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Two-Color Analysis of Epidermal Cell Suspension of Mouse with Anti-DNP and Anti-Thy-1 Antibodies after Skin Painting with DNCB

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ABSTRACT. The epidermal cell suspensions prepared from DNCB painted ear skin of C3H/He mice were double-stained for Thy-1 protein and DNP groups. The cells with both Thy-1 alloantigen and DNP groups in the specimen treated with anti-DNP and followed by anti-Thy-1,2 incubation were detected more frequently than the cells treated with the antibodies in the reverse order. This suggests that DNCB is coupled *in vivo* to the Thy-1 alloantigen on the surface of Thy-1 positive cells.

Key words : Thy-1 positive epidermal cell — Contact sensitivity — Dinitrophenyl group — DNCB

The mammalian epidermis is a heterogeneous epithelium which is composed of ontogenetically and functionally diverse cell populations: keratinocytes, Langerhans cells, intermediate cells, melanocytes, Merkel cells and Thy-1 positive cells. Our previous investigations,[1-3] in which localization of 2,4-dinitrophenyl (DNP) groups in the skin of guinea pigs or mice following painting the skin with 2,4-dinitrochlorobenzene (DNCB) was examined by the immunofluorescent (IF) method using antibody against DNP groups in order to clarify the mechanism of contact sensitivity, showed that DNP groups were distributed on not only keratinocytes but also Langerhans cell and Thy-1 positive cells. The object of the experiment reported here was to examine the relation of DNP groups and Thy-1 alloantigen on the surface of Thy-1 positive epidermal cells.

MATERIALS AND METHODS

Male C3H/He mice, 10 to 15 weeks old, were painted with 0.05 ml of 0.5% DNCB-ethanol solution on ear skin. The ears were obtained 30 minutes after application. Epidermal cell suspensions were prepared from the ear skin as described previously.[3] Smear sections of the single cells were washed three times in PBS and exposed to 1:100 dilution (PBS) of biotin-anti-Thy-1,2 at 4°C over night, followed by a 25-minute incubation with 1:20 dilution of phycoerythrin (PC)-avidin at room temperature. After PBS washes, the cells

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were reacted with 50 μg/ml FITC-anti-DNP antibody for 30 minutes at 37°C, followed by final washes. The specimens were mounted in glycerine buffer. Fluorescence was examined with a Nikon Fluorescence Microscope using a UV filter system.

In the next experiment, two smear sections of single epidermal cells were prepared from each mouse painted with DNCB. A section was exposed to biotin-anti-Thy-1,2 and PE-avidin, followed by incubation with FITC-anti-DNP. Another specimen was treated in the reverse order.

RESULTS

The EC suspensions prepared from DNCB painted ear skin of mice were double-stained for Thy-1 protein (orange) and DNP groups (green) in this order. Incidence of ECs with DNP groups only (DNP^+) was 82.4% on an average (Table 1). 2.0% on an average of the ECs were stained intensely only for Thy-1 protein ($Thy\text{-}1,2^+$), and DNP groups were simultaneously detected with Thy-1,2 on 1.3% of the ECs. The incidence of the cells with two colors in $Thy\text{-}1,2^+$ ECs was only 39.0±5.6%.

A smear section prepared from DNCB painted ear skin was next incubated in biotin-anti-Thy-1,2 and PE-avidin, followed by treatment with FITC-anti-DNP. Another section from the same skin was treated in the reverse order. Two-color cells in the section incubated in the antibodies in the latter order was more frequent than the cells treated with them in the former order (Table 2). There was no significant difference in the incidences of DNP^+ ECs and $Thy\text{-}1,2^+$ ECs between both sections.

TABLE 1. Two-color analysis of epidermal cell suspension of C3H/He mouse with anti-DNP and anti-Thy-1,2 antibodies after skin painting with DNCB

Antigens	Positive cells (%)
	(Mean±S.E.)
DNP^+ only	82.4±1.8
$Thy\text{-}1,2^+$ only	2.0±0.2
DNP^+ + $Thy\text{-}1,2^+$	1.3±0.2
none	14.3±1.8

Data from 6 animals

TABLE 2. Mean frequency (%) and standard error of DNP^+ and $Thy\text{-}1,2^+$ cells in epidermal cells of mice following skin painting with DNCB

Antigen	Treatment	
	Anti-Thy-1,2 → Anti-DNP	Anti-DNP → Anti-Thy-1,2
DNP^+ only	83.7±2.9*	82.7±2.3
$Thy\text{-}1,2^+$ (including DNP^+)	1.8±0.1	1.7±0.1
DNP^+ in $Thy\text{-}1,2^+$	52.8±9.6	77.8±3.4

* Data from 3 animals

DISCUSSION

In the studies reported here, the distribution of DNP groups on ECs prepared from the skin of mice following painting with DNCB were analysed using fluorescent antibody as the green-fluorescent stain for DNP groups and biotinylated antibody counter-stained with PE-avidin as the orange one for Thy-1 protein. Only 39% on an average of the cells with orange fluorescent stain (Thy-1,2$^+$) showed simultaneously green one (DNP$^+$) when the cells were treated firstly with anti-Thy-1,2 and then with anti-DNP. On the other hand, the frequency of Thy-1,2 negative ECs with DNP groups in whole ECs was 82.4%. Regarding the mechanism of this discrepancy, it is possible to offer two explanations which fit the data : 1) DNCB does not react as readily with the components of Thy-1,2$^+$ cells as with those of the other ECs, and 2) binding of the cells with anti-Thy-1 disturb the anti-DNP in reacting with Thy-1$^+$ cells. Thy-1,2$^+$ cells may be more frequent in fact than they look. The latter possibility was next examined.

The order of treatment with anti-Thy-1 and anti-DNP was found to yield the difference in the incidence of Thy-1,2$^+$ cells with DNP groups. Two-color cells in the specimens treated with anti-DNP and followed by anti-Thy-1 incubation were detected more frequently than the cells treated with the antibodies in the reverse order. This shows the possibility that DNCB is directly coupled *in vivo* to the Thy-1 alloantigen on the surface of Thy-$^+$ cells. Further studies are required in these experimental areas.

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