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BRIEF NOTE

CELLULAR LOCALIZATION OF DNP GROUPS IN LYMPHOID TISSUES OF GUINEA PIG FOLLOWING SKIN SURFACE APPLICATION OF DNCB

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In recent years, it was demonstrated by several workers that living lymphoid cells conjugated with simple chemical allergens *in vitro* effectively induce contact sensitivity[1-4]. This indicates that the first steps in the induction phase of contact sensitivity, namely the formation of antigen and its recognition by immunocompetent cells, are accomplished by the conjugation of hapten with lymphoid cells. However, no evidence has been presented which shows that the conjugation actually occurs *in vivo*. We have attempted to perform a histological analysis of lymphoid cells in order to detect the localization of 2, 4-dinitrochlorobenzene (DNCB) when it is introduced percutaneously into body. Fluorescein labelled antibody to 2, 4-dinitrophenyl (DNP) groups was used as a tracer for DNCB.

Female Dunkin Hartley strain guinea pigs were given an application of 0.05 ml of 5 per cent solution of DNCB in ethanol to the right inguinal skin. Both sides of the inguinal lymph node, the mesentric node, thymus, spleen and peripheral blood were obtained at various time intervals following application of DNCB. The cells prepared from these tissues and peripheral blood leucocytes were smeared on glass slides, air dried and fixed in 95 per cent ethanol. Anti-DNP antibody was specifically purified according to Eisen et al.[5] from hyperimmunized sera obtained from rabbits which had been sensitized with DNP ovalbumin as described previously[6]. It was labelled with fluorescein isothiocyanate (FITC) and had the following characteristics: FITC, 30 μg/ml, total protein, 7.5

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mg/ml, F/P, 1.64 (molar) or 4.0×10^{-3} (weight). The conjugate was diluted to less 50 μg antibody/ml and used after twice absorption with guinea pig liver acetone powders. The sections described above were investigated by direct immunofluorescent method using the prepared conjugate. For controls, blocking tests with unlabelled anti-DNP antibody and specific antigens were carried out. Sections treated with FITC labelled anti-ovalbumin rabbit serum, and lymphoid tissue cells from croton oil painted animals when examined with FITC labelled anti-DNP antibody did not show any specific fluorescence.

Fluorescent cells were observed in the lymphoid tissues and peripheral blood obtained from DNCB painted guinea pigs. The cells in most instance were lymphocytic in type exhibiting various staining patterns: homogeneous, coarsely grained or peripheral patterns (Fig. 1).

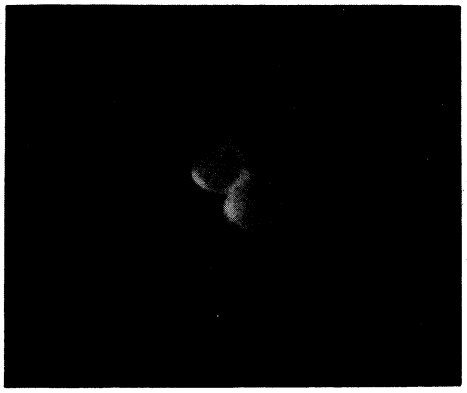

Fig. 1. Lymphocytes obtained from the regional lymph node 6 hours after painting with DNCB, bear peripheral fluorescence (smear, original magnification×400).

The cell nuclei were not stained. Occasionally fluorescence was recognized in areas corresponding to the cytoplasm of polymorphonucler cells and macrophages. The frequency of DNP group-bearing cells per 10^5 cells at various time intervals after painting the right inguinal skin with DNCB are given as mean numbers and ranges in Fig. 2. One hour after painting, the right inguinal nodes contained far more DNP group-bearing cells than the left and mesenteric nodes. The peak frequency of the cells in the regional node was at 12 hours following the exposure to DNCB, and the frequency declined rapidly after that. A few DNP group-bearing cells were still detectable 7 days later. The peak fre-

