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Brief Note
Antigen in Contact Sensitivity : IV. Electron Microscopic Study by Peroxidase Conjugated Antibody Technique on the Distribution of DNP Groups on the Epidermal Cells of Guinea Pigs Following Skin Painting with DNCB

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Key words : Contact sensitivity — Epidermal cell — DNCB — Antigen distribution — Peroxidase conjugated antibody technique

The true nature of the carrier is more controversial. Since contact sensitivity is induced and elicited by an epicutaneous application of the hapten, skin proteins, especially epidermal proteins, may be considered as the most likely carriers. Previous investigation[1] 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 scanning immunoelectron microscopy, showed that DNP-groups were distributed diffusely on the surface of epidermal cells. The purpose of this report is to confirm morphologically keratinocytes by using electron microscopically peroxidase labelled antibody technique, on which DNP-groups are localized.

Male Hartley strain guinea pigs weighing 350-450 g were painted with 0.05 ml of 5% DNCB-ethanol solution on the both sides of ear skin. The ears were obtained 3 hours after painting and epidermal cell suspensions were prepared using 0.5% trypsin solution. The epidermal cells were incubated with anti-DNP antibody (rabbit) at 37°C for 30 minutes as described previously.[2] After washing with phosphate buffer saline (PBS, pH 7.2) three times, the cells were incubated with peroxidase labelled anti-rabbit IgG (Goat) at 37°C for 30 minutes. The cells were washed 3 times in PBS and cell pellets were fixed in 2.5% glutaraldehyde for 1 hour at room temperature. After two additional washes (in PBS and then in Tris HCl buffer ph7.2), the cells were incubated with Graham Karnovsky medium (0.1% 3,3-diaminobenzidine in Tris HCl containing 0.003% hydrogen peroxide) for 15 minutes at room temperature. The cells were washed 2 more times in PBS and were postfixed in 2% osmium tetraoxide for 1 hour at 4°C. Dehydration was carried out in a grade series of ethanol solution and embedded in Epon 812. Thin sections examined under a Hitachi H-500 electron microscopy. The experiments were controlled by conventional blocking techniques using antibody and antigen.

Continuous reaction products were found on the surface of keratinocytes (Fig. 1). The similar distribution of DNP groups on the epidermal cells has also been shown by using immunoferritin electron microscopy.[3] Whether DNP groups distributed on the surface of keratinocytes play actually an important role as antigen in contact sensitivity remains to be clarified.

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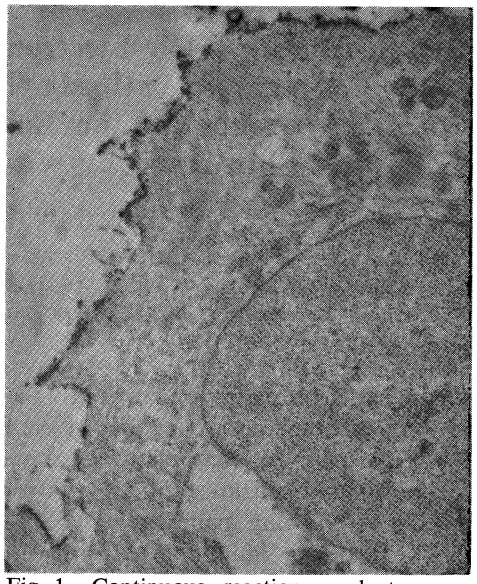

Fig. 1. Continuous reaction products were found on the surface of keratinocyte.

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REFERENCES

1) Oka, D., Nakagawa, S., Ueki, H., Kumon, H. and Ohmori, H. : Antigen in contact sensitivity : II. Scanning immunoelectron microscopic studies on the distribution of DNP groups on the epidermal cells of guinea pigs following skin painting with DNCB. J. Dermatol. **11** : 15-19, 1984
2) Nakagawa, S., Nakaye, M., Oka, D., Takei, Y. and Ueki, H. : Ultrastructural localization of DNP groups on draining lymph node cells of guinea pigs following skin painting with DNCB : II. Immunoferritin electron microscopic study. Kawasaki Med. J. **9** : 181-186, 1983
3) Oka, D., Nakagawa, S. and Ueki, H. : Antigen in contact sensitivity : III. Immunoferritin electron microscopic study on the distribution of DNP groups on the epidermal cells of guinea pigs following skin painting with DNCB. Kawasaki Med. J. **9** : 203-204, 1983