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An Attempt to Suppress the Induction of Contact Sensitivity to 2,4-Dinitrochlorobenzene by Tape Stripping Treatment of Guinea Pig Skin

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ABSTRACT. The effect of stripping treatment with cellophane tape on induction of contact sensitivity (CS) to 2,4-dinitrochlorobenzene (DNCB) was studied in inbred JY1 strain guinea pigs in order to determine whether Langerhans cells (LC) are relevant to production of CS. Tape stripping of ear skin achieved, to a considerable degree but not absolutely, depletion of epidermal LC, as measured by cell surface of LC dedetcted by ATPase staining. However, pretreatment of tape stripping on the induction site of contact sensitization with DNCB did not diminish the rate and intensity of challenge reactions to DNCB.

Key words : Contact sensitivity — Langerhans cell — Tape stripping — ATPase staining — DNCB

Langerhans cells (LC) were found to be characterized by Fc-IgG and C3 receptors and bear surface Ia antigens.[1-3] LC also took up a variety of soluble and particulate antigens and thus had characteristics with the cells of the mononuclear phagocyte system.[4,5] When animals are painted with hapten on skin naturally or artificially depleted of epidermal LC and subsequently challenged with the hapten, they not only are hyposensitive but are tolerant to attempt at sensitization with the hapten.[6,7] These observations indicate that LC plays an essential role in the induction of CS. However, mechanisms in details by which LC is relevant to production of CS are still unknown.

Ultraviolet light irradiation of animal skin results in a transient loss of ATPase-positive, Ia-positive, cells from the epidermis.[8,9] Another method that has been reported to divest skin of LC is repeated stripping with cellophane tape.[10] So we used tape stripping of animal body wall skin in an effort to remove resident LC and to investigate the influence of LC depletion on the induction of CS.

MATERIALS AND METHODS

Animals: Male inbred JY1 guinea pigs were purchased from Kiwa Laboratory Animal Company and animals weighing 250-350 g were used.

Tape stripping and ATPase staining: Dorsal and venteral surface of both sides of ears were stripped by repeated applications (20 times) of cellophane tape. This number of tape applications was sufficient to cause the epidermal surface to glisten.

The ears taken at various intervals after tape stripping were split in the plane of the cartilage which was removed together with subcutaneous tissue.

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The specimens were incubated in 0.7% ethylendimanine tracetic acid (EDTA) at 37°C for 2 hours.[11] After this treatment, the epidermis could be readily separated from dermis with fine forceps. The epidermal sheets were washed in PBS and fixed in cacodilate formaldehyde solution for 20 minutes at 4°C. Following washing twice with distilled water, the specimens were incubated at 37°C for 60 minutes in staining medium consisting of 2.7 ml of ATP stock (50 mg ATP salt, 5 mg glucose, 50 ml distilled water, 40 ml tris buffer, 10 ml 0.1 M $MgSO_4$ $7H_2O$) and 0.3 ml of 2% $Pb(NO_3)_2$ solution. After rinsing with distilled water, the specimens were developed in 1% yellow ammonium sulfide at room temperature for 20 minutes, rinsed in water and mounted under a coverslide with glycerine jelly. ATPase positive cells were counted with a calibrate microscope equipped with reticle in the eyepiece at magnification ×400. At least 10 fields were examined on each sheet and the counts were expressed as a number of cells per squaremillimeter of surface of epidermis.

Sensitization and elicitation of contact sensitivity (CS) : For sensitization, 0.01 ml of 0.5% 2,4-dinitrochlorobenzene (DNCB) ethanol solution was applied epicutaneously through normal or stripped ear skin of JY1 guinea pigs. Tape stripping treatment was carried out immediately before DNCB application. Seven days after the painting of DNCB, skin test was performed by applications of 0.01 ml of 0.1, 0.05 and 0.025% DNCB ethanol solutions on the depilated flank. The contact reactions were read 24 hours later and evaluated according to the following scales : no visible change, 0 ; slight or discret erythema, 0.5 ; moderate erythema, 1 ; confluent erythema, 2 ; intense erythema and swelling, 3. The degree of hypersensitivity was expressed as the total of all three readings in each animal.

RESULTS

Ear skin of normal inbred JY1 guinea pigs were stripped to glistening by using cellophane tape. The density of ATPase-positive cells in the epidermis was determined immediately following tape stripping and 3 and 7 days after the treatment. The density of epidermal ATPase-positive cells clearly fell by the tape stripping treatment (Table 1). The number of the cells in epidermis immediately after the treatment was about 65% of that of normal skin. Apporoximately 80% of the positive cells showed abnormal configuration. Their dendritic processes were grossly attenuated or absent. Three days later, density of the cells came to 50% of control value. These cells scarcely retained abnormal morphology at the time. By 7 days after stripping, the positive cells had returned to 56% of the control density.

TABLE 1. The effect of tape stripping treatment on ATPase-positive cell densities in guinea pig epidermis.

