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ULTRASTRUCTURE OF THREE TYPES OF EPIDERMAL DENDRITIC CELLS IN HAIRLESS MICE*

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ABSTRACT

Electronmicroscopic studies of epidermal dendritic cells in hairless mice were carried out in normal and pigmented dorsal skin, the latter induced by topical application of DMBA. A quantitative study of three types of epidermal dendritic cells was performed in this series. Statistical evaluation disclosed that: (1) the number of Langerhans cells was inversely proportional to that of the melanocytes, and (2) the number of the indeterminate dendritic cells remained constant. A pair of flanking indeterminate dendritic cells which appeared to be divided daughter cells was incidentally found a week after the start of the experiment. Our results suggest that the indeterminate dendritic cell could be an undifferentiated cell which might give rise to either one of the other epidermal dendritic cell types.

Several studies have been carried out on melanogenesis induced in hairless mice by ultraviolet radiation (1, 2) or carcinogens (3–7). Adenosinetriphosphatase (5) and nonspecific esterase (6) were demonstrated in the epidermal dendritic cells of normal hairless mice. These cells are dopa-negative and almost exclusively located in the lowermost layer (4–7). During carcinogen-induced melanogenesis, more than a half of them became melanogenic and dopa-positive, and thus, were interpreted as "amelanotic melanocytes" (6, 7).

The purpose of this paper is to demonstrate the Langerhans cell, the melanocyte and the indeterminate epidermal dendritic cell, and to discuss their relationships on the basis of quantitative changes following the application of dimethylbenzanthracene (DMBA).

MATERIALS AND METHODS

Animals: Hairless mice, ranging in weight from 23 to 28 gm and from 12 to 16 weeks of age were selected from a colony of dark hairless mice. The mice are offspring of C57HR/Ch mutants which were established by crossbreeding C57HR/Ch† with haired C57BL/10-H-2^a (B10.D2) black mice‡ to introduce more pigment capacity into the strain. The animal was tentatively designated as "dark strain", based on the appearance of generalized pigmentation two weeks after the first application of DMBA. The mice were kept in separate cages and received drinking water and food ad lib up to sacrifice.

Carcinogen: 7,12-Dimethylbenz[α]anthracene (DMBA) prepared by the Eastman Kodak Company was dissolved in light mineral oil.

Method: According to a modified method of Klaus and Winkelmann (4), a 1 per cent DMBA solution was applied topically to the dorsal surface of each of the animals with a standard medical dropper. These mice received four drops of the solution two times at an interval of three days. Two mm. punch biopsy specimens were obtained from the back of the animals prior to the application of the solution, on the seventh and fourteenth day, and two months after the initial application of the solution. Each group was composed of five mice, and two blocks were prepared from each of the animals. The tissue was fixed at 4° C for 2 hours in 1 per cent osmium tetroxide in 0.2 M phosphate buffer, pH 7.4, and following dehydration in graded alcohols was embedded in Epon 812 according to the method of Luft (8). Sections were cut on a Porter-Blum MT-2 ultramicrotome and stained on the grid for one hour with a saturated aqueous solution of uranyl acetate followed by 10 minutes' immersion in Reynolds' lead citrate (9). They were examined with a JEM 7 electron microscope at the initial magnification of ×2,500 for counting the number of dendritic cells, and ×8,000 for identifying each of them. Measurements were made on 5 electron micrographs of the epidermis in each block, avoiding the follicular utriculi deliberately. Thus, a total number of micrographs taken was 50 in each group. Each of the micrographs was taken at random in a manner that the skin surface ran approximately parallel with one side of the 5 cm square [plates]. In other

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† Dr. J. M. Knox, Baylor University, supplied the hairless mice.

‡ Dr. and Mrs. T. Tanaka in the Aichi Cancer Institute supplied the haired black mice of B10.D2 strain.

