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BRIEF NOTE

THE INDUCTION OF CONTACT SENSITIVITY TO DNCB WITH DNP LYMPH NODE CELLS KILLED BY SONICATION

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Abstract

Contact sensitization was achieved with dinitrophenylated autologous lymph-node cells killed by sonication as well as living cells. The sensitizing ability of the sonicated cells disappeared after freeze-thawing or heating. Significance of these findings is discussed.

Despite much experimental efforts by various workers on the induction of contact sensitivity, the mechanism still remains unclarified. The widely held concept that small molecular weight contactants must combine with skin proteins to form a complete antigen is unlikely to happen in every case of contact sensitization. Baumgarten and Geczy[1] have successfully induced a contact sensitivity to 2, 4-dinitrochlorobenzene (DNCB) in guinea pigs by injection of *in vitro* dinitrophenylated (DNP) live lymphocytes. This has been confirmed by several investigators.[2,3,4] They have claimed that the sensitization is achieved only with live lymphocytes conjugated with contactants, but not with killed lymphocytes. The objects of the experiment reported here were to show that sonicated DNP lymph-node cells were able to produce a contact sensitivity as well as the live cells.

The guinea pigs used were of the male Hartley strain (350-450g). *In vitro* dinitrophenylated cells were prepared by incubation of normal lymph node cells with 2.5, 10 and 20 mM 2, 4-dinitrobenzene sulfonate ($DNBSO_3Na$) as described elsewhere[5]. The cells were thoroughly washed in PBS (0.01 M phosphate buffer saline, pH 7.2), stained for viability and then adjusted to the desired cell count in PBS. The viability rates of 2.5, 10 and 20 mM-cells were 72, 54 and 51 per cent respectively. The numbers of DNP cells in the lymph-node cells were counted by immunofluorescent method as previously described[5]. A part of cells were killed by sonication, and the sonicated cells were then incubated at 56°C for 30 minutes or frozen at -70°C and melted at room temperature three times.

The live or killed cells were injected intradermally to ears of guinea pigs,

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and their sensitivity was tested by contact with 0.2, 0.09, 0.05 and 0.01 per cent DNCB in ethanol on the flank 14 days later. The intensity of skin reactions was assessed 24 hours after patch testing as described previously[6].

The injections of autologous live lymph-node cells which had been dinitrophenylated *in vitro* using 2.5, 10 or 20 mM $DNBSO_3Na$ produced a contact sensitivity to DNCB in the animals as shown by a positive patch test reaction (Table). The 10 and 20 mM-cells were less effective in inducing the sensitivity than 2.5 mM-cells, though the former contained DNP cells more than the latter. The sensitivity was also induced with the sonicated cells. Heating or freeze-thawing depressed significantly the ability of the sonicated cells to induce contact sensitivity.

TABLE

The Induction of Contact Sensitivity to DNCB with Autologous Lymph-Node Cells of Guinea Pigs Incubated in $DNBSO_3Na$

Lymph node cells incubated with	Cell number injected		Patch test	
	Total	DNP cells	Positive	Mean intensities
20 mM $DNBSO_3Na$	2.0×10^6	9.6×10^5	3/4	0.9
10 mM $DNBSO_3Na$	〃 〃	3.7×10^5	4/5	0.8
2.5 mM $DNBSO_3Na$	〃 〃	0.8×10^5	6/6	1.6
〃 〃 (sonicated)	〃 〃	〃 〃	4/5	1.2
〃 〃 (sonicated and frozen)	〃 〃	〃 〃	2/6	0.2
〃 〃 (sonicated and heated)	〃 〃	〃 〃	0/6	0

It has been found by Baumgarten and Geczy[1] that guinea pigs can be sensitized with live autologous lymphocytes dinitrophenylated *in vitro*, but not with DNP lymphocytes killed by heat. Asherson *et al.*[7] have also shown that cells taken from draining lymph nodes of mice 1 day after painting with oxazolone induce a state of contact sensitivity when injected into syngeneic recipients. The sensitizing effect disappears after freeze-thawing or heating. It has been thought that they are immunizing processes with the injected cells associated with haptens acting as immunogens, and that simple carry-over of antigen is not involved since killed cells are ineffective.

However, autologous DNP lymph-node cells killed by sonication were found to be able to induce contact sensitivity in the present experiment. The sensitizing ability was lost when the cell debris was frozen or heated. These findings indicate that the disappearance of sensitizing ability is not dependent upon the death of DNP cells but on freezing and heating. Miller *et al.*[8] have claimed that antigen associated with products of the major histocompatibility

complex (MHC) is important in the immunogenic lymph-node cells for contact sensitization. A possibility that the freeze-thawing and heating alter the nature of the MHC products is suggested. A relatively low number of DNP cells (2.5 mM-cell) was effective and a larger dose (10 or 20 mM-cell) was less effective in induction of sensitivity. The high concentration of $DNBSO_3Na$ may also change the nature of the products.

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