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LANGERHANS CELLS IN VITILIGO: A QUANTITATIVE STUDY*

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Vitiligo is a common disease of unknown etiology characterized clinically by an acquired and usually progressive loss of pigment which can be histochemically demonstrated by an absence or a marked reduction in the number of 3,4-dihydroxyphenylalanine (dopa)-positive melanocytes. Although this reduction in the population of dopa-positive melanocytes is widely appreciated, the role of the Langerhans cell and its relationship to the melanocyte in this disease are not as yet firmly established. Miescher and Schaaf (1), using gold-impregnation techniques, reported the presence of Langerhans cells in equal numbers in pigmented and nonpigmented epidermis of patients with vitiligo. Becker and co-workers (2) reported that the number of Langerhans cells as well as the number of melanocytes of normal and vitiliginous skin was constant. They concluded from their studies that the Langerhans cell was identical to the melanocyte and that the variation in pigment in the vitiliginous skin was due to a variation in the physiologic function and not to the number of melanocytes.

Recent electron microscopic observations by Birbeck and co-workers (3) indicate that melanocytes are absent from vitiliginous skin and are replaced in their basal-layer position by cells indistinguishable from high-level clear cells or Langerhans cells. These findings have been interpreted to indicate that the Langerhans cell is a postdivisional product of the melanocyte and actually represents the inactive melanocyte observed by other workers with the light microscope.

All previous light microscopic studies of the Langerhans cell in vitiligo have been criticized because the unpredictable gold-impregnation or the supravital dye technique has been used. The enzyme histochemical method for the demonstration of adenosine triphosphatase (ATPase), however, has been recently shown to permit a simple reproducible and probably specific demonstration (4–8) of the Langerhans cell in several mammalian species, and when applied to isolated epidermal sheets (9) is an ideal method for evaluating the Langerhans cell population quantitatively. Because of the conflicting evidence regarding the Langerhans cell-melanocyte population in vitiligo, it was thought that a reappraisal of this problem, utilizing this method, was needed.

MATERIALS AND METHODS

Ten patients (eight women and two men) aged 30 to 66 years with typical vitiligo of long standing participated in this study. Following local anesthesia with 1% solution of xylocaine, 5-mm punch-biopsy specimens were removed from the center of untreated vitiliginous areas and from the closest corresponding site of normal skin of the contralateral side.

After removal of the subcutaneous tissue, pure epidermal sheets were prepared from two portions of each specimen by immersing them in 2 N sodium bromide solution for 4 hours. The epidermal sheets were washed and fixed, as described elsewhere (9), and one portion was incubated for the demonstration of nucleoside triphosphatase using adenosine triphosphate as substrate according to the method of Wachstein and Meisel (10). The other portion was incubated in dopa by the method of Becker (11).

Vertical sections, cut on a cryostat at a thickness of 15 μ, were obtained from involved and uninvolved areas in eight patients. One half of each section was incubated for the demonstration of nucleoside triphosphatase and the other half was incubated in dopa as described previously.

ATPase-positive dendritic cells were evaluated in vertical sections in regard to their morphology and intraepidermal distribution and compared with the intraepidermal distribution of dopa-positive melanocytes. Quantitative cell counts were made on epidermal sheets by means of a reticle fitted into the eyepiece of a microscope with an objective lens of ×43. The size of the field outlined by the reticle was calibrated, the cells in each of 10 randomly chosen fields were counted, and cell populations were expressed as the mean number of cells per square millimeter of epidermis.

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RESULTS

Individual figures obtained from the five regions of the body examined are listed in Table I. The number of Langerhans cells in normal-appearing skin (Fig. 1 *A* and *B*) varied from 464 to 1,009/mm² (mean 697). In involved areas (Fig. 1 *C* and *D*), counts varied from 561 to 958 cells/mm² (mean 731). Tests of significance determined by analysis of variance of the square roots of the observed counts disclosed no statistically significant difference between the Langerhans cell population of involved skin and that of uninvolved skin.

In the five samples obtained from the arm the number of cells varied from 464 to 800/mm² in normal skin and from 537 to 860/mm² in vitiliginous skin. Two samples were obtained from the thigh and one each from the axilla, wrist, and forearm. From these areas the highest counts were obtained from the forearm (1,009 and 958 cells/mm² for uninvolved and involved skin respectively), and the lowest counts were observed from the thigh (648 and 701 cells/mm² respectively).