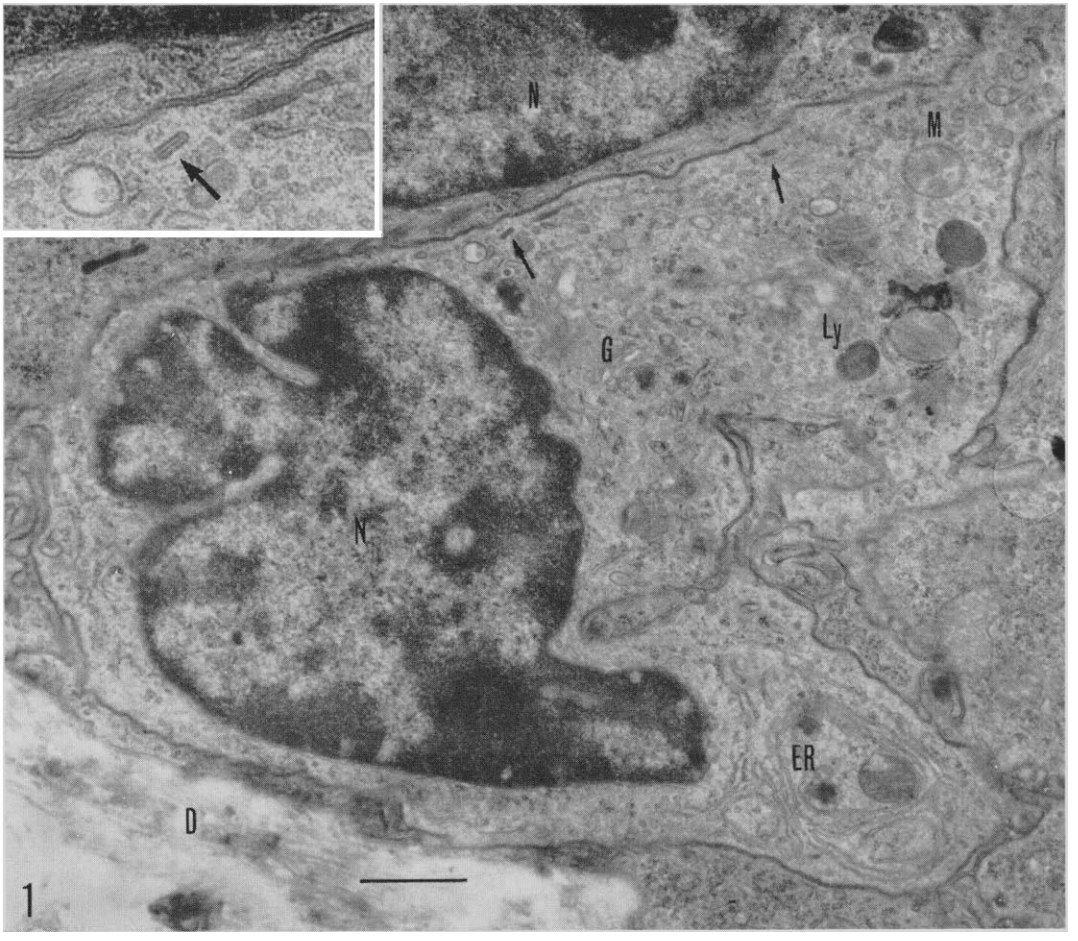

FIG. 1. A basal Langerhans cell in the normal epidermis of the hairless mouse. The cell possesses an indented nucleus (N) and characteristic granules (arrows) (see also the inset). D: Dermis, ER: endoplasmic reticulum, G: Golgi areas, Ly: lysosome, M: mitochondria. ×13,900; inset: ×37,400.

words, a micrograph contained 20 μ of the epidermis in width, and thus the counting of dendritic cells in each block was made on a 100 μ width of epidermis. The dendritic cells, in which the nucleus was not seen, were omitted in counting. The following criteria were adopted to differentiate each of the three types of dendritic cells. The melanocyte was characterized by the presence of premelanosomes in the cytoplasm, and the Langerhans cell by the presence of so-called Langerhans cell granules. The other type of dendritic cell was tentatively distinguished from the former two types by lack of characteristic organelles in the cytoplasm.

RESULTS

Gross changes

The first pigmentation in this series was evident approximately a week after the initial administration of DMBA, and the pigment reached a maximal degree 12 to 14 days after the first application, and then gradually faded over the subsequent two weeks.

