KAWASAKI MED. J. VOL. 11 : NO. 3, 183-185 (1985)

Brief Note

The Effect of DNCB Painting on ATPase Positive Cells in Guinea Pig Epidermis

Accepted for Publication on May 16, 1985

Key words : Contact sensitivity — DNCB — ATPase positive cells — Langerhans cells

Contact sensitivity (CS) to 2,4-dinitrochlorobenzene (DNCB) was achieved in guinea pigs by intradermal injection of epidermal cells prepared from skin painting with DNCB 3 hours before (DNP-EC). However, pretreatment by tape strripping, the method for divesting the epidermis of Langerhans cells (LC), at the induction site of CS impaired the development of CS to DNCB.[1] It has been reported that *in vitro* haptenated epidermal cells containing LC, even if injected intravenously, sensitized animals.[2] Our previous data may be explained by postulating that the antigen presenting function of LC included in DNP-EC is impared. LC are characterized by Fc-IgG and C3 receptors and bear Ia antigens. They also take up a variety of antigens and are able to present various antigens in an immunologic way to T lymphocytes. Adenosine triphosphatase (ATPase) has been demonstrated at the LC surface by a histochemical technique described by Wachstein & Meisen.[3] It has also been previously shown that within the epidermis this enzymatic marker is specific for LC.[4] In this experiment, we examined the configuration and density of LC revealed by

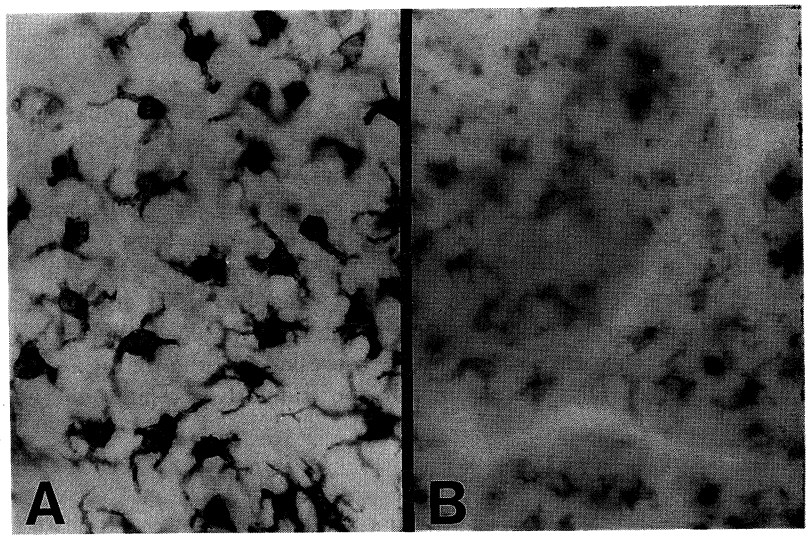

Figure. EDTA separated epidermis, stained for ATPase activity.
A, untreated skin with a normal appearing Langerhans cells.
B, 3 hours before DNCB painted skin. Dendritic processes were grossly attenuated or absent.

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ATPase stain in epidermis prepared from untreated skin and skin painted 3 hours before.

Untreated ears or ones painted 3 hours before with 200μl of 5%DNCB were obtained and split along the plane of cartilage, which was removed together with subcutaneous tissue. Specimens of skin were incubated for 2 hours at 37°C in 0.7%EDTA (ethylene diamine tetraacetic acid). After this treatment, the epidermis could be readily separated from the dermis with fine forceps. The epidermal sheets were stained for ATPase. The number of ATPase positive cells in the epidermis of the untreated skin was $1328\pm32/mm^2$ (Table) and normal appearing Langerhans cells were seen (Fig. A). The number of such cells in the skin painted 3 hours before was $752\pm48/mm^2$ (Table). ATPase positive cells identified at this time exhibited abnormal configurations. Their dendritic processes were grossly attenuated or, more commonly, absent (Fig. B). Bergstresser *et al.*[5] have been reported that at 6 hours after the first application of 25μl of 0.5% DNFB to mouse abdominal skin, the number and configurations of ATPase positive cells were preserved. Within 12 hours, however, pronounced changes occurred. No normal cells were observed and the number decreased. In this study, it is therefore suggested that application of DNCB to the skin induced alteration in epidermal Langerhans cells at an early time.

TABLE. Number of ATPase positive epidermal cells in untreated and 3 hours before DNCB painted skin

Epidermal sheet prepared from	ATPase positive cell density
	Mean $\pm$ S.E./mm^2
Normal skin	1328 ± 32
Skin 3 hours after painting with 5% DNCB ethanol solution	752 ± 48

Irradiation of UVB resulted in diminution of the LC population as has previously been revealed by this technique.[5] However, Aberer *et al.*[6] indicated that diminution of LC is mainly due to a loss of the enzymatic marker of LC and not to their destruction. Since the ATPase stain revealed diminution of LC after the application of a chemical hapten, diminution may be also due to an alteration in the enzymatic marker. When animals were painted with hapten on skin depleted of ATPase positive cells by UVB irradiation, they could not be sensitized to the hapten, but this also induced tolerance.[7] These results suggest that this enzymatic marker represents a function of LC, and that surface application of DNCB may impair the function of epidermal LC.

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