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Antigen in Contact Sensitivity : I. Immunofluorescent Studies on the Distribution of Bound DNCB within the Epidermis of Guinea Pigs Following Skin Painting with the Sensitizer

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ABSTRACT. Cryostat sections, sheet and single cells of epidermis prepared from ear skin of guinea pigs 3 hours after painting with 5% DNCB ethanol solution were examined by using FITC labelled anti-DNP antibody. DNP groups were demonstrated on almost 100% of Malpigian cells of the epidermis, partial distribution of them was seen in horny layer. The number of DNP cells in the single cells was 80.2% on an average and roughly the same as the cells incubated *in vitro* with 30mM solution of $DNBSO_3Na$.
Significance of these findings is discussed.

Key words : contact sensitivity — epidermal cell — DNCB — antigen distribution

The induction of contact sensitivity have been extensively studied. Contact sensitization is easily induced in animals by simply painting the skin with contact sensitizing agents. It has been demonstrated that haptens coupled *in vitro* to syngeneic epidermal cells are effective immunogens.[1-3] The implication of these findings is that hapten binds in a similar way to the surface components of epidermal cells *in vivo* to create antigenic units capable of inducing immunogenic responses. However, whether such conjugation actually occurs *in vivo* remains to be established. Previous investigations[4,5] in which localization of 2,4-dinitrophenyl (DNP) groups in the skin of guinea pigs following surface application of 2,4-dinitrochlorobenzene (DNCB) was examined, showed that DNP groups were clearly demonstrated on the epidermal cells.

The objective of experiments in this reports is to obtain the quantitative data on the number and distribution of epidermal cells taking up contact sensitizer.

MATERIALS AND METHODS

Animals : Male Hartley strains guinea pigs weighing between 350-450g were used for observation of epidermal DNCB distribution. 2.5kg male rabbits were used for preparation of antisera to DNCB.

Production and characterization of anti-DNP antibody : Hyperimmunized

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sera were obtained from rabbits which had been sensitized with DNP_4-ovalbumin conjugate emulsified with Freund's complete adjuvant (Difco, FCA) as described previously[4]. Anti-DNP antibody (anti-DNP) was specifically purified from sera by the immunoadsorbent method according to the Eisen et al.[6] The prepared anti-DNP was characterized by gel diffusion against DNP-protein conjugates and by immunoelectrophoresis with goat anti-rabbit whole serum and goat anti-rabbit IgG serum and was shown to be a DNP specific IgG fraction.

The antibody was labelled with fluorescence isothiocyanate (FITC) by a previously described procedure[4]. It was used after absorption with rabbit liver acetone powder.

Treatment of guinea pigs and preparation of specimens : Guinea pigs were painted with a total of 0.1ml of 5%DNCB-ethanol solution on ear skin. The ear obtained 3 hours after painting with DNCB were carefully shaved and washed with PBS (0.01M phosphate buffer saline, pH7.2). Thereafter the specimen was immediately frozen and cut at 4μm on cryostat and each section was air dried.

The ears which had been painted with 5%DNCB-ethanol solution 3 hours before were split in the plane of the cartilage which was removed together with subcutaneous tissue. Specimens of skin were incubated for 2 hours at 37°C in 0.7%EDTA (ethylenediaminetetraacetic acid). After this treatment, the epidermis could be readily separated from the dermis with fine forceps. After the epidermal sheet was treated with FITC-labell anti-DNP, it was frozen and cut at 4μm on cryostat.

The intact or DNCB painted ears were split as described above. Specimens of the skin were incubated in a 0.02% solution of EDTA at room temperature for 10 minutes and in a 0.25% solution ot trypsin in PBS at 4°C overnight. The dermis was peeled away with fine forceps and the epidermis was diced into fragments 1-2mm^2. The pieces were incubated for 20 minutes in 0.25% trypsin solution at 37°C. Cell aggregates and debris were removed from single cells suspension by filtration through a double layer of guaze. The cells, pelleted by centrifugation at 200G for 6 minutes, were resuspended in MEM containing 10%FCS. The epidermal single cells obtained from intact ears were incubated *in vitro* in 30mM solution of 2,4-dinitrobenzene sulfonic acid sodium salt ($DNBSO_3$ Na) in PBS for 1 hour at 37°C and washed in PBS.

Immunofluorescent method : Unfixed frozen sections, epidermal sheet and single cells were washed three times with MEM containing 10%FCS and incubated with 10% normal rabbit sera. Thereafter these specimens were washed and incubated with 50μg/ml of FITC labelled anti-DNP for 60 minutes at 37°C. The specimens were washed 3 times in PBS and mounted in glycerine buffer. Fluorescence was examined with Nikon Fluorescence Microscope using UV filter system. Percentage of staining cells in the single cells was determined by examination of the microscope field in fluorescent light and in conventional light alternation.

For control, the blocking test by unlabelled antisera and specific antigen were carried out.