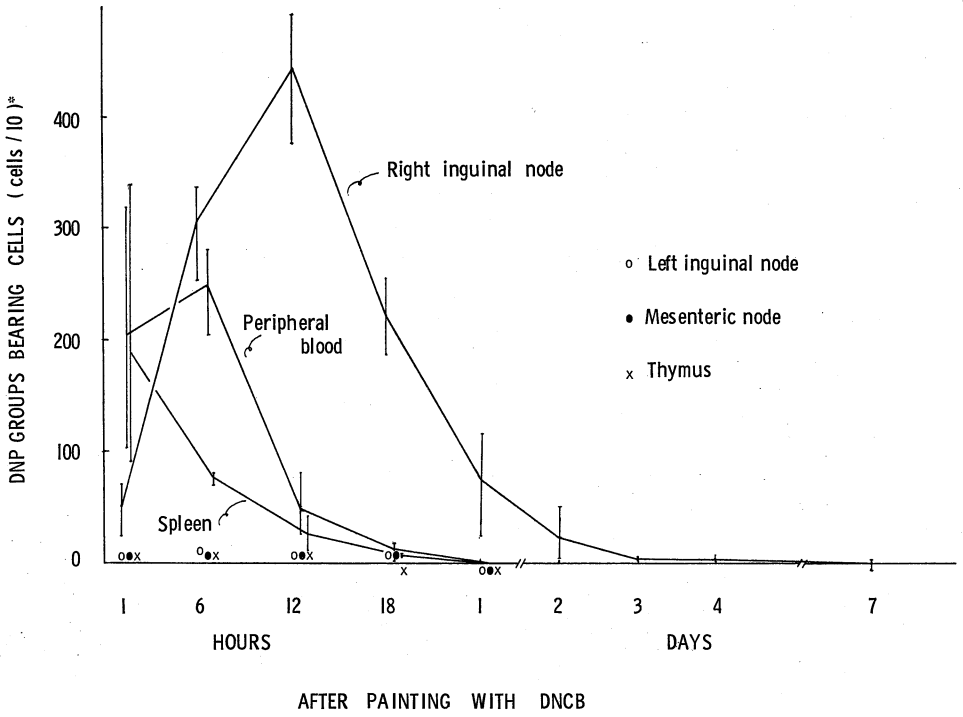

Fig. 2. Frequencies of DNP groups bearing cells in lymphoid tissues and peripheral blood at various times after painting with 5 per cent solution of DNCB to the right inguinal skin of normal guinea pigs. *Mean numbers and ranges of frequencies from three to five animals are shown.

quencies in the spleen and peripheral blood, although variable for individual animals, occurred at 1-6 hours. Thereafter, they definitely decreased and no cell could be detected at 24 hours or later. In the first 18 hours, DNP group-bearing cells were observed in the contralateral and mesenteric nodes and thymus, but they were far less frequent than those of the draining node, spleen and peripheral blood.

These time courses are at variances with those reported by Geczy and Baumgarten, who showed that regional lymph nodes and spleen exhibited maximal radioactivity at 24 hours and that lymphocyte rich fraction of peripheral blood contained relatively large amount of radioactivity from 24 to 192 hours after surface application of DNCB.[7] Similar results to our experiments have been demonstrated by other workers. It has been reported by Parker and Turk that there is no real change

in the radioactive content of regional lymph nodes in the first 24 hours and it drops sharply in the second 24 hours[8]. Witten and March have shown that radioactivity in buffy coat preparation of peripheral blood increases up to 8 hours and thereafter declines quickly[9]. It should be pointed out that these workers examined the distribution of DNCB by quantitative analysis of the radioactive content in the lymphoid tissues, a methodology which differed fundamentally from ours.

It has heen demonstrated in electromicroscopic examination using the immunochemical peroxidase procedure with anti-DNP antibody that DNP groups are localized on the membrane of lymphocytes[10]. Regarding the mechanism of surface binding of DNP groups to lymphocytes, it is possible to offer two explanations, which fit the data: (1) DNCB reacts directly *in vivo* with protein constituents of the cell membrane, and (2) that binding is dependent on the presence of antigen-binding-receptors which have been demonstrated on lymphocytes by Davie and Paul[11]. Antigen binding cells were prepared according to the method of Davie and Paul by incubating lymph node cells in DNP bovine serum albumin (BSA). Furthermore, cells were also obtained from regional lymph nodes of guinea pigs injected intradermally with DNP-BSA conjugates with the expectation that such cells would bind DNP-BSA *in vivo* but DNP-BSA was not detected on the surface of these cells by the immunofluorescent method (unpublished data). It would appear that *in vivo* or *in vitro* binding of DNP-BSA to the cells occurs in amounts too low to be detected by our method. These experiments provide support for the first possibility described above.

In the studies, therefore, evidence is presented which shows that direct conjugation of DNCB with lymphoid cells actually occurs *in vivo* during contact sensitization by painting with DNCB. This finding adds considerable weight to the concept that the first steps of induction phase of contact sensitivity are accomplished by direct conjugation of hapten with lymphoid cells.

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