0022-202X/80/7401-0040$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 74:40-42, 1980

Vol. 74, No. 1
Printed in U.S.A.

PUVA-induced Repigmentation of Vitiligo: Scanning Electron Microscopy of Hair Follicles

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PUVA-induced repigmentation of vitiligo was studied using both the split-dopa reaction and scanning electron microscopy. Proliferation of hypertrophic, Dopa-positive melanocytes were observed in the lower portion of some hair follicles, whereas other giant melanocytes were observed along the middle portion. The existence of a melanocyte reservoir in human hair follicles is postulated.

Previous histoenzymological (Split dopa) and ultrastructural (transmission EM) have suggested the following hypothesis with regard to PUVA-induced repigmentation of vitiligo: first, that proliferation of follicular melanocytes occurs, followed by their migration along the hair follicle to the infundibulum and, finally, into the adjacent achromic epidermis, where they propagate centrifugally [1].

In order to test this hypothesis, we studied the hair follicles from repigmented skin of 2 patients with vitiligo who were being treated by oral photochemotherapy. These hair follicles were split with sodium bromide before being tested.

MATERIALS AND METHODS

Patients (Table I)

Two patients with vitiligo vulgaris were investigated. Vitiliginous macules involved 30% (case 1) and 40% of the body surfaces. None of them showed features of trichrome vitiligo [4] or associated disorders. In both patients, there was more than 75% improvement (more than 75% of total area of original depigmentation was repigmented).

Treatment

The regime of progressive UVA dosage, as well as that of 8-MOP have previously been published [5].

Biopsies

Six or 7-mm punch biopsies were taken under local 1% lignocaine anesthesia. From each patient, 3 biopsies were obtained: from the center of the islands of repigmentation, from the junction of repigmentation and achromic skin, and from neighbouring, healthy skin.

Split-Dopa Studies

The 3 biopsies were incubated in a 2 N NaBr solution at 37° for 45 min and the separated layers thus obtained were then incubated with 0.1% dopa solution in 0.1 M sodium phosphate buffer (pH 7.4) for 4 hr at 37°. After the dopa reaction had been completed, the tissues were fixed in 10% neutral formalin and dehydrated in alcohol. The epidermis was placed upside down on a glass slide and both the epidermal basal layer and hair follicles were examined.

Scanning Electron Microscopy (SEM)

Specimens which had previously been studied under the light microscope were then processed for scanning electron microscopy.

The samples were air dried and a small portion fixed on standard mounting blocks by means of double-face tape, with the surface to be studied facing upwards. The tissue surfaces were uniformly coated in a vacuum evaporator with about 200 Angstroms of gold palladium to ensure good electrical conductivity in SEM. The samples were examined with an Cambridge Stereoscan 600 scanning electron miscroscope.

TABLE I. *Clinical data—Treatment schedule*

Case No.	Age/sex	Skin type [2]	Duration of vitiligo	Vitiligo type [3]	Clinical course	Dose of 8-MOP (mg)	UVA (J/cm²)	Biopsy site
1	32/F	II	2	Absolute	Stable	40	119	Breast
2	30/M	IV	8	Absolute	Stable	50	139	Abdomen

RESULTS

Split Dopa (Figure, A and B)

In the deep portion of some of the hair follicles which converge onto the repigmented areas or their immediate vicinity there was considerable proliferation of giant dopa-positive cells. These cells are larger than the epidermal melanocytes observed in the center of the repigmented islands.

Other dopa-positive cells could be identified in the infundibulum, extending up from the middle part of the follicle. These were located in the outer sheath of the root.

No giant dopa-positive cells were observed in hair follicles from normal control skin.

SEM (Figure, C and D)

In the lower portion of some follicles, numerous large cells with a round body and short dendrites were observed.

Similar cells were also detectable along the middle part of the hair follicles. These were quite large, since their cell body, which was globular or oval, with 4 to 8 generally thin, long dendrites, covered half or two-thirds of the diameter of the hair follicle. Some dendrites were curled up on the body, whereas other extended along the major axis of the hair follicles.

These cells were clearly prominent on the hair follicles and were not surrounded by basement membrane, which had probably been removed during the separation procedure.

In the vicinity of the infundibulum of these hair follicles, giant dendritic cells were observed in the epidermis. However, no such cells were observed in the hair follicles of normal control skin.

COMMENTS

Dendritic cells observed on hair follicles under SEM correspond, by their localization, to the dopa-positive cells observed under the light microscope. Their histoenzymological and morphological features are characteristic of melanocytes.

These observations favor our hypothesis regarding PUVA-induced repigmentation of vitiligo, since no follicular melanocytes were observed in normal skin, even after PUVA.

Manuscript received February 22, 1979; accepted for publication August 1, 1979.

