Demonstration of B Lymphocytes in Rat Thymic Lymph Follicles*

Kenjiro Matsuno,¹ Novoru Yamanaka² and Masahiko Kotani¹

Department of Anatomy (Prof. M. Kotani),¹ Kumamoto University School of Medicine, Kumamoto, and Department of Pathology (Prof. K. Kikuchi),² Sapporo Medical College, Sapporo, Japan

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Summary. By using immunoperoxidase techniques, it was demonstrated that the lymph follicles, formed in the thymic medulla of castrated DA rats after 20 injections with typhoid-paratyphoid bacilli at 10 day intervals, were composed mainly of B lymphocytes.

It seems that myasthenia gravis and many other autoimmune diseases are closely associated with the formation of lymph follicles in the thymus (Sloan, 1943; Castleman, 1955; Okabe, 1966; Habi, Kameya and Tamaoki, 1971). An increase in the number of B lymphocytes in the thymus of myasthenia patients has been reported (Abdou et al., 1974; Staber, Fink and Sack, 1975). Previously we have reported that a large number of lymph follicles with germinal centers can be experimentally formed in the thymus of castrated DA strain rats after prolonged and intensive stimuli with bacterial antigens (Matsuno et al., 1979). By using immunoperoxidase techniques, the present study demonstrates that these thymic lymph follicles are composed mainly of B lymphocytes.

MATERIAL AND METHODS

Three male rats of DA strain, 9 weeks of age, were castrated 5 days before the first injection of typhoid-paratyphoid bacilli (TAB). Twenty intraperitoneal injections of 2 ml saline suspension of TAB (typhoid bacilli 2 × 10⁸, paratyphoid A bacilli 0.5 × 10⁸ and paratyphoid B bacilli 0.5 × 10⁸) were carried out at 10 day intervals in the same way as reported previously (Matsuno et al., 1979). Each ml of TAB vaccine (Chiba Prefectural Serum Institute, Ichikawa) was washed three times in 20 ml physiologic salme before use. Three control rats of the same age were castrated but injected with saline only in the same manner. The animals were sacrificed 5 days after the last injection.

One lobe of the thymus from the experimental and control rats was excised and cut serially on a cryostat at 6 μm. The initial 2 sections were employed to determine

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Fig. 1. A lymph follicle with the germinal center (G) in the medulla (M) is indicated by arrows. C cortex. Methyl green-pyronin stain. ×240

Fig. 2. A following section to Figure 1, stained by the immunoperoxydase technique with anti-rat B lymphocyte-specific serum. The surface membrane of the majority of cells in the follicle is stained distinctly, on the contrary to cells outside the lymph follicle. ×240

Fig. 3. A following section to Figures 1 and 2. Cells stained by the immunoperoxydase technique with anti-rat T lymphocyte-specific serum were few in the lymph follicle (arrows), although cells outside the lymph follicle are stained positively. ×240
the presence of cells bearing surface-membrane immunoglobulins (B lymphocytes) by the immunoperoxydase assay using anti-rat B lymphocyte serum (ABLS). The following 2 sections were employed to determine the presence of lymphocytes with T lymphocyte-specific antigens by the immunoperoxydase assay using anti-rat T lymphocyte serum (ATLS). The final 2 sections were fixed in Carnoy's fluid and stained with methyl green-pyronin for histologic examinations of germinal centers. This sequence was repeated until the entire lobe was examined. ABLS was prepared by immunizing rabbits with purified B cells from rat spleen and absorbed with rat red blood cells, rat thymocytes, and syngeneic sarcoma cells (YAMANAKA et al., 1981). ATLS was prepared by immunizing rabbits with purified T cells from rat mesenteric lymph nodes and absorbed with rat red blood cells and syngeneic sarcoma cells as reported previously (ISHII et al., 1976).

RESULTS

Many lymph follicles with germinal centers were found in the thymic medulla of rats treated with TAB bacilli in sections stained with methyl green-pyronin (Fig. 1). As shown in Figure 2, the surface membrane of the majority of cells in these follicles was stained distinctly with the ABLS. Cells stained with the ATLS were few in the lymph follicles (Fig. 3), although such cells were numerous surrounding the lymph follicles. In addition, cells stained with the ABLS were often found scattered or in small groups in the medulla of the thymus outside the lymph follicles.

On the contrary, no lymph follicles with germinal centers were found in the thymus of control rats. Cells stained with the ABLS were also found sporadically in the medulla of the control rats, but this very rarely.

DISCUSSION

ABLS used in this study recognizes rat B lymphocyte-specific antigens and ATLS recognizes T lymphocyte-specific antigens. The majority of the cells of lymph follicles, formed in the thymic medulla of castrated rats following 20 injections with TAB bacilli at 10 day intervals, were stained with the ABLS, but not with the ATLS. This indicates that the lymph follicles formed in the thymus were composed mainly of B lymphocytes as are those in other lymphoid tissues, such as lymph nodes and spleen. An increase in the number of B lymphocytes in the thymuses of myasthenia gravis patients has been described using both a surface-membrane immunoglobulin marker technique (ABDOU et al., 1974) or a rosetting technique with sheep-erythrocyte-antibody-complement complexes (STABER, FINK and SACK, 1975). The latter study localized the B lymphocytes to the follicles of the thymus. These results along with the present data may support a hypothesis by DATTA and SCHWARTZ (1974) that local antigenic stimuli by some agents may induce the disease of myasthenia gravis. MARSHALL and WHITE (1961) showed that lymph follicles can be induced in the guinea-pig thymus by direct injection of TAB antigens.
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ラッット胸腺リンパ濾胞を構成するBリンパ球

松野健二郎，山中登，小谷正彦

精巣摘出を行なった DA 系ラットの胸腺に、腸チフス－パラチフス菌を 10 日間隔で 20 回注射したのち、胸腺の凍結切片を作製し、その一部をカルノア液に固定、メチールグリノ－ピロクエン染色を施すと、胸腺中心をもったリンパ濾胞が形成されたことが分る。これにつづく切片を抗ラット B リンパ球血清 または抗ラット T リンパ球血清を用いて、ベルオキシダーゼによる酵素抗体法で染色することによって、リンパ節や脾臓におけるリンパ濾胞と同じように、胸腺に形成されたリンパ濾胞は主として B リンパ球から構成されることがわかった。

REFERENCES


