Kawasaki Med. J. Vol. 3: No. 2, 67-71, 1977

THE DISTRIBUTION OF DNP GROUPS IN LYMPH NODES FOLLOWING APPLICATION OF DNCB TO GUINEA PIG SKIN

Shojiro NAKAGAWA, Shinichi FUJITA* and Kihei TANIOKU

Department of Dermatology, Kawasaki Medical School, Kurashiki, 701-01,
**Department of Dermatology, Red Cross Hospital, Okayama, 700, Japan.*

Accepted for Publication on Oct. 12, 1976

Abstract

The distribution of DNP groups in lymph nodes was investigated at various intervals following application of DNCB to guinea pig skin. DNP groups were detected in the cells in sinuses of lymph nodes obtained 12 and 24 hours after painting. The draining lymph nodes contained a far greater number of cells than the contralateral nodes. The significance of these findings is discussed.

INTRODUCTION

Although there is general agreement that lymphocytes sensitized specifically by antigens are generated in regional lymph nodes, the mechanisms by which immunologically competent cells recognize the antigenic determinants of contact senstivity are largely unknown.[1,2] In order to clarify the mechanisms, it is of prime importance to determine the localization and the fate of antigens when introduced percutaneously into body. Experiments of this nature have been greatly facilitated by incorporation of isotopes into the antigens and then have demonstrated the accumulation of radioactivities in draining lymph nodes by quantitative analysis.[3,4,5,6,7] However, relative few studies have been reported concerning the histological localization of the antigens in lymph nodes.

In the present study, 2, 4 dinitrochlorobenzene (DNCB) was applied to skin surface of guinea pig and the distribution of the compound was investigated in lymph nodes draining the site. Fluorescéin labelled antibody to 2, 4 dinitrophenyl (DNP) groups was used as a tracer of DNCB.

MATERIALS AND METHODS

Male albino guinea pigs, weighing 300 to 400g, were used. The animals were given an application of 0.5ml of 5 per cent solution of DNCB in ethanol to skin in the right foot. Both sides of popliteal and inguinal lymph nodes were removed at various intervals after painting. Paraffin sections were made from the nodes according to previously described procedure.[8)]

Fluorescein labelled antibody was prepared from rabbit antisera to DNP groups as described previously.[8)] A fluoresein to protein ratio of it was estimated to be 1.3. The conventional direct immunofluorescent procedure was carried out. Thereafter, the sections were stained again with hematoxilin eosin. Blocking tests using the unlabelled antibody and specific antigens were performed.

RESULTS

Following the application of DNCB to skin of normal guinea pigs, histological analysis of lymph nodes for localization of DNP groups was

