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Brief Note

Antigen in Contact Sensitivity : VI. Immunofluorescent Study on the Distribution of DNP Groups on Langerhans Cells and Dendritic Thy-1 Positive Cells in Epidermis of Mice Following Skin Painting with DNCB

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Key words : DNCB — Contact sensitivity — Epidermal Langerhans cell — Epidermal Thy-1 positive cell — Antigen distribution — Immunofluorescence

According to the hypothesis of mechanism in induction of contact sensitivity (CS), hapten applied to human or animal skin enters the skin where it binds skin components and becomes a complete antigen. This complete antigen is recognized by immunocompetent lymphocytes. The recognition by the lymphocytes requires the initial uptake and processing by a macrophage-like antigen presenting cells that present immunologically relevant moieties to the lymphocytes. Although the exact nature of this antigen has not been determined, recent reports have focused on the importance of epidermal components with respect the formation of complete antigen as carrier substances.[1-4] The mammalian epidermis is a heterogeneous epithelium which is composed of ontogenetically and functionally diverse cell populations; keratinocytes, melanocytes, Langerhans cells and Merkel cells. Recently a new small subpopulation (0.8 to 2.7%) of epidermal cells was discovered in mice and shown to have abundant expression of the Thy-1 alloantigen on the cell surface.[5,6]

Our previous investigation,[7] in which localization of 2,4-dinitrophenyl (DNP) groups in the skin of guinea pigs following painting with 2,4-dinitrochlorobenzene (DNCB) was examined by immunofluorescent method using anti-DNP antibody, showed that DNP groups were distributed on/in epidermal cells. The cells on which DNP groups were detectable (DNP cell) were shown to account for approximately 90 per cent of the epidermal cells.[8] This indicates that the majority of the DNP cells are keratinocytes. The object of the experiments reported here is to determine whether DNP groups are also localized on LC and dendritic Thy-1 positive cells (Thy-1 cell) in the epidermis of mice following skin painting with DNCB. The immunofluorescent study using antibodies against DNP groups and I-A or Thy-1 antigens were carried out for this purpose.

Anti-DNP antibody was prepared and labeled with fluorescein isothiocyanate (FITC-anti-DNP) as described previously.[8] Monoclonal anti-mouse I-A^k antibody (anti-I-A^k) and monoclonal antimouse Thy-1,2 antibody (anti-Thy-1,2) were purchased respectively from Cadarlane Lab. Limited and Miles Lab. Inc,. Tetramethylrhodamine isothiocyanate labeled anti-mouse IgG/IgM antibody (TRITC-anti-mouse IgG/IgM) was obtained from Tago Inc. C3H/He mice were painted with 0.05 ml of 0.5 or 5% DNCB ethanol solution on ear skin. The ears were obtained 10 minutes after application and frozen in acetone dry

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ice chamber (-70°C) immediately after that. Epidermal cell suspensions were also prepared from the DNCB painted tail skin as described by Stingl *et al.*[9]

To determine whether one cell type expressed both DNP groups and I-A^k or Thy-1,2 antigens, unfixed frozen sections and single epidermal specimens were first exposed to anti-I-A^k or anti-Thy-1,2 followed by TRITC-anti-mouse IgG/IgM and then to FITC-anti-DNP. Figures 1 and 2 were obtained by