Electronmicroscopic observations

The epidermal dendritic cells were easily distinguished from flanking keratinocytes by virtue of a clear cytoplasm devoid of tonofilaments and the absence of desmosomes along the plasma membrane. Two types of nonkeratinizing cells were present in the suprabasal and basal layer of the normal epidermis of hairless mice. About half of these dendritic cells were positively identified as Langerhans cells due to the presence in the cytoplasm of

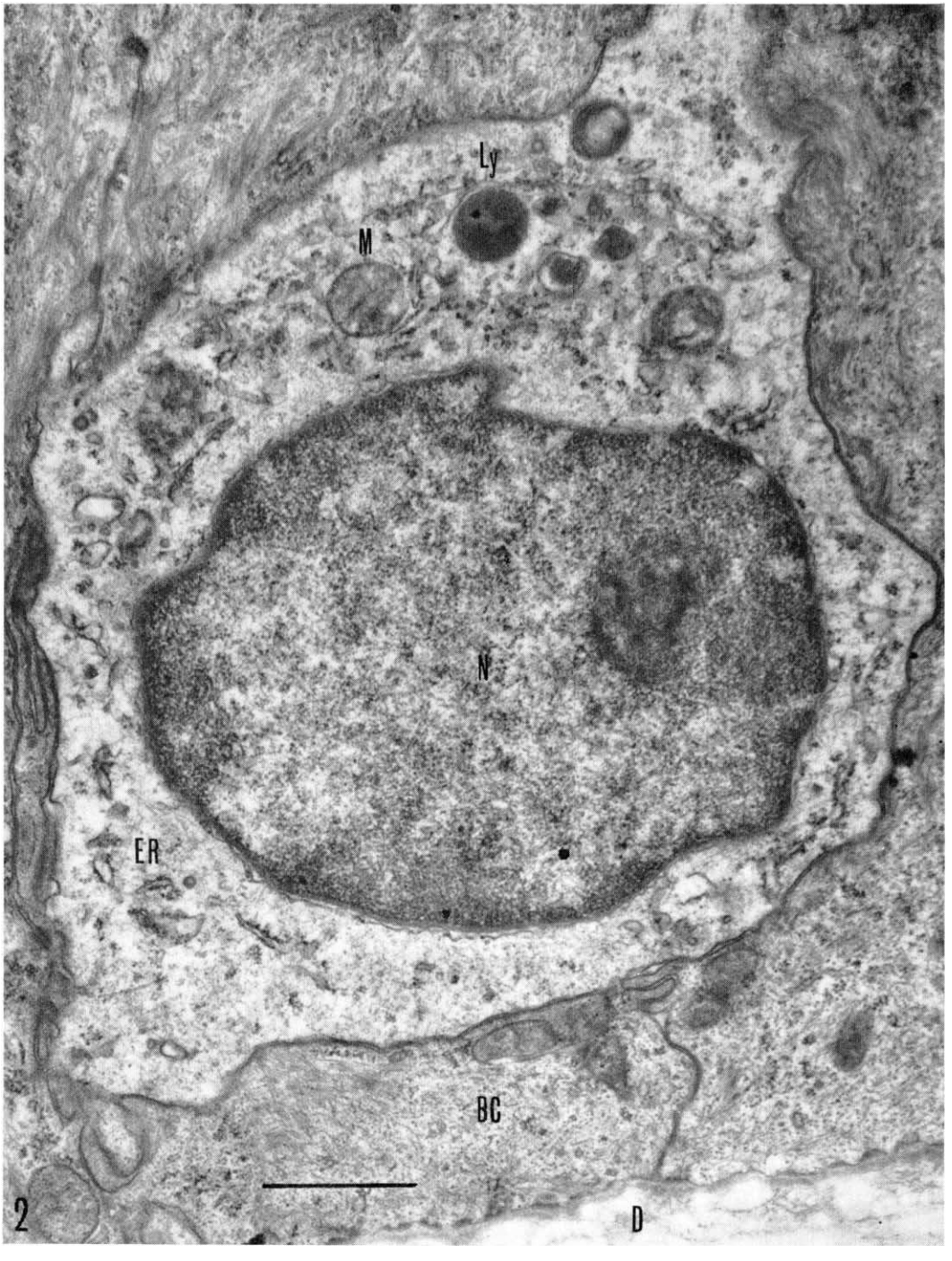

FIG. 2. An indeterminate epidermal dendritic cell in the suprabasal layer of the normal epidermis. Note that there are neither premelanosomes nor Langerhans granules in the cytoplasm. D: Dermis, ER: endoplasmic reticulum, Ly: lysosome, M: mitochondria. ×21,800.