Examination of dopa-treated epidermal sheets revealed the absence of dopa-positive melanocytes from the involved areas of six patients. In the remaining four patients, dopa-positive melanocytes were present but in greatly reduced numbers. In uninvolved skin they appeared to be present in normal numbers, varying from 732 to 1,715/mm² (mean 1,315). Both the highest and the lowest count were obtained from the arm, which was the most frequently examined site. Although the sample size is small, the distribution of dopa-positive melanocytes is in close agreement with the findings of Szabó (12) and of Staricco and Pinkus (13).

The intraepidermal distribution of ATPase-positive dendritic cells in vertical sections, although similar in normal-appearing and vitiliginous epidermis of individual patients, varied considerably from patient to patient. In most areas the majority of cells occupied a suprabasilar position and were equally distributed throughout the stratum malpighii. However, in some sections, particularly those from thin epidermis such as the inner aspect of the arm (Fig. 2 *A* and *B*) or the axilla, ATPase-positive dendritic cells were located predominantly in the basal layer.

TABLE I

Cell counts in involved and uninvolved skin in vitiligo

Patient	Site	Langerhans cells, no./mm²		Dopa-positive melanocytes, no./mm²	
		Normal skin	Vitiliginous skin	Normal skin	Vitiliginous skin
1	Arm	464	537	1,318	Absent
2	Arm	488	561	1,228	Absent
3	Arm	683	860	1,715	16
4	Arm	729	806	732	Absent
5	Arm	800	779	1,342	16
6	Forearm	1,009	958	1,460	Absent
7	Wrist	716	713	1,286	Absent
8	Axilla	718	624	1,308	22
9	Thigh	648	701	1,155	Absent
10	Thigh	714	774	1,603	448
Mean		697	731	1,315	0–448

Dopa-positive melanocytes were not observed in any vertical sections of involved skin, whereas in normal-appearing skin they were confined to and almost entirely lined the dermoepidermal junction.

DISCUSSION

The quantitative and morphologic similarities of the Langerhans cell population of involved and uninvolved skin of vitiligo and the lack of any numerical correlation between the Langerhans cells and the dopa-positive melanocytes indicate that the Langerhans cell is not changed in this disease and support the concept of Wolff and Winkelmann (8) that these two cell lines are perhaps distinct and unrelated, contrary to the view proposed originally by Breathnach (14) and by Zelickson (15) that Langerhans cells represent postdivisional products of melanocytes.

The histochemical localization of ATPase appears to be a unique property of Langerhans cells of man as well as several other mammalian species. Electron microscopic studies of guinea pig epidermis (7) have shown that histochemically demonstrable ATPase is limited solely to this cell. Similar electron microscopic studies in man have not been made, but it appears likely that the Langerhans cell of man also can be identified specifically with

