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IMMUNOHISTOCHEMICAL STUDIES  
ON THE LOCALIZATION OF ANTIGEN-BEARING  
PHAGOCYTES AND LYMPHOCYTES IN MOUSE  
LYMPH NODES AFTER THE GERMINAL  
CENTER FORMATION

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The antigen, 2, 4-dinitrophenylated and alumprecipitated bovine serum albumin, was inoculated into the foot pads at 2 weeks after the primary inoculation of the antigen. At 2 and 3 days after the second inoculation, many anti-T cell serum negative lymphocytes, possibly B cells, were found in the lumen and the endothelium of the post capillary venules, and they predominantly gathered around the venules. These gathering areas of possibly B cells were encountered frequently in contact with the cortical lymphatic nodules containing the germinal centers. From 4 to 14 days after the second inoculation, a number of antigen-bearing phagocytes appeared in the cortical marginal zones, which were located between the germinal centers and the marginal sinuses. These findings suggest that B cells are largely recruited from the blood to the paracortex, and also the antigen-bearing phagocytes directly present the antigen to the cortical lymphocytes for the acceleration of the antibody production after the formation of the germinal centers.

Key words: antigen-bearing phagocytes, lymphocytes, germinal centers, mouse lymph nodes

INTRODUCTION

It has been known that there is a correlation between the development of the germinal centers and the generation of immunological memory lymphocytes.<sup>13)</sup> Therefore, the mechanism of the intranodal immune responses after the germinal center formation is thought to be different from that before their formation. There is a difference in functional properties between B memory cells and unprimed B cells.<sup>23)</sup> Furthermore, it was shown that the follicular localization of the soluble antigen was

rapid in pre-immunized rat lymph nodes.<sup>19)</sup> In this paper, the behavior of the immunocompetent cells, not only the antigen-bearing phagocytes but also T cells and B cells, was observed immunohistochemically in subsequence to the rechallenge of aggregated antigen into the mouse lymph nodes with the germinal centers.

#### MATERIALS AND METHODS

Mice: Fifty-four male and female BALB/c mice were used.

Preparation and administration of antigen: 2, 4-dinitrophenylated bovine serum albumin (DNP-BSA) was prepared with 2, 4-dinitrobenzenesulfonic acid sodium salt (Tokyo Kasei Kogyo Co., Ltd.) and crystalline BSA (Sigma Chemical Co., U.S.A.).<sup>7)</sup> DNP and BSA in the prepared DNP-BSA were in the molar ratio of 3-6: 1. Then DNP-BSA was precipitated with alum (DNP-AP-BSA).<sup>12)</sup> A half mg of DNP-AP-BSA in 0.05 ml physiological saline was inoculated into each foot pad of mice in subsequence of the first inoculation of the same dose of DNP-AP-BSA 2 weeks earlier, when the mice were aged 6 to 10 weeks. Thereafter, 3 to 6 mice were bled in each time sequence at 3, 6 hours, 1, 2, 3, 4, 7, 10 and 14 days after the second inoculation. Also, 3 to 6 mice were bled in each time sequence at 14, 21 and 28 days after the primary inoculation.

Immunohistochemistry: Popliteal lymph nodes were fixed in periodate-lysine-paraformaldehyde and embedded in paraffin. Four  $\mu$ m thick sections were made and exposed to rabbit anti-DNP-BSA serum, which was prepared in our laboratory, or rabbit anti-mouse T cell serum (Cedarlane Lab. Ltd., U.S.A.) to detect the antigen or cells, applying the enzyme-linked antibody methods.

#### RESULTS

The germinal centers were observed in every popliteal lymph node of the mice in the present experimental series. Some germinal centers contained crescent-shaped networks of DNP-BSA, whose convex side was always arched along the marginal sinus side of the germinal center. Between the germinal centers and the marginal sinuses, the so-called marginal zones were found, however voluminously the germinal centers developed in the lymphatic nodules.

The DNP-BSA-bearing phagocytes were detected both in the lymphatic nodules and the paracortex (fig. 1). They were observed throughout the experimental periods after the second inoculation; however, the DNP-BSA-bearing phagocytes in the lymphatic nodules were smaller in number and ingested a smaller amount of DNP-BSA from 3 hours to 3 days after the second inoculation than in later periods.