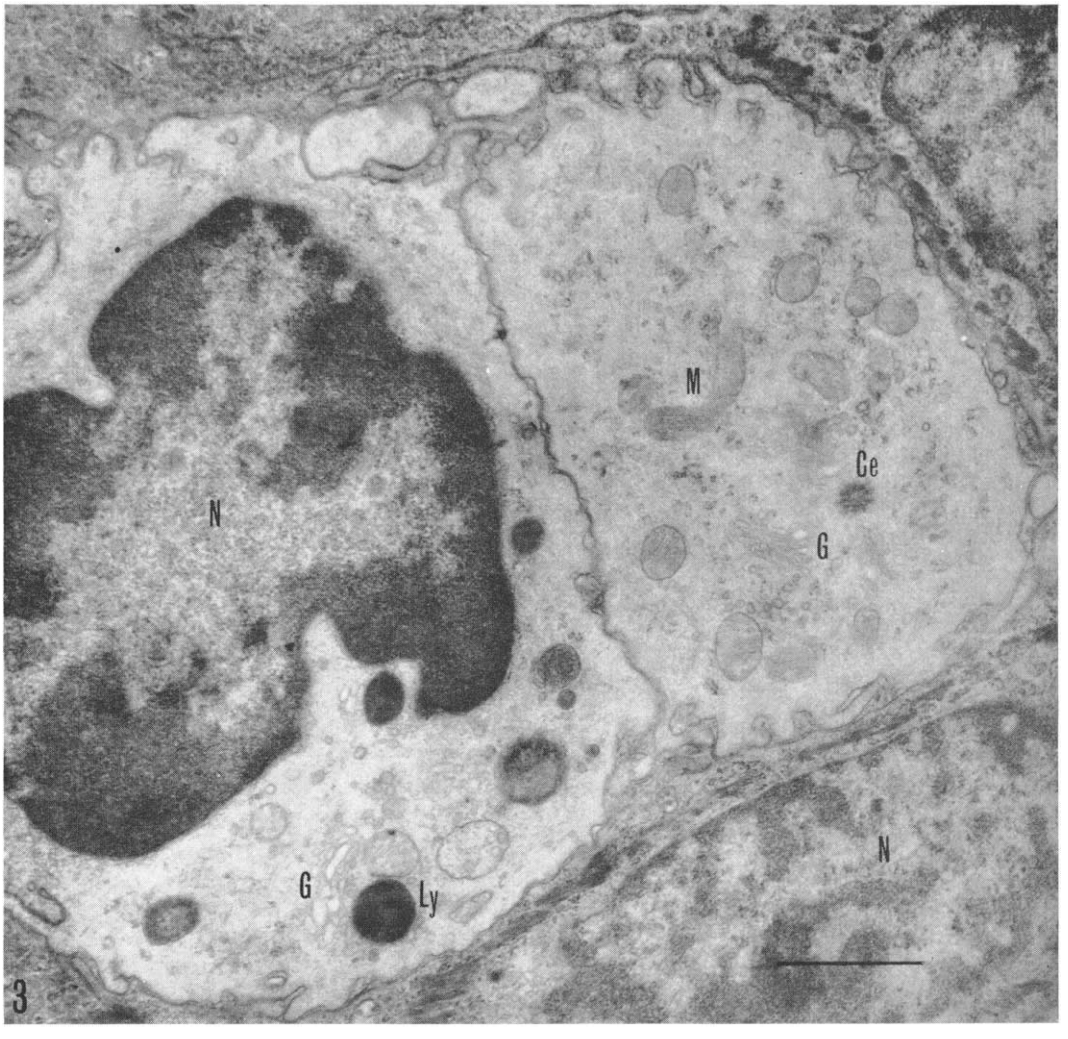

FIG. 3. A pair of the indeterminate dendritic cells on the seventh day after the first application of DMBA. They appear to be two daughter cells following a mitosis. Ce: Centriolar structures, G: Golgi areas, Ly: lysosome, N: nucleus. ×18,700.

organelles characteristic of this type of cell (Fig. 1). The other half of the dendritic cells were composed of the indeterminate dendritic cells (10) devoid of any characteristic organelles (Fig. 2).

