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BRIEF NOTE

ANTIGENICITY OF DNP-LYMPHOCYTE IN CONTACT SENSITIVITY

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Despite the research effort expanded so far on induction of contact sensitivity, the mechanism is still unclarified. The widely held concept that low molecular weight contactants combine with skin proteins to form a complete antigen unlikely accounts for every case of contact sensitization. Recently some investigators have claimed that a contactant will attach to viable lymphocytes *in vitro* and that the combination forms an immunogenic unit[1,4,5,9]. It has been also demonstrated that the combination actually occurs *in vivo* during contact sensitization[7]. These findings indicate that the cell membrane of lymphocyte play an important role as a carrier substance in development of contact sensitivity. In the present work, we attempted to determine the antigenicity of hapten binding lymphocyte in contact sensitivity.

Hartley strain guinea pigs were initially applied epicutaneously 0.1 ml of 50% solution of 2, 4-dinitrochlorobenzene (DNCB) in acetone to skin on the nape. Shortly thereafter, the previously painted area was injected intradermally with 0.1 ml of Freund's complete adjuvant (FCA). For control, animals were painted with 0.1 ml of 5% solution of urushiol in ethanol and injected with FCA. A cell suspension was prepared by teasing the lymph nodes of normal guinea pigs in PBS (phosphate buffer saline), lymphocytes then being separated from the suspension by sodium metrizoate Ficoll gradient centrifugation. The lymphocytes were suspended in PBS containing 2.5, 10 and 30 mM, 2, 4-dinitrobenzene sulfonic acid sodium salt ($DNBSO_3Na$). The suspensions were incubated at 37°C for 10 minutes and washed sufficiently in PBS. A part of the cell suspensions was sonicated. The *in vivo* dinitrophenylated (DNP) lymphocytes were obtained from the regional lymph nodes of guinea pigs which had

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been painted both sides of inguinal skin with 0.2 ml of 5% DNCB-ethanol solution 12 hours before as described elsewhere[7]. DNP-conjugate of the extract of guinea pig epidermis (GPE) was prepared by the procedure described previously[8]. Nine days after sensitization, 0.1 ml of 50 μg DNP-GPE and 0.1 ml of the cell suspension (6×10^5 cells) were injected intradermally on the shaved back skin. The reactions were read 24 hours later. The diameter of erythematous induration was measured in millimeters.

Figures show the means of the measurements of the skin reactions in each animal group and standard errors of the means. Positive delayed reactions were detected on the intradermal injections of either DNP-lymphocytes or DNP-GPE in the animals sensitized with DNCB and FCA (Figure 1). The histological study of the reactions to DNP-lymphocytes revealed a small mononuclear cell infiltration and vasodilatation in the dermis, in the same way as is seen in delayed sensitivity skin reaction. The differences in the reactions to either living and killed (sonicated) DNP-lymphocytes or *in vivo* and *in vitro* formed DNP-lymphocytes were

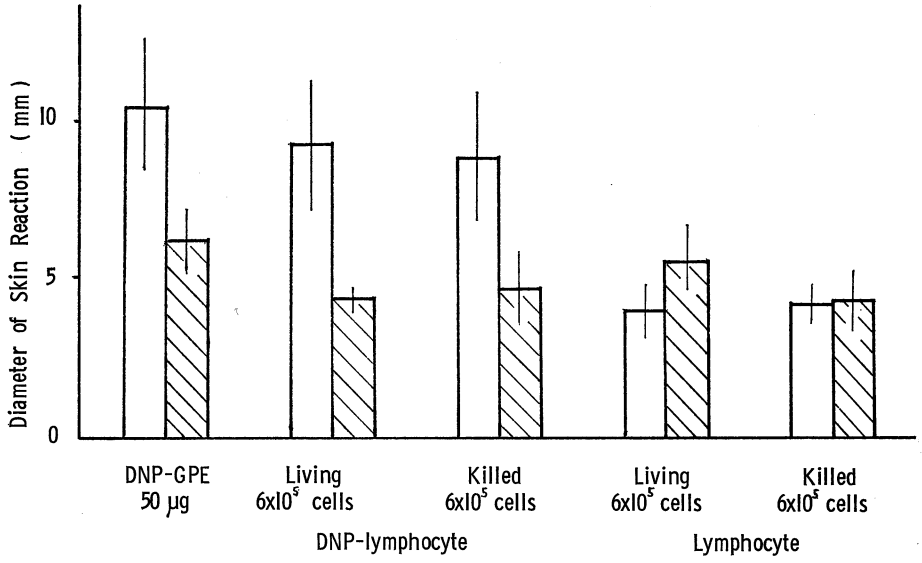

Fig. 1. Delayed reations to living and killed DNP-lymphocytes in the animals sensitized with DNCB and FCA (open columns) and in the animals with urushiol and FCA (hatched columns). DNP-lymphocytes were prepared by incubation of lymphocytes with 30 mM $DNBSO_3Na$. Results presented as the means of diameters in mm of erythematous indurations observed 24 hours after intradermal injections of antigens in each animal group and standard errors (99% confidence level).

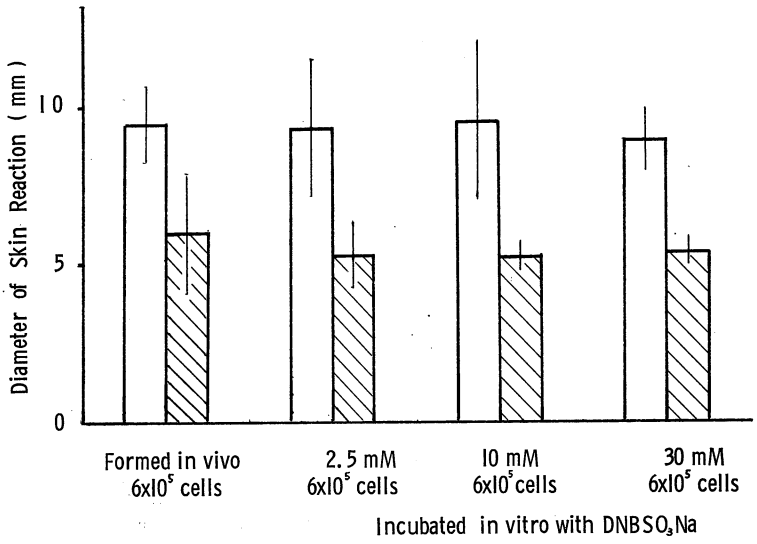

Fig. 2. Delayed reactions to *in vivo* and *in vitro* formed DNP-lymphocytes. Results presented in the same way as Fig. 1.

not statistically significant (Figure 1 and 2). The incidences of DNP-lymphocytes formed *in vitro* and *in vivo* were determined by immunofluorescent method as described previously[7]. Percentages of DNP-lymphocytes formed *in vitro* by incubation with 2.5, 10 and 30 mM $DNBSO_3Na$ were 4.1, 18.5 and 85.5% respectively. Frequency of DNP-lymphocytes formed *in vivo* was 4.8%.

The present work indicates that cellular antibodies capable of reacting with DNP-lymphocytes as well as DNP-epidermal proteins are produced in the animals with contact sensitivity to DNCB. Our results, together with the previous investigations[1-6,9], suggest that lymphocytes play an important role as a carrier substance in the development of contact sensitivity.

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