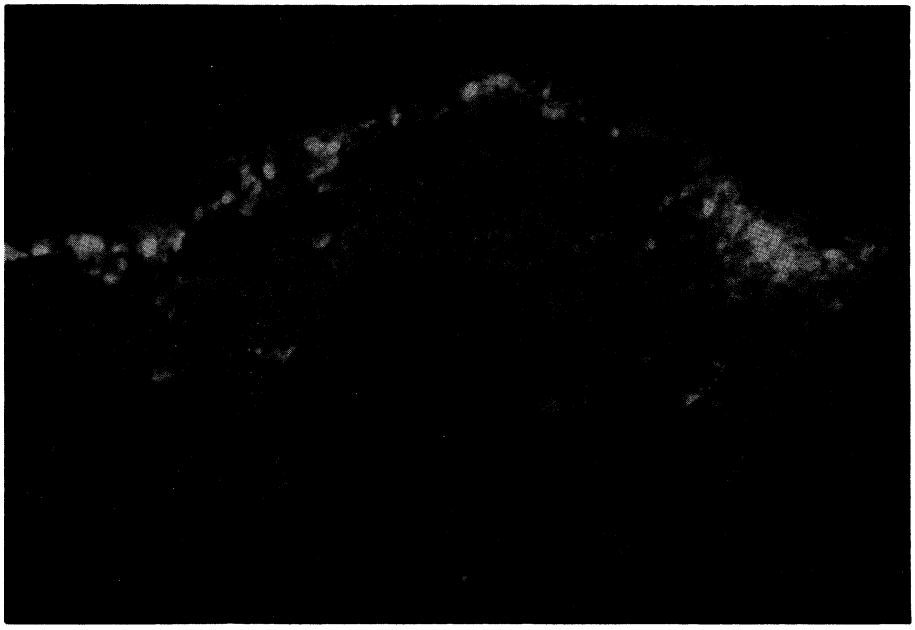

Fig. 1. Section prepared from the right popliteal lymph node of guinea pig 24 hours after painting with 5% solution of DNCB in ethanol, stained with fluorescent antibody to DNP groups. Fluorescence is present in the cells in subcapsular sinus (original magnification ×120).

carried out by immunofluorescent method. Numerous fluorescent cells were observed in subcapsular sinus of draining nodes as shown in Fig. 1 and 2. They were also seen in intermadiate and medullary sinuses. It was difficult to identify the cell population of them. Some of them seemed to be polymorph nuclear cells.

Further examinations were performed to confirm specificity of the immunofluorescent staining. Absence of specific staining was noted in the specimens which had been obtained from croton oil painted animal and then treated with fluorescein labelled anti-DNP antibody. The sections from DNCB painted animals were examined with fluorescein labelled anti-human IgG antibody without any staining.

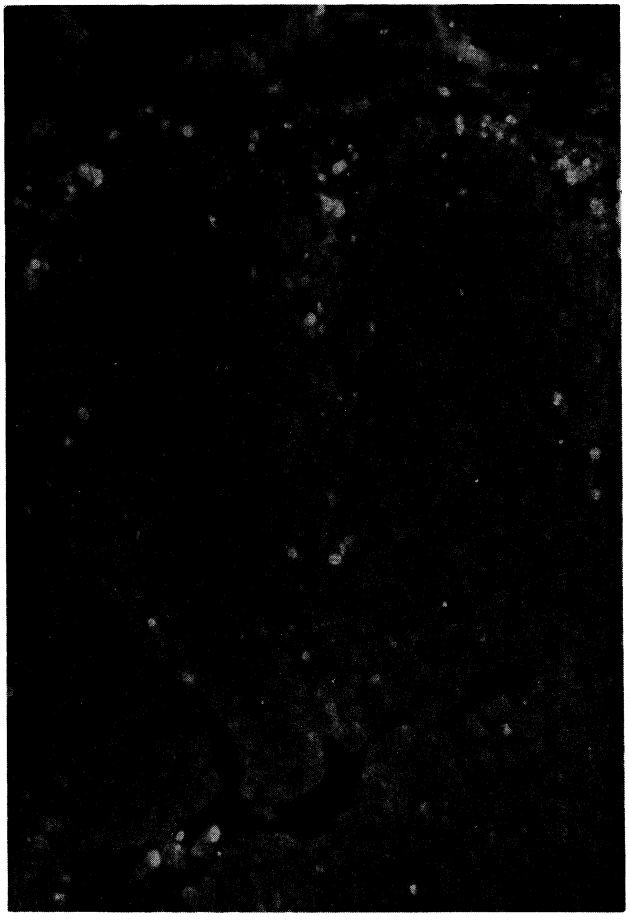

Fig. 2. Section of right inguinal node 24 hours after painting. Some fluorescent cells are also observed in intermediate and medullary sinuses (×120).

Fluorescent cells were demonstrated in the draining nodes 12 and 24 hours after painting. In one of five animals, a few fluorescent cells were detected in the contralateral inguinal node. The results are summarized in Table. Three to five animals were examined at each test interval.

TABLE

Distribution of DNP groups in lymph nodes after surface application of 5% DNCB to the right foot skin of guinea pigs

Hours after painting	Right nodes		Left nodes	
	Popliteal	Inguinal	Popliteal	Inguinal
0.5	−	−	−	−
6	−	−	−	−
12	+	+	−	−
24	+	+	−	+~−
48	−	−	−	−
72	ND	−	ND	ND

ND, not done.

