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Brief Note
Antigen in Contact Sensitivity : V. Immunofluorescent Study on the Distribution of DNP Groups on the Epidermal Langerhans Cells of Guinea Pigs Following Skin Painting with DNCB

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Key words : DNCB — Contact sensitivity — Epidermal Langerhans cell — Antigen distribution — Immunofluorescence

According to the hypothesis of mechanism in induction of contact sensitivity (CS), hapten applied to the 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 lymhocytes requires the initial uptake and processing by a macrophage like antigen

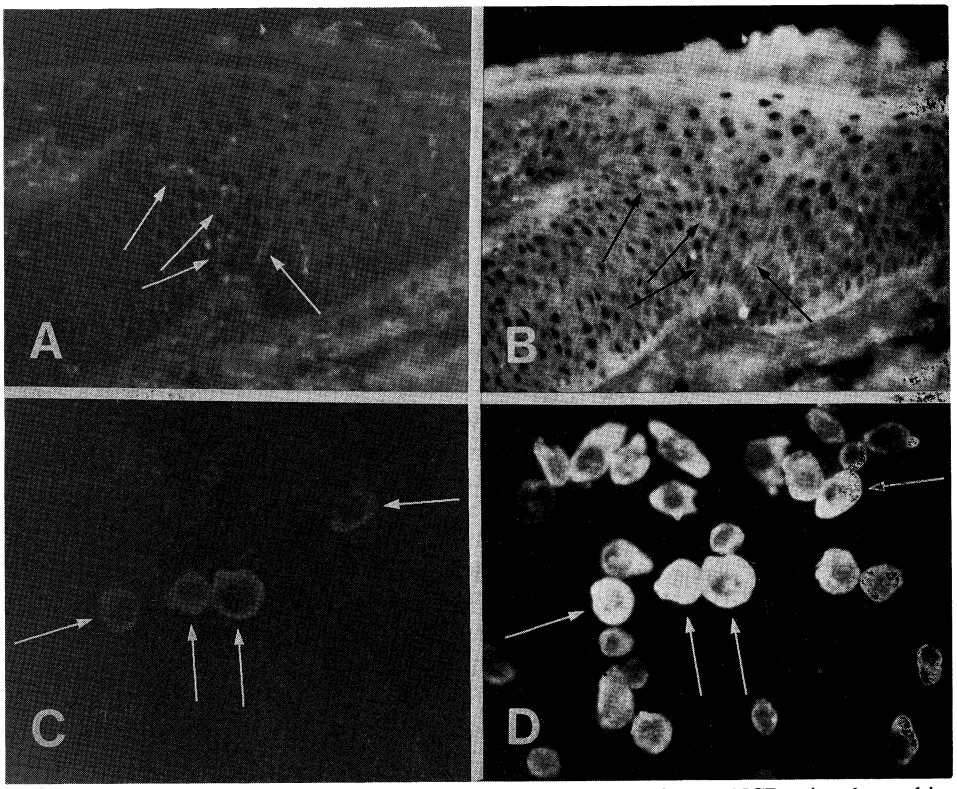

Fig. 1. Unfixed frozen section and single epidermal specimen from DNCB painted ear skin were exposed first to anti-Ia followed by TRITC-anti-Gp IgG and then to directly FITC-anti-DNP. A and C, Rhodamine excitor demonstrates Ia positive cells (LC) (arrows). B and D, Fluorescein excitor shows DNP groups distribution. The cells with Ia antigen exhibited DNP groups (arrows in B and D).

岡　大介，中川昌次郎，大田知子，植木宏明

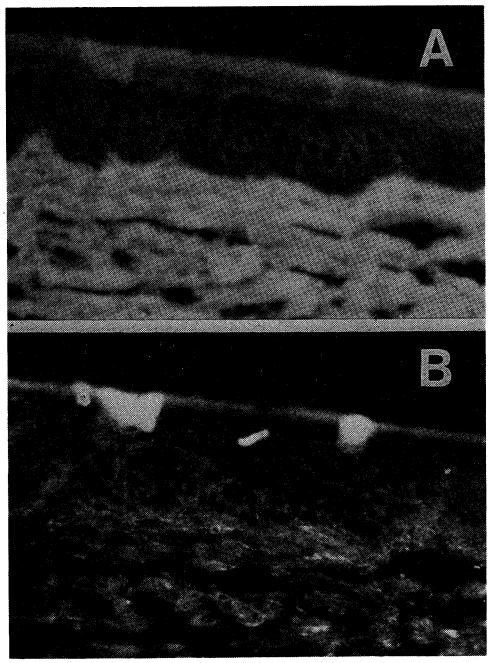

Fig. 2. Experimental controls. The unfixed frozen section from strain 2 guinea pig skin painted with 3% oxazolone-ethanol solution was treated stepwise with anti-strain 13 Ia serum followed by TRITC-anti-Gp IgG and then FITC-anti-DNP. Neither rhodamine excitor (A) nor fluorescein excitor (B) show specific staining in the epidermis.

presenting cells that present immunologically relevant moieties to the lymphocytes. Although the exact nature of this antigen has not been determined, recent evidences have focused on the importance of epidermal components with respect to formation of a complete antigen as carrier substance.[1–3] Some studies suggest that the carrier substance may be surface proteins of Langerhans cell (LC), which comprises a small subpopulation (2 to 8%) of epidermal cells.[4]

Our previous investigation,[5] 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 detectable DNP groups (DNP cell) were shown to account for approximately 90 per cent of the epidermal cells.[6] This indicates that the majorities of the DNP cells are keratinocytes. The object of the experiment reported here is to determine whether DNP groups are also localized on epidermal LC of guinea pigs following skin painting with DNCB. The immunofluorescent study using antibodies against DNP groups and Ia antigens were carried out for this purpose.

Anti-DNP antibody was prepared and labeled with fluorescein isothiocyanate (FITC-anti-DNP) as described previously.[6] Strain 2 guinea pig anti-strain 13 Ia antiserum (anti-Ia) was prepared according to the procedure described by Chiba *et al.*[7] Tetramethylrhodamine isothiocyanate labeled anti-guinea pig

IgG (TRITC-anti Gp IgG) was obtained from Cappel Laboratories Inc.

Strain 13 guinea pigs were painted with 0.05 ml of 5% DNCB ethanol solution on ear skin. The ears were obtained 10 minutes after application and frozen in acetone dry ice chamber (−70°C) immediately after that. Epidermal cell suspensions were also prepared from the DNCB painted ear skin as described by Stingl *et al.*[8]

To determine whether one cell type expressed both DNP groups and Ia antigen, unfixed frozen sections and single epidermal specimens were first exposed to anti-Ia followed by TRITC-anti-Gp IgG and then to FITC-anti-DNP. Figure 1 was obtained by photographing a single field, first using the rhodamine excitor, then using the fluorescein excitor. It illustrates that both DNP groups and Ia antigen occurred simultaneously on one cell. This indicates that DNP groups are distributed on/in Ia positive epidermal cells which were shown to be LC,[9,10] and supports a possibility that DNCB bound LC stimulates immunocompetent lymphocytes.[4] The lymphocytes, after being stimulated by these LC, proliferate and differentiate into effector T cells, and the guinea pigs in consequence become hypersensitive to DNCB. Further studies must be done in this experimental area.

Daisuke OKA, Shojiro NAKAGAWA,
Tomoko OOTA and Hiroaki UEKI
Department of Dermatology,
Kawasaki Medical School,
Kurashiki 701-01, Japan

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