The paracortex was the only place where the anti-T cell serum positive lymphocytes (ATPL) were predominantly located, and the anti-T cell serum negative lymphocytes (ATNL) were scattered at 1 day after the second inoculation (fig. 2). Both ATPL and ATNL were also detected around the post capillary venules (PCV) (fig. 3). At 2 days after

the second inoculation, there were some PCV around which a number of ATNL were accumulated. Furthermore, at 3 days after the second inoculation, the accumulations of ATNL were encountered around a lot of PCV. These growing accumulation areas came into contact with the cortical lymphatic nodules which contained the germinal centers (fig. 4). The ATNL in these accumulation areas consisted mainly of small lymphocytes (fig. 5), and the small ATNL were also observed in the lumen and the endothelium of the PCV (fig. 5). These accumulations were observed subsequently in the later periods after the second inoculation.

At 4 days after the second inoculation, a number of DNP-BSA-bearing phagocytes migrated directly from the marginal sinuses into the marginal zones. From 7 to 14 days after the second inoculation, the entries of the DNP-BSA-bearing phagocytes into the marginal zones were more prominent. These phagocytes engulfed a large amount of DNP-BSA and lined up along the marginal sinuses (fig. 6). And occasionally, they were in clusters in the marginal zones (figs. 7 & 8). The entries of the DNP-BSA-bearing phagocytes into the marginal zones and the amount of DNP-BSA engulfed by them increased after the second inoculation in comparison with those after only a single inoculation. From 7 to 14 days after the second inoculation, clusters of two or three DNP-BSA-bearing phagocytes also formed around the PCV in the paracortex.

The medullary cords, where the plasma blast cells and the plasma cells were mainly distributed, grew in size during the experimental periods. The plasma blast cells were located in the areas in close proximity to the paracortex, while the mature plasma cells were situated in the areas near the hilus. Some lymphocytes in mitosis were observed in the medullary cords.

#### DISCUSSION

It has been known that the PCV form a recirculating pathway of T cells.<sup>8)</sup> In addition, it has been shown that intravenously injected B cells, which were autoradiographically labeled, appeared in the rat thoracic duct.<sup>11)</sup> The labeled B cells were also observed morphologically to pass the PCV of the lymph nodes of normal animals,<sup>9&18)</sup> T cell deprived mice<sup>14)</sup> and B cell deprived mice.<sup>24)</sup>

In the present observation, it was suggested that a large number of anti-T cell serum negative lymphocytes (ATNL), possibly B cells, migrated from the blood to the lymph nodes across the PCV of the immunized mice. It was indicated that the recirculating B memory cells had progressively accumulated in the lymph nodes containing the specific antigen.<sup>21)</sup> Therefore, the present ATNL, possibly B cells, around the PCV might be the immunological memory lymphocytes. It was also naturally thought that the new unprimed ATNL, possibly B cells, might be supplemented from the blood to the paracortex to cooperate with the antigen-bearing phagocytes and T cells.

In the present study, it was also observed that the antigen-bearing phagocytes

frequently entered the marginal zones of the lymphatic follicles directly from the marginal sinuses in the immune responses after the germinal center formation. Although both B cells and T cells acquire an immunological memory.<sup>1&16)</sup> There is a difference between the antigen binding affinity of B cells and that of T cells after the antigen stimulation. The antigen binding affinity of B cells increases during the course of immune response.<sup>5)</sup> while T cells do not increase the affinity for antigen with time after immunization.<sup>3)</sup> Accordingly, the direct entries of antigen-bearing phagocytes to the marginal zones are probably due to the increase of the affinity to bind the antigen-bearing phagocytes and the ATNL, possibly B cells.

