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Changes in Epidermal Langerhans Cells Following the Tuberculin Reaction in Guinea Pigs

Akira KOHCHIYAMA, Daisuke OKA and Hiroaki UEKI

Department of Dermatology, Kawasaki Medical School, Kurashiki 701-01, Japan

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ABSTRACT. Changes in Langerhans cells (LCs) with the tuberculin reaction were studied using EDTA (ethylenediaminetetraacetic acid) separation and ATPase (adenosine triphosphatase) staining techniques in guinea pig epidermis following intradermal PPD injections. The densities of ATPase positive LCs decreased significantly ($p<0.001$) from 24 hours to 7 days after injection with a maximum decrease at 72 hours. In addition, these cells became rather swollen or enlarged and lost their characteristic dendritic processes. The significance of these changes is discussed.

Key words : Epidermal Langerhans cells — Tuberculin reaction — ATPase staining

Epidermal LCs have been implicated as pivotal antigen processing cells in the induction and expression of delayed type hypersensitivity (DTH) responses, especially in contact hypersensitivity reactions.[1—3] It has been reported that allergic contact hypersensitivity reactions in animal skin induce numerical and morphological changes in epidermal LCs.[4—6] Certain treatments e.g., irradiation with ultraviolet-B (UVB) and/or UVA,[7—9] topical and/or systemic administration of glucocorticosteroids[10] and repeated stripping with cellophane tape[10—13] also result in changes in LCs. In this study, we report changes in LCs in guinea pig epidermis associated with the classical DTH reaction induced by PPD injections (tuberculin reaction) using the highly specific ATPase staining technique in EDTA separated epidermal sheets.[14,15]

MATERIALS AND METHODS

Animals : Male, Hartley strain, albino guinea pigs weighing 400–500 g were used.

Tuberculin reactions : Guinea pigs were sensitized by subcutaneous injection with 2 mg of dried BCG vaccine in phosphate-buffered saline (PBS). Seven days after injection, 0.1 μg / 0.1 ml of purified protein derivatives of tubercle bacilli (PPD) in PBS was injected intradermally into the ear skin. For evaluation of the tuberculin reaction, the diameter of palpable, infiltrative erythema was measured in millimeters at 24, 48, 72 hours, 4, 7 days after elicitation.

Biopsies : At 24, 48, 72 hours, 4, 7 days after elicitation, the ears were amputated at the base and their four edges were cut away to leave 10 mm diameter central portions of erythema. As controls, skin biopsy samples were

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taken from the ears of non-treated animals. From 3 to 5 biopsy samples were obtained from each experimental group.

Identification of epidermal LCs : Epidermal LCs were identified by staining EDTA separated epidermal sheets for ATPase activity.[14—15] Briefly, skin samples were incubated for 2.5 hours in 20 mM EDTA in PBS at 37°C. After incubation, epidermal sheets were peeled off from the dermis with fine forceps. Only the epidermis obtained from the dorsal surface of the ear was used. These epidermal sheets were rinsed twice in trismal buffer (6.84% sucrose), pH 7.3, at 4°C, and then fixed in cacodylatebuffered formaldehyde for 20 minutes at 4°C. After rinsing, they were immersed in ATP-pb solution for 20 minutes at 37°C, rinsed twice, and immersed in a 1% solution of 22.3% ammonium sulfide for 20 minutes at room temperature. After rinsing, they were mounted dermal (dark) side up in PBS/glycerol.

Enumeration of ATPase-positive cells : In each animal, ATPase-positive dendritic cells were counted in more than 10 randomly chosen fields of at least 3 specimens by means of an ocular square grid at ×400 magnification. Cell densities were reported as the total number of ATPase-positive cells/mm^2 skin ± SE, and student's t-test was used to assess differences among groups.

RESULTS AND DISCUSSION

Subcutaneous injection of 2 mg of dried BCG vaccine in PBS induced sensitization in all of the animals used in this study. The time course of the response after elicitation was assessed from the diameters of palpable, infiltrative erythema. Erythema of over 10 mm in diameter were observed at 24 and 48 hours with a maximum response at 48 hours. Mean diameters were : 12 mm at 24h, 13 mm at 48h, 9 mm at 72h, 6 mm at 4d and 5 mm at 7d.

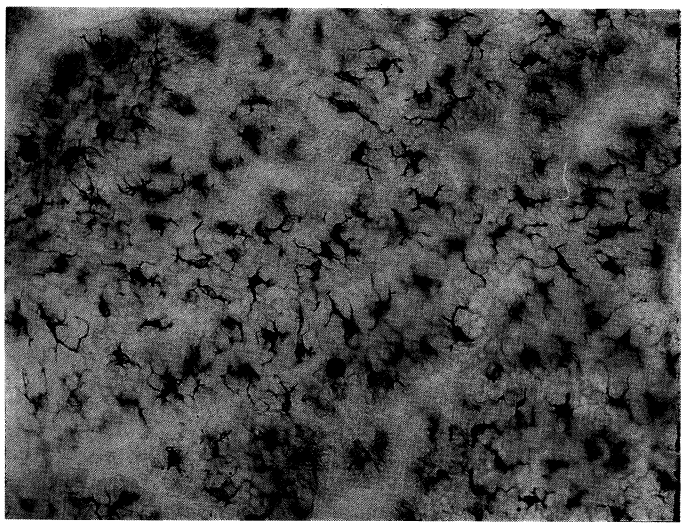

Fig. 1. Epidermal LCs in control guinea pig. Regularly distributed highly-dendritic cells are observed (ATPase staining, ×200).

TABLE 1. Surface densities of ATPase positive LCs following the tuberculin reaction in guinea pig epidermis

Time[a)]	Cells/mm²[b)] (Mean±SE)	Student's t-test[c)]
0h	1405 ± 40.6	
24h	780 ± 20.5	$p<0.001$
48h	778 ± 20.8	$p<0.001$
72h	770 ± 19.5	$p<0.001$
4d	832 ± 19.5	$p<0.001$
7d	827 ± 21.8	$p<0.001$

a) Time after elicitation of the tuberculin reaction.
b) More than 10 fields counted in at least 3 specimens.
c) Each group was compared with a control group.

Fig. 2. Epidermal LCs after elicitation of the tuberculin reaction. These cells decreased significantly and became rather swollen or enlarged and lost their dendritic processes (ATPase staining, ×200).

Using the EDTA separation and ATPase staining techniques, LCs could be visualized well in control guinea pig ear skin. These cells exhibited a regular distribution, and fine dendritic processes radiated out from a central body (Fig. 1). Then mean density and standard error was 1405±40.6 cells/mm² (Table 1).

Elicitation with 0.1 μg of PPD on the ears resulted in a statistically significant decrease ($p<0.001$) in the densities of ATPase-positive LCs from 24 hours to 7 days with a maximum decrease (770 ± 19.5 cells/mm²) at 72 hours (Table 1). In addition, obvious morphological changes were also observed. Compared with the LCs in control animals, these cells became rather swollen

or enlarged and rounded because of the disappearance of dendritic processes (Fig. 2).

It has been suggested that LCs serve as target cells in contact hypersensitivity reactions[16] and immune complex reactions.[17] In the present study, a significant reduction and obvious morphological changes in epidermal LCs were observed following the tuberculin reaction in guinea pig skin. Although it is possible that these changes were induced by mere inflammatory reactions, it may be suggested that the LCs were affected as the target cells in the tuberculin reaction. Further studies should be done with respect to these problems.

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