A week after the initial application of DMBA, the epidermis became acanthotic. The Langerhans cells decreased in number, and were no longer observed in the basal or lowermost layer, but in the higher levels. A predominant number of the dendritic cells were composed of indeterminate dendritic cells. Incidentally, a pair of flanking indeterminate dendritic cells was found, which seemed to be divided daughter cells (Fig. 3). They possessed numerous mitochondria, well-developed Golgi areas and centriolar structures. A few melanocytes were detected in the basal layer (Fig. 4). These melanocytes contained the premelanosomes in various stages of development and the melanosomes. They also possessed abundant branched mitochondria and endoplasmic reticulum, in addition to the well-developed Golgi areas, as did the indeterminate dendritic cells at the same stage. However, no significant changes of the mitochondrion were observed in any of

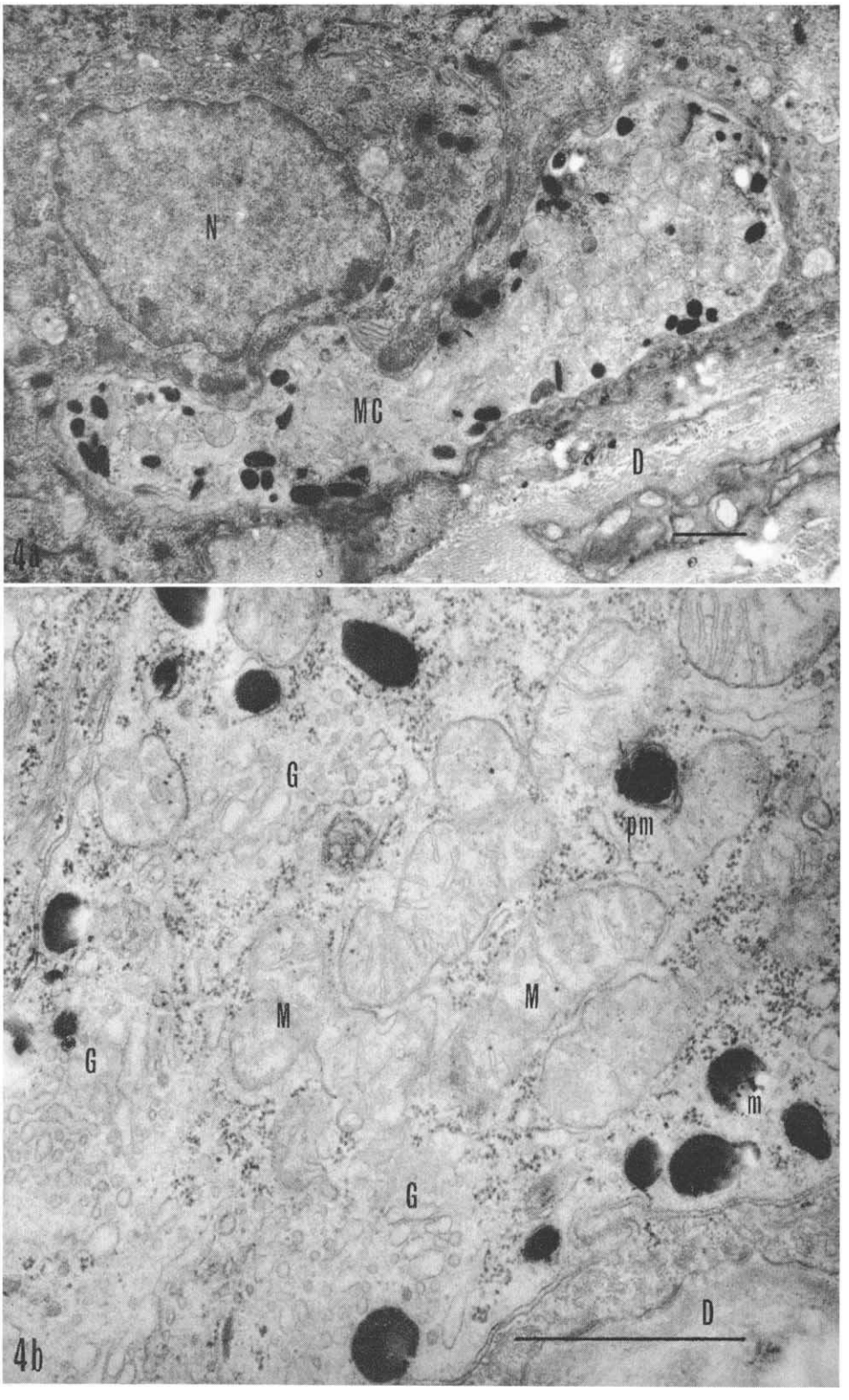

FIG. 4. A melanocyte on the seventh day after the first application of DMBA. (a) A whole view of the melanocyte (MC). D: dermis, N: nucleus. ×10,600. (b) Higher magnification of an area from the Fig. 4a. A considerable number of premelanosomes (pm) and melanosomes (m), well-developed Golgi areas (G), and branched mitochondria (M) can be seen. D: Dermis. ×33,300.

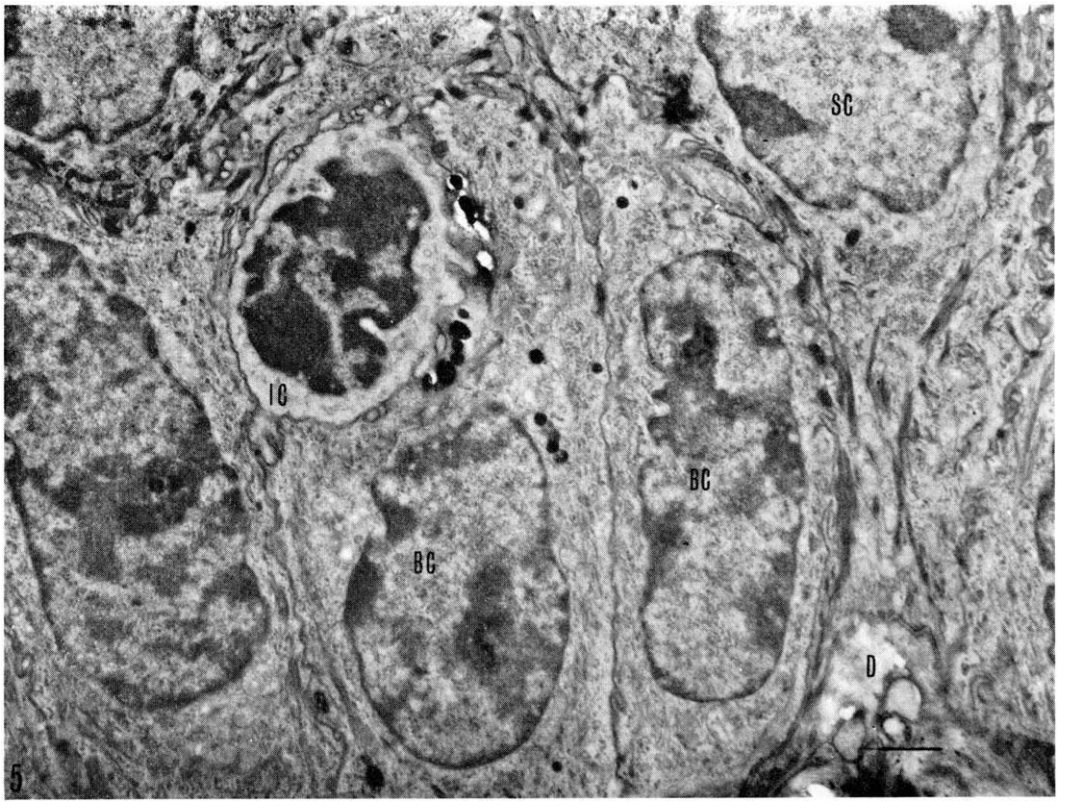

FIG. 5. An indeterminate dendritic cell two months after the experiment started. Most melanosomes (m) are distributed within an adjacent basal cell (BC). D: Dermis, SC: squamous cell. ×9,300.

