of autoimmunity.