Moreover, the development of B memory cells took a long time and needed a lot of antigen in contrast with that of T memory cells.<sup>4)</sup> It was reported that the autoradiographically labeled antigen formed a crescent of label in the superficial area of germinal centers and was retained for a long time.<sup>15)</sup> The marginal zones were located in close proximity to the crescent-shaped networks of antigen-antibody complexes, and always existed between the marginal sinuses and the networks.<sup>17)</sup> These antigen-antibody complexes are also reported to be effective in the stimulation of B memory cell development.<sup>13)</sup> Therefore, it is inferred that B cells in the marginal zones are the lymphocytes which acquire the immunological memory by associating with the networks of the antigen-antibody complexes in the germinal centers.

Histologically, the marginal zones of the lymph nodes are separated from the paracortex, the thymus dependent area, which suggests that T cells are not required in the acquisition of the immunological memory of B cells<sup>6&22)</sup> or that the degree of their requirement is very low,<sup>10)</sup> even though the compensatory necessity of T cells must be taken into consideration.<sup>2&20)</sup>

Further studies are needed to demonstrate the acquisition of the immunological memory of the lymphocytes in the marginal zones. It seems that the studies on the lymphocytes in the marginal zones will clarify the proper function of the germinal centers in the secondary immune responses.

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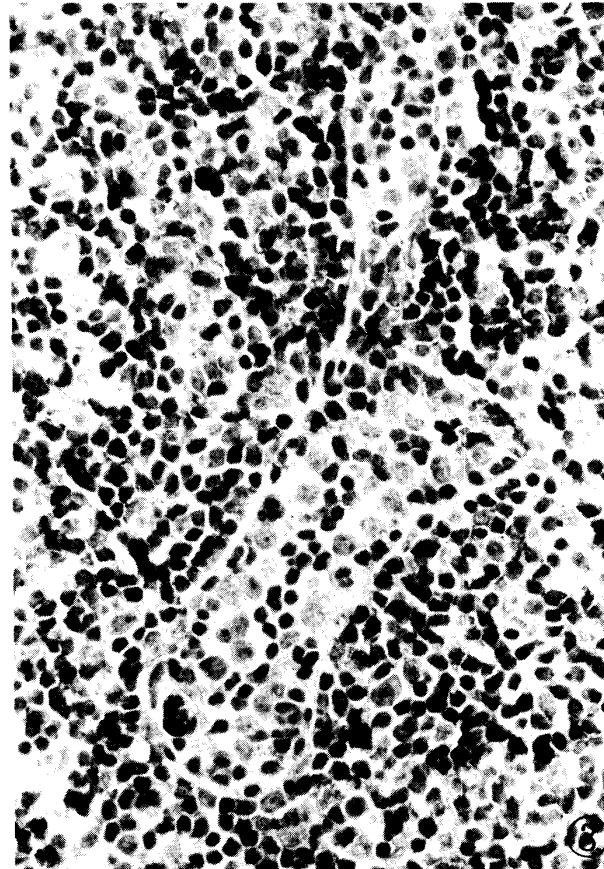
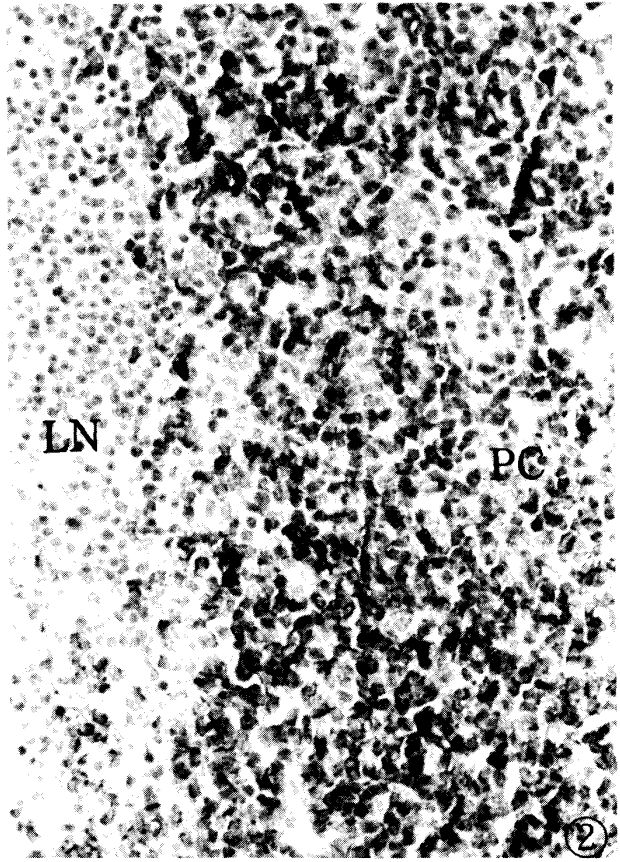
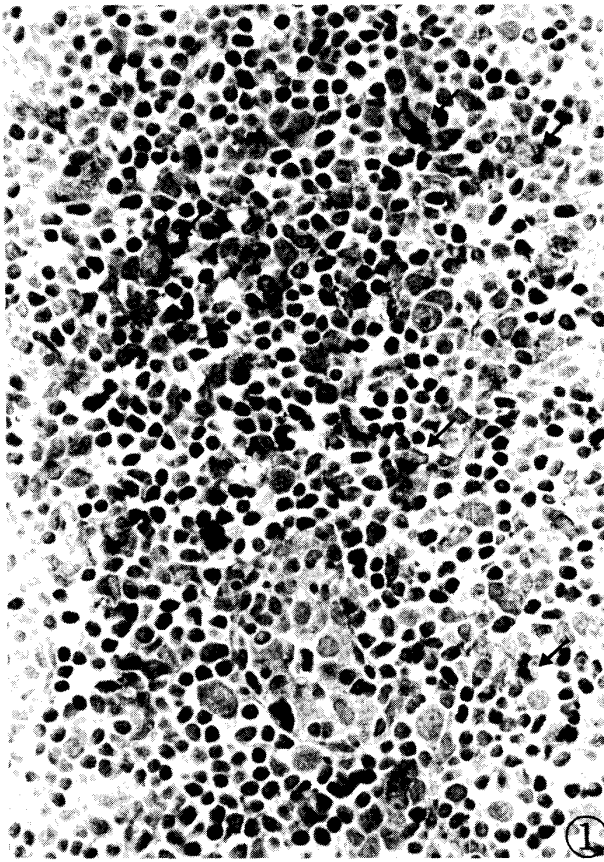
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## EXPLANATION OF PLATES