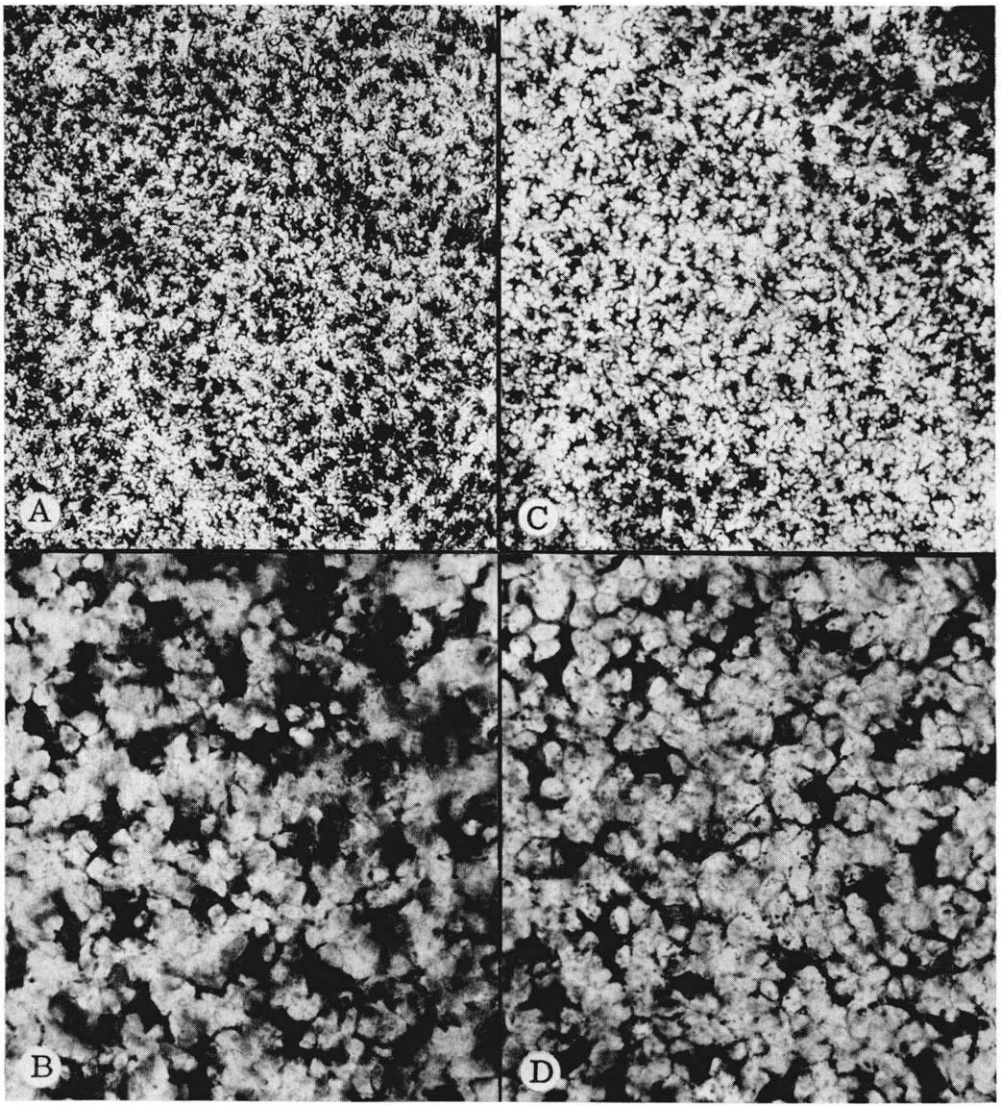

FIG. 1. Epidermal sheets. Distribution and density of Langerhans cells in vitiligo. *A* and *B*, Uninvolved skin. (ATPase; ×115 and ×365.) *C* and *D*, Involved skin. (ATPase; ×115 and ×365.)

this technique. Keratinocytes did not exhibit this enzymatic reaction. ATPase-positive cells did not contain melanin, and serial sections treated by the dopa technique revealed ATPase cells to be dopa-negative.

Our findings are in good agreement with those of Ferreira-Marques (16), who studied gold-impregnated vertical sections of normal human epidermis and from an extrapolation of vertical sections found 670 Langerhans cells/mm^2. Using the same method, Miescher and Schaaf (1) found typical gold-positive dendritic cells in apparently equal numbers in involved and uninvolved skin of patients with vitiligo.

Recently Riley (17) reported that the ATPase-positive dendritic cell population of isolated epidermal sheets of normal-appearing skin was equal to that of vitiliginous skin. The counts of ATPase-positive cells (1,150 to 1,850/mm^2) were higher than those in the present study. We cannot account for the differ-