RESULTS

Unfixed frozen sections prepared from the ear skin of normal guinea pigs 3 hours after surface application of 5%DNCB in ethanol were stained with fluorescent anti-DNP. The specific fluorescence was observed in the epidermis

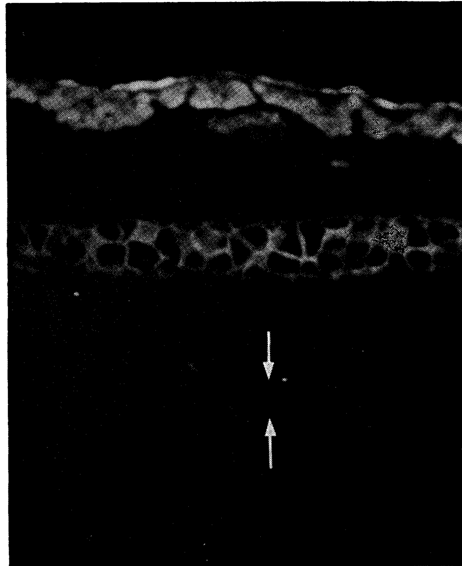

Fig. 1. Cryostat section of ear skin obtained from guinea pig 3 hours skin painting with 5% DNCB ethanol solution followed by treated with FITC labelled anti-DNP. Fluorescence is noted diffusely in the epidermis, but not in the other side of ear which was not applied with DNCB (arrows, ×150).

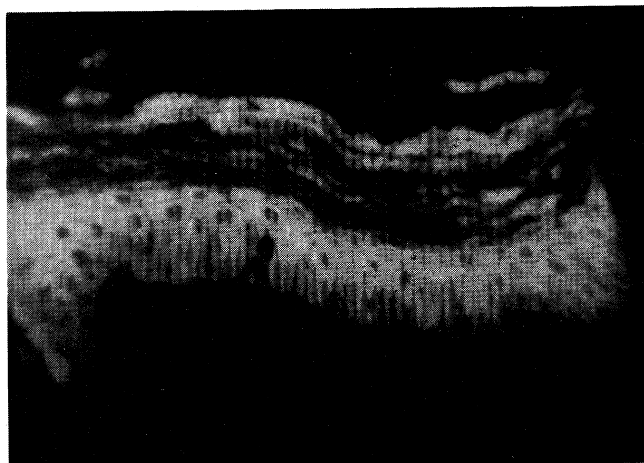

Fig. 2. Fluorescence is distributed in areas corresponding to cytoplasm and cell membrane of Malpigian cells. Horny layer is partially fluorescent (×300).

of DNCB-painted skin, but not in the other side of ear which had not been applied with DNCB (Fig. 1). Fluorescence was distributed diffusely in the Malpigian layer and observed in the areas corresponding to the cytoplasm of epidermal cells and cell membrane. The nuclei of the epidermal cells were not stained (Fig. 2). Horny layer was partially fluorescent.

In the epidermal sheet prepared from DNCB painted ear skin, the epidermis was diffusely stained and DNP-group was also distributed in the areas corresponding to cytoplasm and cell membrane (Fig. 3). The sections were

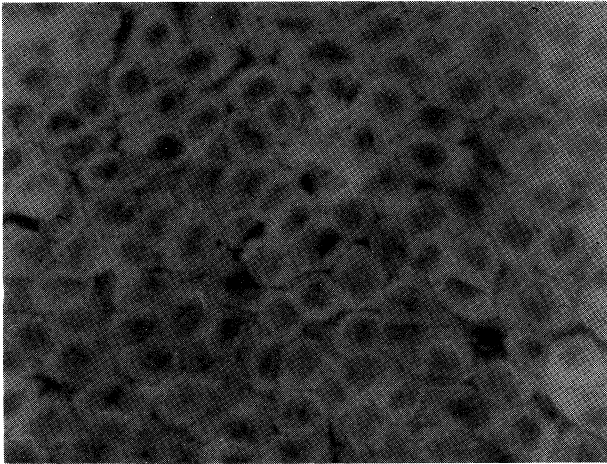

Fig. 3. Epidermal sheet prepared from DNCB painted ear skin and followed by treated with FITC labelled anti-DNP. Fluorescence is observed in areas corresponding to cytoplasm and cell membrane (×300).

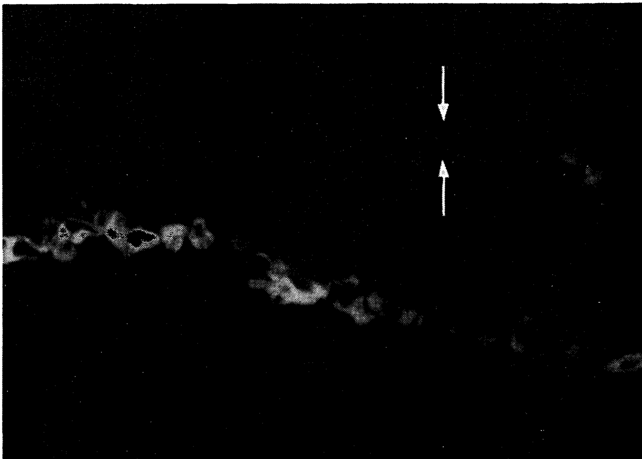

Fig. 4. Cryostat section prepared from epidermal sheet which had been treated with FITC anti-DNP and frozen. The lowest layer of epidermis is stained. Note no fluorescence in horny layer (arrows).

prepared from the epidermal sheet which had been treated with fluorescent anti-DNP and thereafter frozen. DNP-group distribution was only seen in the lowest layer of epidermis as shown in Figure 4.

