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BRIEF NOTE

THE DISTRIBUTION OF DNP GROUPS IN THE DRAINING LYMPH NODES OF BALB/c AND ATHYMIC NUDE MICE FOLLOWING SKIN PAINTING WITH DNCB

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The previous data showed that within the lymph node draining site of 2, 4-dinitrochlorobenzene (DNCB) application a small population of cells were present with 2, 4-dinitrophenyl (DNP) groups on their surface in guinea pigs[1,2]. The DNP groups bearing cells (DNP cells) were detected by a immunofluorescent method using anti-DNP antibody. The draining lymph node cells were found to release *in vitro* specific DNP factors which can effectively block the anti-DNP antibody reacting with DNP groups on the lymph node cells and to cause possibly reduction of the DNP cell number in the draining nodes detectable by the immunofluorescent method[2,3]. The objects of the experiments reported here are to investigate DNP cells in athymic nude mice following skin painting with DNCB.

Animals used were BALB/c and nude mice aged two months. An application of 0.05 ml of 5 % DNCB-ethanol solution was given to both sides of inguinal skin. Inguinal lymph nodes were obtained at various time intervals after painting with DNCB, and smear sections of the lymph node cells were prepared. DNP cells were detected by the immunofluorescent method using fluorescein isothiocyanate (FITC) labelled antibody to DNP groups as described previously[2]. The percentage of the stained cells was determined by examination of the microscopic field in fluorescent light and convential light alternately. Figure 1 shows the frequences of DNP cells in regional lymph nodes of BALB/c and nude mice following skin painting with DNCB. Nude mice had clearly more DNP cells than BALB/c mice,

Mice were painted with 0.05 ml of 5 % DNCB on the inguinal skin 1 hour before harvesting the lymph node cells. The draining lymph node cells were cultured in Eagle's minimal essential medium containing 10 % foetal calf serum and antibiotics for 48 hours, as described by Zembala and Asherson[4]. *In vitro* dinitrophenylated cells were prepared by incubation of lymph node cells from normal BALB/c mice in 2.5 mM 2, 4-dinitrobenzene sulfonate in

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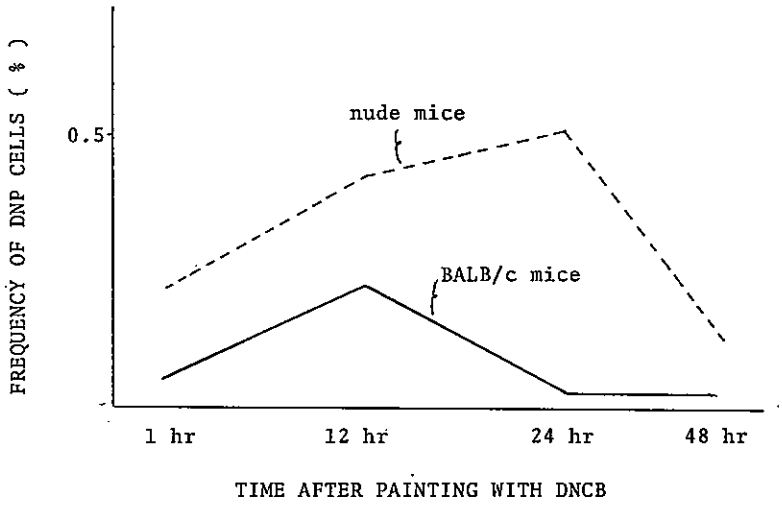

Fig. 1. Frequencies of DNP cells in the draining lymph nodes of BALB/c and nude mice at various times after painting the skin with DNCB. Mean percentages from four animals are shown.

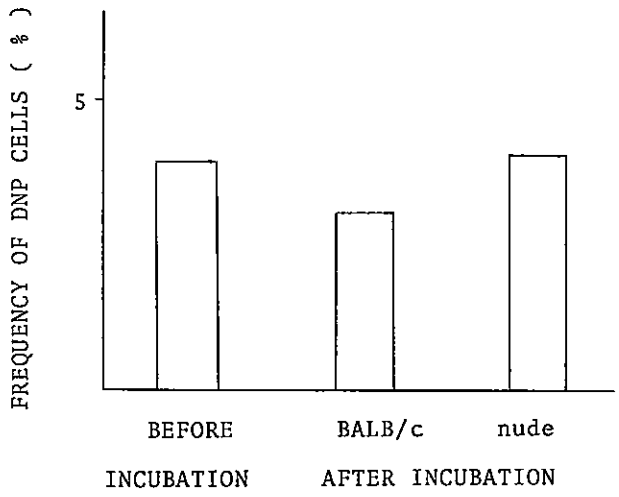

Fig. 2. Frequencies of detectable DNP cells prepared *in vitro* before and after incubating with the culture supernatants of the draining lymph node cells obtained from BALB/c and nude mice 1 hour after painting the skin with DNCB.

PBS at 37°C for 1 hour, spun down and washed sufficiently. These *in vitro* prepared DNP cells were incubated in the culture supernatants at 37°C for 1 hour, and subsequent DNP cells were assessed by the immunofluorescent method. The incubation of *in vitro* prepared DNP cells with the supernatant from BALB/c mice caused less frequent DNP cells detectable by the immunofluorescent method as compared to those before incubation or after the incubation with the supernatant from nude mice (Fig. 2). These results indicate that the draining lymph node cells from BALB/c mice liberate the specific DNP factors as guinea pigs, but those from the nude mice do not. It is reasonable to assume that T lymphocytes in the lymph node draining site of DNCB painting play some role to release the factors. It is suggested that the factors may block the immunologically competent cells recognition of DNP groups.

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