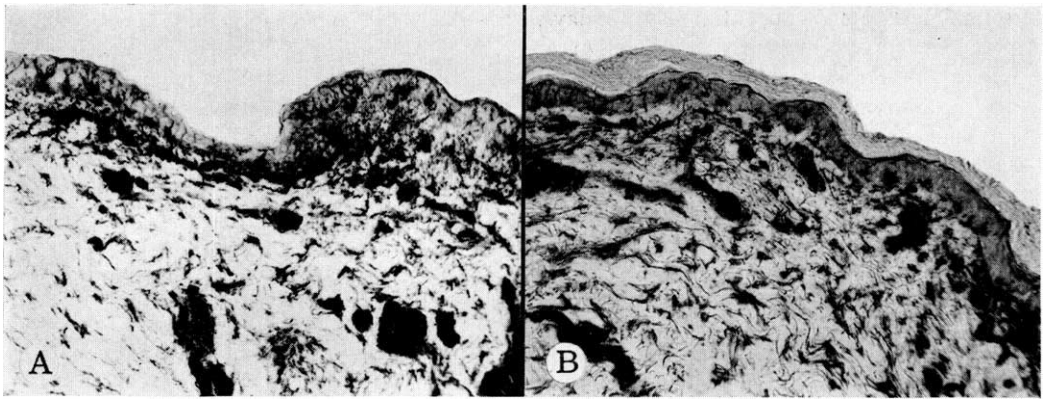

FIG. 2. Vertical sections demonstrating intraepidermal distribution of Langerhans cells in thin epidermis of inner arm. (ATPase; ×100.) Note presence of several cells close to basement membrane. *A*, Uninvolved skin. *B*, Involved skin.

ence in Langerhans cell counts in the two studies except by differences in technique. On vertical section, Riley observed that the number of suprabasal ATPase-positive cells was increased significantly in vitiliginous epidermis, a finding we could not confirm.

Birbeck and co-workers (3) also thought that gold-positive cells were increased in vitiliginous areas in comparison with normal control material and that, in normal control skin, these cells were seldom found close to or within the basal cell layer. They encountered difficulty in demonstrating gold-positive dendritic cells in normal-appearing skin of patients with vitiligo. Electron microscopic studies by the same workers indicated that melanocytes were absent, and cells indistinguishable from Langerhans cells were found on the basement membrane in number and position similar to those of melanocytes of normal epidermis.

The variations in all of these studies of the Langerhans cells in vitiligo may be the result of differences in their intraepidermal distribution. Our observations indicate that this distribution varies considerably and that, for example, in regions with thick epidermis this cell is located in a predominantly suprabasal position, whereas in regions of thin epidermis, Langerhans cells frequently occupy a position close to or on the basement membrane in both normal and vitiliginous skin.

Although the sample size in the present study was small, considerable variation in the Langerhans cell population was encountered among the 10 patients and among the various regions examined. For example, the population in the arm varied from 464 to 860 cells/mm^2 among the five patients studied. This is in contrast to guinea pig epidermis (9), in which the Langerhans cell population appears to be highly constant from region to region as well as from animal to animal. Our knowledge of the regional distribution and normal variation of the Langerhans cell population in human epidermis must be increased; but to date in a given individual, Langerhans cell counts from some areas are reasonably reproducible.

Our findings in dopa-incubated material agree with those of other workers (2, 3). Dopa-positive melanocytes were absent from or greatly reduced in number in involved skin and appeared to be present in normal numbers in normal-appearing skin.

The concept that the Langerhans cell represents a distinct and separate cell line is supported by studies of guinea pig epidermis, which show a highly constant Langerhans cell population in contrast to the dopa-positive melanocyte population, which varies greatly in distribution (9). Recent studies of guinea pig epidermis have also shown that the Langerhans cell is not affected by melanogenic stimuli such as ultraviolet light or carcinogens (18) but may be affected by other factors such as stripping off the stratum corneum of guinea pig epidermis (19), in which event the Langerhans cells, independently of pigment cells, are almost entirely shed within 3 to 4 days after injury but repopulate the epidermis

in normal numbers in approximately 15 days. It does not appear likely that stimulation of the Langerhans cell population could produce specific alterations in the melanocyte population and consequently affect the process of vitiligo.

SUMMARY

Langerhans cells, demonstrated histochemically by the method for adenosine triphosphatase, were studied in skin specimens from involved and corresponding uninvolved areas in 10 patients with vitiligo. Cell counts of isolated epidermal sheets revealed regional and individual variations but were similar for the uninvolved and involved areas of the same patient. The mean count of Langerhans cells was 731/mm² in involved areas and 697/mm² in uninvolved areas. Studies of vertical sections showed variations in the intraepidermal distribution of ATPase-positive dendritic cells, which appeared to be related to epidermal thickness.

The quantitative and morphologic similarities of Langerhans cells in uninvolved and involved skin from patients with vitiligo as well as the lack of any numerical correlation between Langerhans cell and melanocyte populations indicate that the Langerhans cell is not an etiologic factor in this disease and that these two cell lines are distinct and perhaps unrelated.

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