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Brief Note

The Induction of Tolerance in Contact Sensitivity by the Injection of the Regional Lymph Node Cells from Mice 1 Day after Painting the UV-B Irradiated Skin with DNFB to Recipients

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Key words : Contact sensitivity — Tolerance — Regional lymph node — DNFB — ultraviolet ligh-B

Contact sensitivity has been found to be produced by taking the regional lymph node cells (RLNC) from mice one to three days after painting the skin with oxazolone and injecting them into recipients.[1—3] On the other hand, when mice are painted with 2,4-dinitrofluorobenzene (DNFB) on the skin depleted of epidermal Langerhans cells by ultraviolet light (UVL) irradiation and subsequently challenged with the allergen, they not only become hyposensitive but also tolerant to attempts at sensitization with DNFB.[4,5] In the preliminary experiment reported here, we would like to report that the RLNC taken from the mice painted the UVL irradiated skin with DNFB 1 day after painting were capable of inducing tolerance of contact sensitivity to DNFB in the recipients injected with the cells.

C3H/HeN mice were obtained from Japan Cler Inc. The 8-to 12-week-old mice were age-matched. 0.5% DNFB ethanol solution (25 μl) was painted to normal or ultraviolet light-B (UV-B) irradiated inguinal skin of mice. Both sides of inguinal skin was exposed for 90 seconds on each day of 4 successive days. The irradiance of UV-B (280 to 370 nm) was 270 mJ/cm^2 each day. The draining lymph nodes were taken 1 day after painting and cell suspensions were prepared by teasing them in PBS (0.01M phosphate buffer saline, pH 7.2). The cells were washed in PBS three times and injected subcutaneously to footpads of recipient mice (1×10^7 cells). Contact sensitivity was tested by contact with 0.2% DNFB ethanol solution on ear 6 days later on one side of ear. Twenty-four hours after antigen challenge, the degree of ear thickness was measured and results were expressed the increase of thickness (ear increment). Positive controls consisted of normal mice sensitized on body wall skin in the manner described. Negative controls consisted of unsensitized mice whose ears were challenged with the appropriate antigen. Each panel consisted of 5 age- and sex-matched animals. To assess whether animals were tolerant, two groups of animals were painted with the sensitizing dose of 0.5% DNFB ethanol solution (25 μl) on normal axillar skin after reading contact reactions. Six days after that, the other side of ear, intact, was challenged and contact reactions were read as above.

We firstly tried to confirm the effect of UV-B treatment on ability of skin to support DNFB sensitization. DNFB application to UV-B treated skin proved a poor immunogenic maneuver (Animal group 2 in Fig. 1). We also studied whether the RLNC taken from donor mice painted the UV-B irradiated

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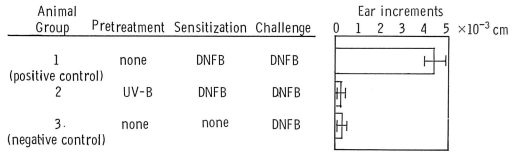

Fig. 1. Effect of UV-B skin irradiation on sensitization to DNFB. Shaved inguinal skin was exposed to UV-B on 4 successive days. The irradiated skin was painted with DNFB and ear was challenged 5 days later. Ear swelling was read on the next day. Positive controls consisted of normal mice sensitized on normal body wall skin with DNFB. Negative controls consisted of unsensitized mice whose ear was challenged with the appropriate antigen.

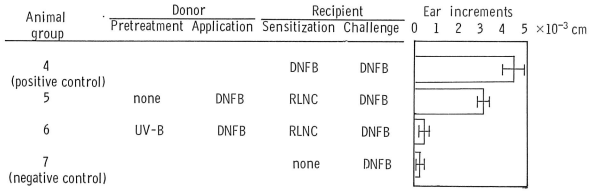

Fig. 2. The production of contact sensitivity to DNFB by injection of regional lymph node cells (RLNC), taken 1 day after painting with DNFB on to normal or UV-B irradiated skin, into the footpads of recipients.

skin with DNFB 1 day after painting have capacity to induce contact sensitivity to DNFB in recipients injected the RLNC. The injection of RLNC taken 1 day after painting with DNFB from intact animals (1d RLNC) was able to induce a contact sensitivity in the recipient animals as shown by a positive patch test reactions (Group 5 in Fig. 2). On the other hand, the donor cells from the mice which were irradiated with UV-B and then painted with DNFB to the treated skin (UV-B 1d RLNC) were significantly less effective in induction of contact sensitization (Group 6). In order to determine whether tolerance was induced in the mice that were not efficiently sensitized by application of DNFB through UV-B treated skin (Group 2) or by injection of UV-B 1d RLNC (Group 6), the animals were painted with a sensitizing dose of DNFB through normal skin. As shown in Figure 3, Group 10 was hyposensitive (50% of normal controls) and Group 9 was almost unresponsive to a second

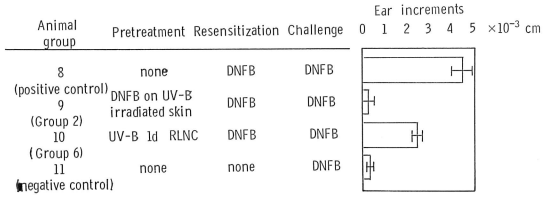

Fig. 3. Assessment of tolerance in contact sensitivity to DNFB in mice sensitized by application of DNFB through UV-B irradiated skin and by injection of RLNC taken 1 day after painting with DNFB on to UV-B irradiated skin into the footpads of recipients.

application of DNFB.

In the present experiment, it was shown that the draining lymph node cells taken from the mice painted the UV-B irradiated skin with DNFB 1 day after painting were able to induce tolerance of contact sensitivity to DNFB in recipient animal injected with the cells. Most recently, Granstein *et al.*[6] have demonstrated that murine epidermis contains antigen presenting cells (APC) that are required for activation of suppression and that these APC are resistant to UVL radiation. It might be expected that the APC contained in the lymph node draining the site of DNFB application activate the suppression system of contact sensitivity to DNFB. Further studies must be done in this experimental area.

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