J. P. Ortonne is in receipt of a Research Fellowship from "Ministère des Universités."

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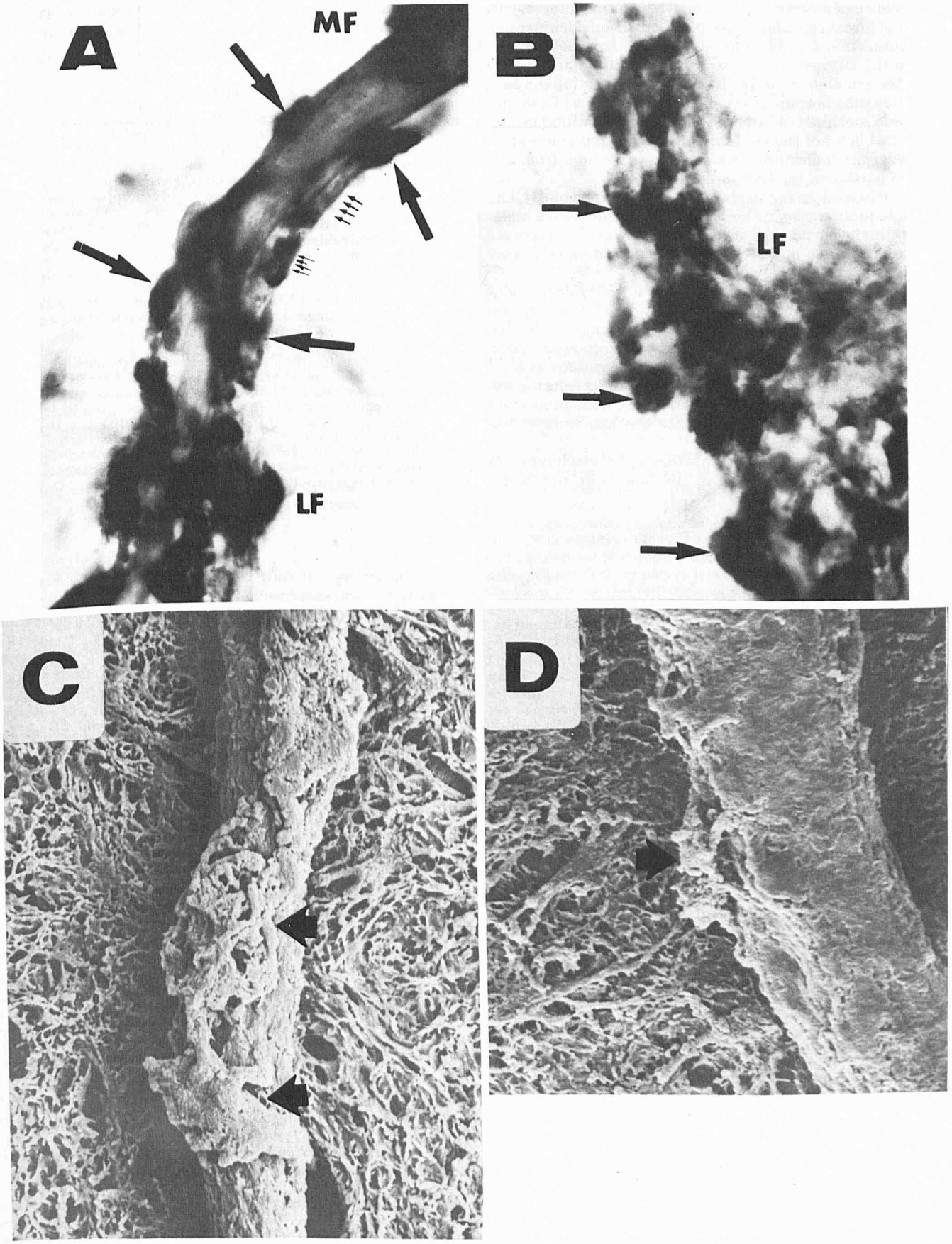

A, split dopa: lower portion (*LF*) and middle portion (*MF*) of hair follicles: *arrows* point towards dopa-positive cells. *Small arrows* (outer root sheat) (× 980). *B*, Split dopa: lower portion (*LF*) of hair follicle: considerable proliferation of dopa positive cells (*arrows*) (× 980). *C*, Scanning electron microscope: 2 giant dendritic cells (*arrows*) are observed along the middle portion of this hair follicle (× 2800). *D*, Scanning electron microscope. Dendritic cell (*arrow*) with an oval body and several dendrites (× 2800).

The presence of amelanotic melanocytes on the outer sheath of the root of human hair follicles has already been documented. Under certain circumstances (epidermal regeneration after dermabrasion [6], ultraviolet ray exposure [7]) these amelanotic melanocytes are able to divide in the middle part of the hair follicle. They then become gradually pigmented, transform into hypertrophic melanocytes and migrate from the infundibulum into the basal layer of the epidermis. Thus the repigmentation of the epidermis following dermabrasion originates from the amelanotic portion of the hair follicle.

This hypothesis does not explain our observations of: (1) The considerable proliferation of hypertrophic, dopa-positive melanocytes in the lower portion of hair follicles. (2) The presence of hypertrophic, dopa-positive melanocytes in the middle portion of the hair follicle.

From our observations, the existence of a reservoir of melanocytes in human hair follicles must be postulated. Proliferation of these melanocytes occurs under certain circumstances (disappearance of the epidermal melanocytic system stimulation (PUVA in this instance). Ultrastructural observations in C 57 black mice suggest that melanocytes dedifferentiate during the catagen and telogen phases of the hair cycle, then proliferate by mitosis, redifferentiate and populate the hair bulb at the onset of the anagen phase [8].

In our study, we were unable to explain the mechanism of the proliferation of melanocytes in the hair bulb, nor is the mode of action of PUVA on hair follicles known. Therefore, further studies are needed to elucidate these observations.

The technical assistance of Miss Agnès Micoud is gratefully acknowledged.

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