## PLATE I

- Fig. 1 DNP-BSA-bearing phagocytes (arrows) observed in the paracortex one day after the second inoculation Counter stained with hematoxylin  $\times 440$
- Fig. 2 Anti-T cell serum positive lymphocytes observed predominantly in the paracortex (PC) Anti-T cell serum negative lymphocytes were found mainly in the lymphatic nodule (LN) and were scattered in the paracortex. One day after the second inoculation. Counter stained with hematoxylin  $\times 340$
- Fig. 3 Anti-T cell serum positive and negative lymphocytes around the post capillary venule in the paracortex One day after the second inoculation. Counter stained with hematoxylin  $\times 440$
- Fig. 4 Anti-T cell serum negative lymphocytes accumulating around the post capillary venule in the paracortex (PC) The accumulation area is in contact with the lymphatic nodule containing the germinal center (GC). Three days after the second inoculation Counter stained with hematoxylin  $\times 220$



## PLATE II

- Fig. 5 Anti-T cell serum negative lymphocyte accumulation around the post capillary venule in the paracortex. Anti-T cell serum positive lymphocytes are hardly observed in the accumulation area. Three days after the second inoculation. Counter stained with hematoxylin.  $\times 680$
- Fig. 6 DNP-BSA-bearing phagocytes migrating into the marginal zone (MZ). Some are found near the germinal center (GC). Seven days after the second inoculation. Counter stained with hematoxylin.  $\times 440$
- Fig. 7 Accumulation of DNP-BSA-bearing Phagocytes in the marginal zone (MZ). GC : germinal center. Seven days after the second inoculation. Counter stained with hematoxylin.  $\times 220$
- Fig. 8 Accumulation of DNP-BSA-bearing Phagocytes along the marginal sinus. Seven days after the second inoculation. Counter stained with hematoxylin.  $\times 530$