DISCUSSION

In the study reported here, evidence is presented which shows that after epicutaneous application of DNCB to guinea pigs, DNP groups are localized on/in the cells in lymph sinuses of draining nodes. DNP groups were detected in the nodes 12 and 24 hours after painting. Relative similar results to our experiment have been demonstrated by other workers. Geczy and Baumgarten[6] have reported that the draining lymph nodes exhibit maximal radioactivity at 24 hours after exposure to radioactive 2, 4 dinitrofluorobenzene (DNFB). It has also been written by Parker and Turk[3] that there are much radioactive contents of regional nodes in the first 24 hours and they drop sharply in the second 24 hours. In the immunofluorescent study on cellular localization of DNP groups in lymphoid tissues,[9] we have demonstrated that the frequencies of DNP groups bearing cells in draining nodes increase up to 12 hours and thereafter decline.

It has also shown in the work[9] that the majority of the cells are lymphocytes and the others polymorph nuclear cells and macrophages. The electromicroscopic study using peroxidase labelled anti-DNP antibody has shown DNP groups on the surface of lymphocytes.[10] It is of interest to call attention to recent investigations which show that *in vitro* conjugation of DNCB or DNFB to lymphocytes induce contact

sensitivity against these chemicals.[7,11,12] These results indicate that the initial steps of sensitization are accomplished by contact between lymphoid cells and chemical allergens. The present observation is considered to show a possibility that the contact between lyphocytes and DNCB actually occurs in regional lymph nodes.

Acknowledgment

This study was supported in part by Kawasaki Medical School Grant No. 51206 for Project Research.

REFERENCES

1) Turk, J. L.: Delayed hypersensitivity. The significance of changes in local lymphoid tissue during the induction of delayed hypersensitivity. pp. 138, North-Holland Publishing Company, Amsterdam, 1967
2) Fujita, S.: Contact dermatitis and the regional lymph nodes. Nishinihon J. Dermat. 31: 240-246, 1979 (in Japanese)
3) Parker, D. and Turk, J. L.: DNP conjugates in guinea-pig lymph nodes during contact sensitization. Immunology 18: 855-864. 1970
4) Hall, J. G. and Smith, M. E.: Studies on the afferent and efferent lymph of lymph nodes draining the site of application of fluorodinitrobenzene (FDNB). Immunology 21: 69-79, 1971
5) Godfrey, H. P., Baer, H. and Watkins, R. C.: Delayed hypersensitivity to catechols. V. Absorption and distribution of substance related to poison ivy extracts and their relation to the induction of sensitization and tolerance. J. Immunol. 106: 91-102, 1971
6) Geczy, A. F. and Baumgarten, A.: Distribution of contact sensitizer, 1-fluoro- 2, 4-dinitrobenzene, in the tissues of the guinea pig. Immunology 22: 381-392, 1972
7) MacFarlin, D. E. and Balfour, B.: Contact sensitivity in the pig. Immunology 25: 995-1009, 1973
8) Nakagawa, S., Ueki, H. and Tanioku, K.: The distribution of 2, 4 dinitrophenyl groups in guinea pig skin following surface application of 2, 4 dinitrochlorobenzene: An immunofluorescent study. J. Invest. Dermat. 57: 269-277, 1971
9) Nakagawa, S. and Amos, H. E.: Cellular localization of DNP groups in lymphoid tissues of guinea pig following skin surface application of DNCB. Kawasaki Med. J. 3: in press.
10) Tada, H., Nakagawa, S., Tanioku, K. and Amos, H. E.: Ultrastructural distribution of DNP groups on lymphoid cells in guinea pigs following skin surface application of DNCB. in preparation.
11) Baumgarten, A. and Geczy, A. F.: Induction of delayed hypersensitivity by dinitrophenylated lymphocytes. Immunology 19: 205-217, 1970
12) Polak, L. and Macher, E.: In vitro sensitization to dinitrochlorobenzene in guinea pigs. Nature 252: 748-749, 1974