Specific fluorescence was seen in peripheral or homogenous patterns in the single cells which were prepared from DNCB painted ear skin (Fig. 5). Percentage of staining cells was 80.8% on an average, and the viability rate of the cells

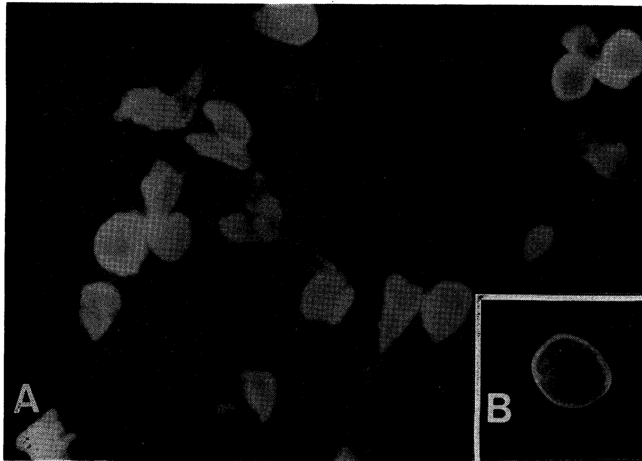

Fig. 5. Single cells prepared from DNCB painted ear skin and followed by treated with FITC labelled anti-DNP. Fluorescence is seen in homogenous or peripheral patterns (A, B).

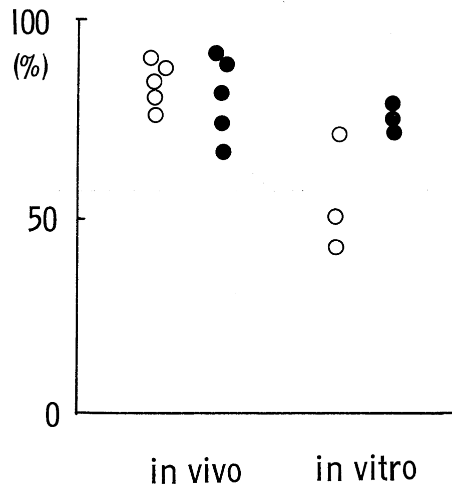

Fig. 6. Frequencies of DNP cells in epidermal single cells prepared from guinea pig ear skin 3 hours after painting with 5%DNCB-ethanol solution, *in vitro* or incubated with 30mM solution of $DNBSO_3$ Na, *in vitro* (closed circles), and viability rates of these cells (open circles).

was 83.8% (Fig. 6). On the other hand, the frequency of them in the single epidermal cells which had been incubated *in vitro* in 30mM solution of $DNBSO_3Na$ was 80.3% and viability rate was 55.3%.

DISCUSSION

The cryostat sections and sheet preparations of epidermis obtained from ear skin of guinea pigs 3 hours after painting with 5%DNCB-ethanol solution were examined by using fluorescent anti-DNP antibody. DNP-group were demonstrated on the almost 100% of Malpigian cells of the epidermis. Partial distribution of the groups was seen in horny layer. Previous work[4] has shown that the localization of DNP groups in the horny layer occurs mainly 6 hours or later after skin painting with DNCB.

When immunofluorescent technique was carried out on the single cells prepared from the epidermis of DNCB painted ear skin, the number of cells on which DNP groups detected (DNP cell) was 80.2% on an average and was roughly the same as the single cells incubated *in vitro* with 30mM solution of $DNBSO_3Na$. The distribution of DNP cells was not shown on approximately 20% of them. It is reasonable to suggest that most part of the cells without DNP group are horny cells.

The frequency of DNP cells in epidermal cells incubated with 30mM $DNBSO_3Na$ solution was also similar to those in lymph node cells conjugated *in vitro* with 30 mM $DNBSO_3Na$ solution[7]. It was shown that the percentage of DNP cells was proportional to the concentration of $DNBSO_3Na$[7].

Important questions raised by this study are whether the epidermal cells with DNP groups play a role in induction of contact sensitivity and if so, what role they do. It has been demonstrated that contact sensitization can be achieved by injection of single cells of epidermis conjugated *in vitro* with sensitizers[1-3]. It is suggested that epidermal cells taken from DNCB painted skin can be also effective immunogen. Further studies have to be done in this experimental area.

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