the keratinocytes. The premelanosomes and melanosomes looked ovoid or rod-shaped, the length varying from about 0.1 to 0.5 microns and the width from 0.07 to 0.25 microns (Fig. 4).

On the fourteenth day after commencement of the experiment, the basal dendritic cells were mainly melanocytes, and partly indeterminate dendritic cells. The latter cells were also found in the suprabasal layer. Only one Langerhans cell was observed in the whole micrograph taken in this stage.

Two months later melanocytes were no longer seen in the basal layer. The indeterminate dendritic cell appeared to be predominant in number, but some of them seemed to be the effete melanocytes, where melanosomes were distributed within the basal cell adjacent to the dendritic cell (Fig. 5).

The results of the quantitative study of the epidermal dendritic cells carried out on hairless mice are presented in Table I, and summarized in Table II. Statistical evaluation revealed the following: (1) the number of indeterminate dendritic cells was significantly larger than that of the Langerhans cell or of the melanocytes (2) the number of either one or two of the three types of dendritic cells changed significantly, depending on the stage in which the specimens were taken, and (3) no significant difference was found between any two of the stages with respect to total number of epidermal dendritic cells (Table III). Further counts revealed the following: (1) the number of melanocytes was greatest two weeks after the start of the experiment, and significantly larger one week after the start than before the experiment or two months later (2) the number of Langerhans cells was significantly larger in the normal epidermis than in any other stage, and significantly smaller two weeks after the start of the ex-

TABLE I

Quantitative changes of three types of epidermal dendritic cells before and after topical application of DMBA

Five micrographs of the epidermis 20 microns in width were taken at random in each block, and thus counting was made on 100 microns of epidermis. L: Langerhans cells, I: indeterminate dendritic cells, M: melanocytes.

The start of the experiment

Before					One week after					Two weeks after					Two months after				
Block No.	Dendritic cells				Block No.	Dendritic cells				Block No.	Dendritic cells				Block No.	Dendritic cells			
	L	I	M	Sum		L	I	M	Sum		L	I	M	Sum		L	I	M	Sum
1	0	1	0	1	11	0	1	0	1	21	0	3	0	3	31	0	3	0	3
2	1	1	0	2	12	1	3	0	4	22	0	2	2	4	32	0	1	0	1
3	2	1	0	3	13	0	3	0	3	23	0	1	0	1	33	0	4	0	4
4	1	3	0	4	14	0	0	2	2	24	0	1	1	2	34	1	2	0	3
5	1	0	0	1	15	0	1	0	1	25	0	2	1	3	35	1	1	0	2
6	1	1	0	2	16	0	2	1	3	26	0	3	2	5	36	0	1	0	1
7	1	2	0	3	17	1	1	0	2	27	0	1	2	3	37	1	1	0	2
8	2	1	0	3	18	0	1	1	2	28	1	2	0	3	38	0	2	0	2
9	1	0	0	1	19	1	1	0	2	29	0	1	1	2	39	1	1	0	2
10	1	1	0	2	20	0	1	0	1	30	0	1	1	2	40	1	1	0	2
Sum	11	11	0	22	Sum	3	14	4	21	Sum	1	17	10	28	Sum	5	17	0	22

TABLE II

Summarized result of the epidermal dendritic cells before and after the topical application of DMBA

The start of the experiment	Epidermal dendritic cell types			Sum
	Langerhans cell	Indeterminate dendritic cell	Melanocyte	
Before	11	11	0	22
One week after	3	14	4	21
Two weeks after	1	17	10	28
Two months after	5	17	0	22
Sum	20	59	14	93

TABLE III

Analysis of variance table estimated from Tables I and II

Source of variance	Sum of square	Degree of freedom	Mean square
Experiment group: G	1.025	3	0.342
Block within group: B(G)	9.233	26	0.355
Dendritic cell type: D	29.850	2	14.925*
Interaction: D × G	13.750	6	2.292*
Estimate of error: E	41.067	82	0.501
Total: BDG	94.925	119	—
Estimate of error: E'†	50.300	108	0.465

* shows a significant difference at a level of 0.01.

