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Brief Note

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

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Key words : contact sensitivity — epidermal cell — DNCB — antigen distribution — immunoferritin electron microscopy

Despite much experimental efforts by various workers on the induction of contact sensitivity, its mechanism is still unclear. It has been generally considered that small molecular weight contactants must combine with carrier proteins to form complete antigens. Since the epicutaneous application of contactants can induce contact sensitivity, skin proteins, especially epidermal

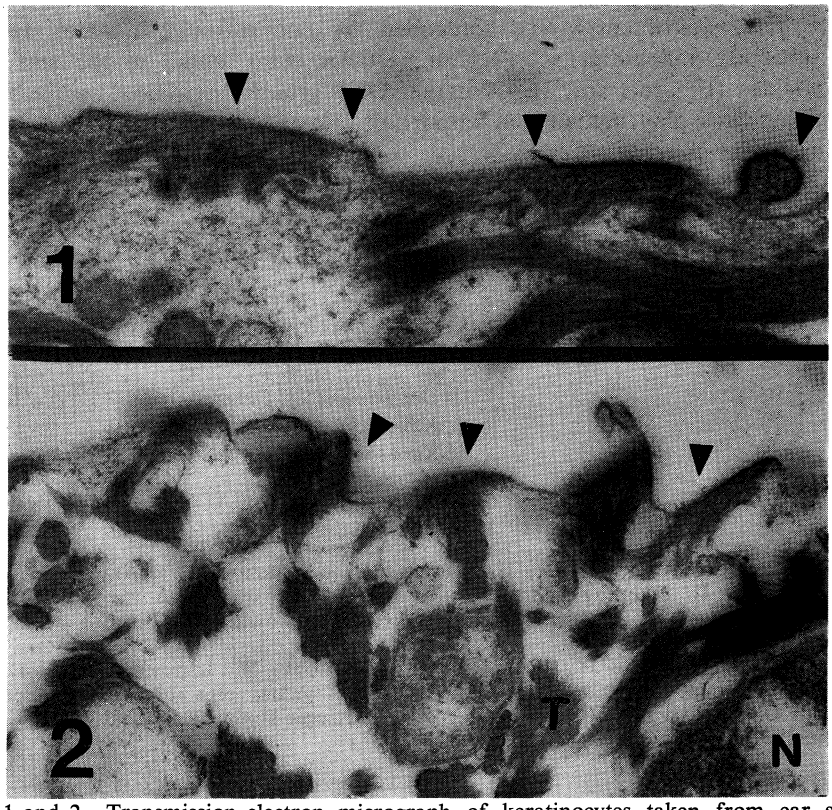

Fig. 1 and 2. Transmission electron micrograph of keratinocytes taken from ear skin of guinea pig 3 hours after painting with DNCB incubated with ferritin-anti-DNP conjugate. Note ferritin particles on the surface of the cells (arrows). T, tonofilament N, nucleus.

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proteins, may be considered as the most likely carriers.

Previous investigations[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 the experiment in this report is to confirm morphologically keratinocytes by using immunoferritin electron micrscopy, on which DNP groups are localized.

Male Hartley strain guinea pigs weighing 350–450 g were painted with 0.05 ml of a 5 per cent DNCB-ethanol solution on both sides of ear skin. The ears were obtained 3 hours after painting and epidermal cell suspensions were prepared using 0.5% solution of trypsin. The epidermal cells were incubated with ferritin labelled anti-DNP antibody (rabbit) at 37°C for 30 minutes as described previously.[2] After washing with phosphate buffer saline three times, cell pellets were fixed in 2.5% glutaraldehyde and then 2% osmium tetraoxide for 1 hour at 4°C respectively, dehydrated in graded ethanol and embedded in Epon 812. Thin sections were stained with methanolic uranyl acetate and lead citrate and examined under a Hitachi H-500 electron microscopy. The experiments were controlled by conventional blocking technics using antibody and antigens. Ferritin particles were found on the surface of keratinocytes (Figure 1 and 2). 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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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** (in press)

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** (in press)