Epidermal sheets	Cell densities (cells ± S.D./mm²)	Morphology
normal skin	1198 ± 83	normal
immediately after stripping	779 ± 169	abnormal
3 days after stripping	595 ± 41	normal
7 days after stripping	672 ± 60	normal

Since tape stripping treatment was shown to deplete the ATPase-positive cells in epidermis, we next investigated the effect of the treatment on induction of CS with DNCB. Application of DNCB to normal skin resulted in strong sensitization (Table 2). DNCB application to the skin stripped immediately before also showed the similar degree of contact sensitization.

TABLE 2. Effect of tape stripping treatment on the development of contact sensitivity by painting with DNCB.

Sensitization	Treatment	Contact sensitivity to DNCB		
		Positive		Mean intensity
		0.1%	0.05%	
DNCB	none	7/7	2/7	0.9
DNCB	stripping(0d)	7/7	4/7	1.2

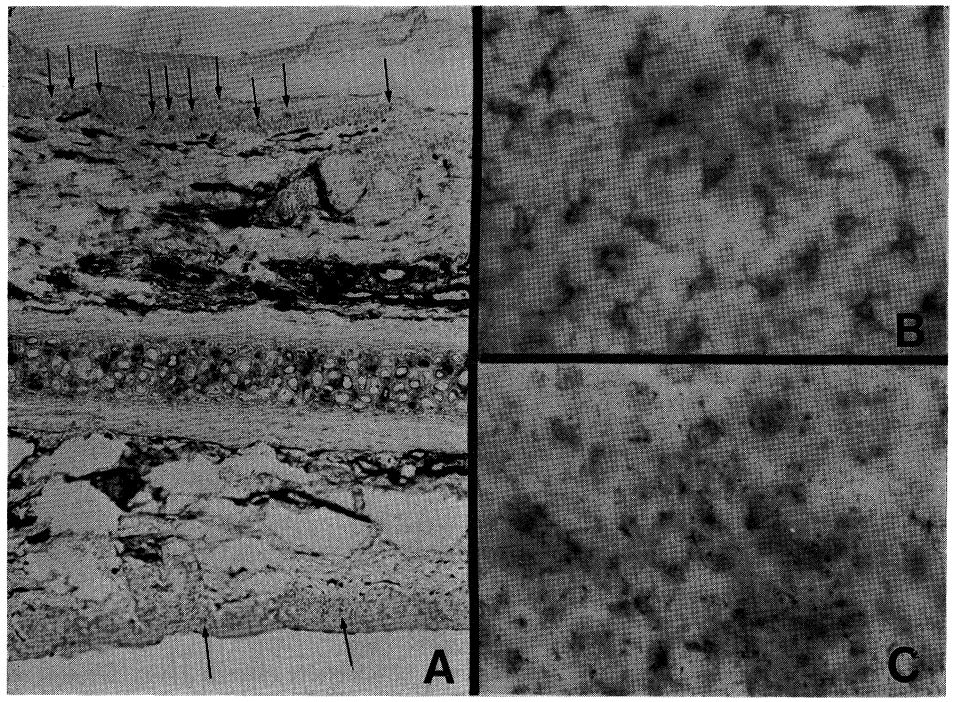

Fig. A, Unfixed frozen section. ATPase positive dendritic cells (arrows) in normal epidermis of dorsal surface of guinea pig ear (top) and in contralateral surface of ear which had been stripped immediately before (bottom). B and C, Epidermal sheet preparations showing ATPase positive cells. B, Normal epidermis of dorsal surface of guinea pig ear. C, Contralateral ear of same animal immediately after stripping.

DISCUSSION

Lessard *et al.*[10] have demonstrated that repeated tape stripping of guinea pig skin removes the stratum corneum, exposing the suprabasal layer. Most of this suprabasal portion of the epidermis is then eliminated as a compact parakeratotic layer, carrying resident Langerhans cells (LC) with it. During the next few days, the underlying newly regenerated epidermis is devoid of

identifiable LC Streilen *et al.*[11] also have reported that tape stripping of murine abdominal wall skin achieves almost complete depletion of epidermal LC within a few hours of application, as measured by cell surface ATPase and expression of Ia antigens. Quantitative counts of LC detected by ATPase staining in our present experiment revealed that tape stripping reduced significantly LC in the epidermis immediately after stripping and in the newly regenerated epidermis 3 and 7 days after the treatment.

Pretreatment of tape stripping immediately before on the induction site of contact sensitization with 2,4-dinitrochlorobenzene (DNCB) did not diminish the rate and intensity of challenge reactions to DNCB. This result is contrast with the findings of Kotake *et al.*[12] who reported that contact sensitivity (CS) reaction to 2,4-dinitrofluorobenzene (DNFB) was suppressed in mice sensitized with the chemical through stripped skin. The differences which might explain this disparity are: they used mice and DNFB whereas we sensitized guinea pigs with DNCB, they applied animals with DNFB one day after tape stripping whereas we painted animals with DNCB immediately after stripping. Further experiments have to be done in this experimental area.

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