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Brief Note

Antigen in Contact Sensitivity : VII. The Induction of Contact Sensitivity and Delayed-Type Skin Reaction Using *in Vitro* Haptenated Epidermal Cells in Guinea Pigs

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Key words : DNCB — Contact sensitivity — Delayed-type skin reaction — Langerhans cell

According to the hypothesis of contact sensitivity mechanism, a hapten applied to human or animal skin enters the cutis where it binds skin components, and becomes a complete antigen. This antigen is recognized by T lymphocytes. Although the exact nature of this antigen has not been determined, recent reports have focused on the importance of epidermal components with respect to the formation of complete antigens as carrier substances. As techniques for sensitizing animals with *in vitro* haptenated epidermal cells (EC) have recently been described,[1,2] we attempted to clarify the antigens in contact sensitivity (CS) using the conjugates.

EC suspensions were prepared from inbred JY1 strain guinea pigs according to the technique described by Sting *et al.*[3] The ECs were dinitrophenylated by incubation with 20 mM 2,4-dinitrobenzene sulfonate as described previously.[4] 5×10^6 DNP-ECs were injected intradermally into both sides of the ears of JY1 guinea pigs, and skin testing with 0.2, 0.1, 0.05, 0.025% DNCB ethanol solutions was carried out 14 days later. The contact reactions were evaluated 24 hours later according to the scale described previously,[5] and the degree of hypersensitivity was taken to be the total of all four readings in each animal. Student's t-test was used to assess differences in reactivity. A p value of less than 0.05 was considered to be significant. CS was efficiently produced when DNP-ECs were administered (Group 1 in Fig. 1). Killing of DNP-EC by sonication showed a decrease of its antigenicity in CS (Group 2). DNP-ECs

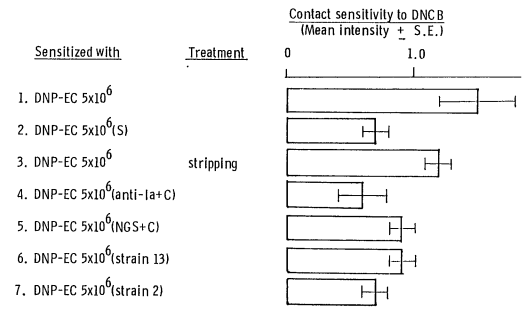

Fig. 1. The induction of contact sensitivity to DNCB by intradermal injection of DNP-ECs in JY1 strain guinea pigs. S, sonication, C, complements, NGS, normal guinea pig serum.

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were injected through skin that had been stripped with cellophane tape immediately before injection as reported previously.[5] The induction of CS to DNCB was not impaired by the treatment (Group 3). This is contrary to the result of experiment using *in vivo* dinitrophenylated EC.[5] The antigenicity of DNP-EC was declined when the ECs had been treated with anti-Ia alloantiserum and complements before their haptenation and administration (Groups 4 and 5).[5] DNP-ECs prepared from strain-13 and 2 guinea pigs also produced CS but were less effective than the DNP-ECs from JY1 strain guinea pigs (Groups 6 and 7).

The guinea pigs sensitized epicutaneous application of 5% solution of DNCB (25 μl) were tested by intradermal injection of 5×10^6 DNP-ECs on the 14th day after sensitization. Delayed-type skin reaction (DSR) was definitely detected on the intradermal injection of the conjugates 24 hours after challenge (Group 1 in Fig. 2). Sonication of DNP-EC revealed a significant decrease of its antigenicity in DSR (Group 2). DSR induction did not occur when the ECs had been treated with anti-Ia and complements before their haptenation and administration (Groups 3 and 4). DNP conjugates prepared from ECs of strain-13 and -2 guinea pigs were used for intradermal test as antigens. The animals sensitized with DNCB did not develop positive DSR to them (Groups 5 and 6).

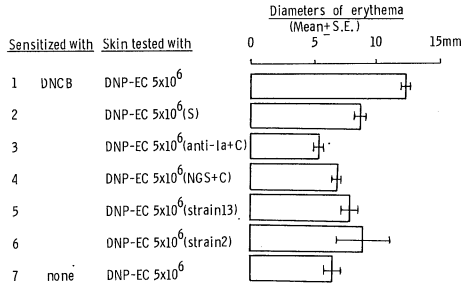

Fig. 2. Delayed-type skin reaction 24 hours after intradermal injection of DNP-EC to JY1 guinea pigs which 14 days earlier had been applied epicutaneously with 5% DNCB (25 μl).

Experiments using anti-Ia alloantisera and complement indicate that the induction of CS and DSR is at least in part due to the presence of Ia-positive cells (presumably Langerhans cells) among the DNP-ECs. Although the precise role of Langerhans cells in induction of CS has not been determined, recent reports have focused on the importance of epidermal Langerhans cells with respect to antigen presentation to immunologically competent lymphocytes.[1,6-8] The role of Langerhans cells has been also recognized in the eliciting phase of CS.[9,10] Several findings have been reported which suggest that T lymphocytes recognize the hapten molecules conjugated to membrane component in association with Ia antigen on the surface of Langerhans cells.[8,11,12] One of the important questions raised here is whether the immunologically relevant hapten determinants are covalently coupled to the Ia antigens or the hapten is coupled to different membrane antigens. We favor the latter possibility from our present observation that DNP conjugate prepared from EC of strain-

13 guinea pigs did not induce neither CS nor DSR in JY1 strain guinea pigs. Both JY1 and strain-13 guinea pigs have been shown to have common Ia guinea pig leukocyte antigens.[13] So if Ia antigen modified directly by DNP play an important role as antigen for productions of CS and DSR, DNP conjugate prepared from EC of strain-13 guinea pigs should be also effective to induce CS and DSR in JY1 guinea pigs. Further studies should be done in this experimental area.

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