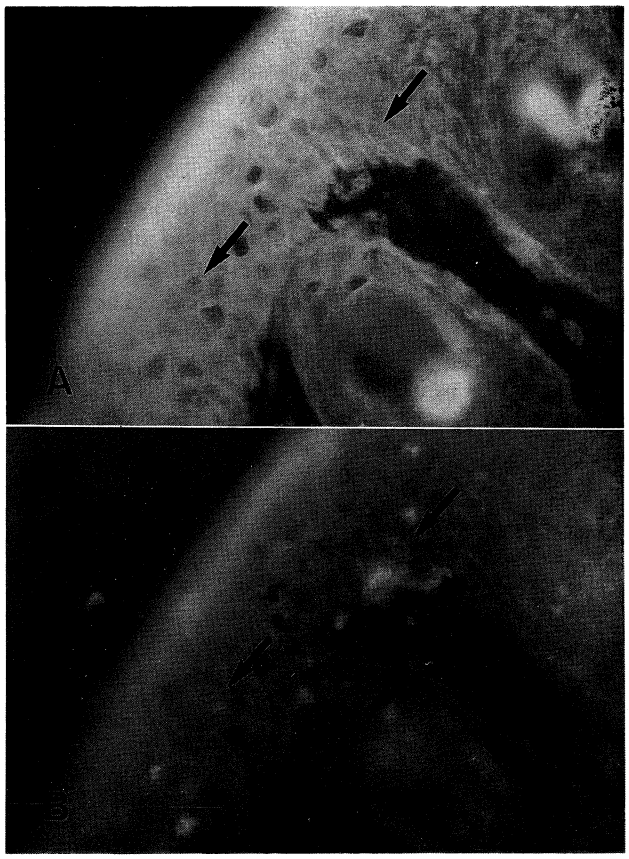

Fig. 1. Unfixed frozen section prepared from DNCB painted C3H/He mouse skin was exposed first to anti-I-A^k followed by TRITC-anti-mouse IgG/IgM and then to directly FITC-anti-DNP. A, Fluorescein excitor shows DNP group distribution. The cells with I-A^k antigen exhibited DNP groups (arrows). B, Rhodamine excitor demonstrates I-A^k positive cells (Langerhans cell, arrows).

photographing a single field, first using the rhodamine excitor, then using the fluorescein excitor. They illustrate that both DNP groups and I-A^k or Thy-1,2 antigen occurred simultaneously on one cell. This indicates that DNP groups are distributed on/in I-A^k positive or Thy-1,2 positive epidermal cells. I-A positive epidermal cells were shown to be LC.[10,11] The result supports a possibility that DNCB bound LC stimulates immunocompetent lymphocytes, and the

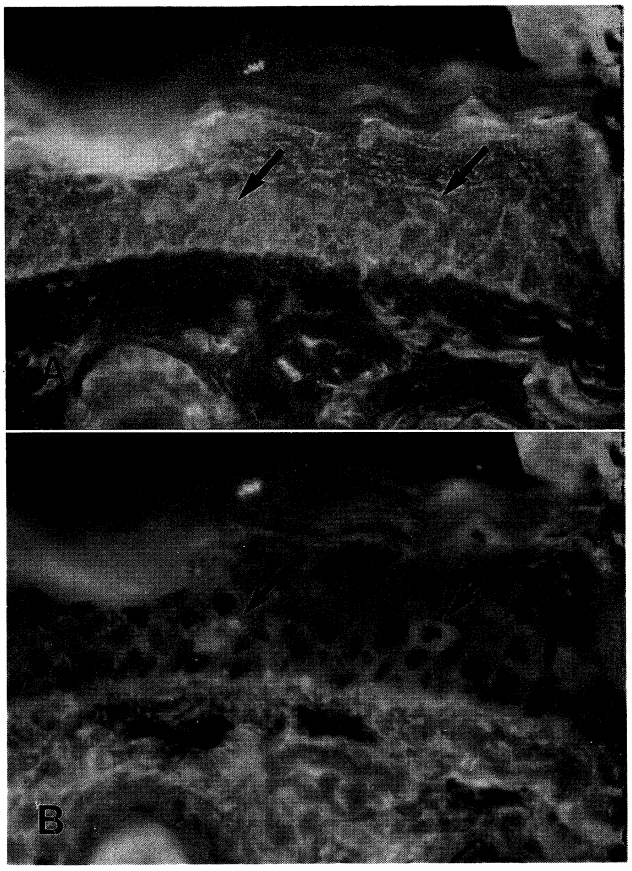

Fig. 2. Unfixed frozen section obtained from DNCB painted C3H/He mouse skin was exposed first to anti-Thy-1,2 followed by TRITC-anti-mouse IgG/IgM and then to directly FITC-anti-DNP. A, Fluorescein excitor shows DNP groups. The cells with Thy-1,2 antigen exhibited DNP groups (arrows). B, Rhodamine excitor demonstrates Thy-1,2 positive cells (arrows).

lymphocytes, after being stimulated by the LC, proliferate and differentiate into effector cells.[4] Mice in consequence become hypersensitive to DNCB. As the function of the Thy-1 positive epidermal cells has not be clarified yet,[12] the role of the DNCB bound Thy-1 positive cells in induction of CS is not clear. Further studies must be done in these experimental areas.

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