† shows a final estimate of error which was set up with a combination of B(G) and E.

periment than two months later (3) no significant difference between any two stages was found in the number of the indeterminate dendritic cells (4) the number of indeterminate dendritic cells was significantly larger than that of any other dendritic cells after the start of the experiment and (5) the number of melanocytes was significantly larger than that of Langerhans cells two weeks after the start of the experiment, and significantly smaller before the experiment and two months later.

DISCUSSION

Electronmicroscopic studies of the normal epidermis in a dark strain of hairless mice disclosed that the Langerhans cell and the in-

determinate dendritic cell in the suprabasal and basal layer were approximately the same ratio in number. The latter cell seems to be identical with one first recognized by Horiki (11) in 1966, and recently designated as either the indeterminate epidermal dendritic cell by Zelickson and Mottaz (10) or α-dendritic cell by Mishima and Kawasaki (12).

The existence of the basal Langerhans cell has been reported in depigmented skin areas in vitiligo (12, 13), in the white forelock region in human piebaldism (14), in the depigmented epidermis of the halo nevi (15) and in white epidermis of the recessively spotted guinea pig (16). There is controversy about the nature and significance of the Langerhans cell (17, 18). It has been proposed that it is an effete melanocyte (19, 20), a division product of the melanocyte (21), an intraepidermal nerve ending (22), a melanocyte precursor (16), or a post melanin-synthetic cell (23). In 1966, Mishima (24) found melanosomes in the lysosome-like vacuoles within the cytoplasm of Langerhans cells, and suggested that it would indicate the transformation of melanocytes into Langerhans cells. On the other hand, Zelickson (23) reported that Langerhans cells were present not only in the epidermis but also in the dermis. Moreover, Langerhans granules were found within macrophages in lesions of histiocytosis X (25–28), and in those of some other diseases (28). Another idea proposed is that epidermal Langerhans cells constitute an intraepithelial phagocytic system (28). These studies have resurrected lingering doubts that the Langerhans cells do belong to the melanocyte lineage, and the doubts have been reinforced by evidence that the melanocytes were not demonstrated in skin developed from neural crest-free grafts but Langerhans cells were observed (29).

As the existence of the indeterminate epidermal dendritic cell has been recognized (10–12, 15, 30), the debates have been focused on the relationships among the three forms of dendritic cells. Zelickson and Mottaz (10) found the indeterminate dendritic cell in the epidermis of normal and vitiliginous human skin, and mentioned the following four possibilities: (1) it represented a form of premelanocyte in which melanin synthesis could be induced; (2) an effete melanocyte which had ceased its activity; (3) undifferentiated cells which could give rise to either one of the other epidermal dendritic cell types, or finally (4) a completely unrelated cell.

Swanson *et al.* (15) found an indeterminate dendritic cell flanking a Langerhans cell in the depigmented epidermis of a halo nevus. One of the authors (30) also reported the same findings in a tumor of melanoacanthoma. In this series of experiments, the authors chanced to observe a pair of the flanking indeterminate dendritic cells which appeared to be divided daughter cells.

In addition, the quantitative study of the epidermal dendritic cells before and after topical application of DMBA showed the following results: (1) the number of Langerhans cells was reversely proportional to that of the melanocytes over a period of active melanogenesis, and (2) the number of the indeterminate dendritic cells remained constant. The same results were reported by Zelickson and Mottaz (10) in human normal and vitiliginous epidermis.

Although no direct relationships were obtained among the three types of dendritic cells, our results imply that the indeterminate dendritic cell may be an undifferentiated cell which could give rise to either one of the other epidermal dendritic cell types, or that the melanocyte and Langerhans cell could transform into each other through the indeterminate dendritic cell.

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