Light and Electron Microscopic Observations on the Vascular Pattern of the Thymus of the Mouse*

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The thymus is one of the subjects that have received the most intensive investigation in recent years, and it has become clear that the thymus plays an important role in development of the immune system. The present state of the knowledge of the thymus studies is surveyed repeatedly and no repetition is needed (see, for instance: GOOD and GABRIELSEN 1964; DEFENDI and METCALF 1964). A remarkable progress in the studies of thymic function also appears to give a new impetus to the study of thymic structure. This is also the case with the vascular pattern of the thymus. The vascular pattern of the thymus was previously reported in detail, particularly for the mouse (SMITH, THATCHER, KRAEMER and HOLT 1952). Recently electron microscopy was also applied to the study of this subject. As pointed out by SMITH, THATCHER, KRAEMER and HOLT (1952), the thymic cortex is characterized by a fine network of numerous capillaries and the medulla by a preponderance of veins and fewer arteries. Therefore, in considering the vascular pattern of the thymus particularly in relation to the functional role, one should pay attention mainly to capillaries in the cortex and to veins in the medulla. In the cortex, the capillaries were examined with the electron microscope in particular connection with the question whether or not a blood-thymus barrier, if present, is morphologically confirmed. As an explanation of immunological unresponsiveness of the thymus to antigens in the blood stream the concept of a vascular barrier was suggested (MARSHALL and WHITE 1961). The electron microscopical findings of the thymic capillaries also were in favor of the concept of the barrier (CLARK 1963, WEISS 1963, GAUDECKER and HINRICHSEN 1965, KAMEYA and WATANABE 1965). More recently, however, the presence of the barrier was not confirmed electronmicroscopically (CLARK 1964), and further close investigation into this problem is required. In the medulla, the veins, particularly larger ones, are surrounded by a wide perivascular space which is considered to serve as passageway of thymic lymphocytes which are formed in the cortex and migrate to the medulla (SAINTÉ-MARIE and LEBLOND 1958, 1964; HINRICHSEN 1965). For this reason, relationship of the perivascular space to the surrounding medullary parenchyma is of special interest in connection with transfer of thymic lymphocytes. However, while the vascular pattern in the cortex has often been observed with the electron microscope, that in the medulla has been relatively neglected. For above reasons, it was decided to re-examine the vascular pattern of the mouse thymus by light and electron microscopy.

Material and Methods

Mice used in this study were young adults of both sexes of Japanese dd strain, ranging in

* Dedicated to the memory of the late Professor Masaji SEKI
age from 5 to 10 weeks. For light microscopy the thymuses were fixed in Bouin fluid or 10% formal. In a few cases, just after sacrifice, India ink was injected from the left ventricle, and the thymuses were fixed in situ and then removed. After embedded in paraffin, the thymuses were serially sectioned at 6 μ. The sections were stained usually with hematoxylin and eosin and sometimes with periodic acid Schiff (PAS) and hematoxylin. For electron microscopy thymic tissues were fixed in 2% osmium tetroxide buffered at pH 7.6 in 0.1M phosphate buffer containing 15 mg/ml sucrose, dehydrated and embedded in Epon mixture. Thin sections were cut with glass knives on an LKB Ultratome, stained successively with methanolic uranyl acetate and lead citrate, and examined in an Akashi Tronscope 50R.

Observations

1. General vascular pattern

The main vascular system of thymus was traced in serial sections and the distributing pattern was examined by use of serial sections of the injected organ (Fig. 1). In the thymus of the adult mouse, the main blood vessels enter and leave the organ in the interlobular septa. The interlobular septa project from the capsule into the cortex almost as far as the cortico-medullary boundary and produce lobulation of the thymus. The thymus of the mouse has relatively simple lobulation as compared with that of other laboratory rodents. The interlobular arteries enter the medullary parenchyma at the end of the septa and immediately branch into smaller arteries or arterioles. The arterioles run outwards and give off capillary branches in the deep cortex. The cortical capillaries are directed almost radially, and make a coarse, anastomosing network. In the outer cortex, often immediately beneath the capsule, the surface capillaries form loops, then returning inwards. In the deep cortex and peripheral medulla the returning capillaries gradually drain into smaller venous vessels. In the medulla the venules are collected into the veins. The veins gradually lead into larger veins. The large veins finally leave the medulla through the interlobular septa.

2. Fine vascular pattern, especially relationship to the surrounding parenchymal tissue

As described above, most of the cortical vessels are capillaries, and most of the medullary vessels are venules and veins.

Capillaries in the cortex: The capillaries have essentially the same structure as those in
other tissues (Fig. 2). The endothelial cells contain a few mitochondria, a moderate amount of RNP particles and ergastoplasmic reticulum, and a considerable number of small vesicles. Many vesicles are also associated with the luminal and basal plasma membranes, suggesting pinocytosis. The cells have frequent microvillous projections on the luminal surface. The endothelial cells form a continuous lining without fenestrations or pores. Just beneath the endothelium lies a continuous basement membrane of moderate density in a pericapillary...
The space around the capillary wall, though narrow, often contains amorphous, sometimes mottled ground substance of low electron density. Pericytes and delicate collagen fibrils are also present. Outside the pericapillary space is the cortical parenchyma. The cortical parenchyma, as well known, is composed of epithelial reticular cells, lymphocytes and macrophages (M) are seen in both the pericapillary spaces and the intercellular meshes of the cortical parenchyma. ×3,960
phages. Cytological features of these cellular elements are previously described in detail (Clark 1963, Hoshino 1963). The epithelial reticular cells possess very elongated cytoplasmic processes which are connected together to form a cellular reticulum. The pericapillary space is usually bordered by a layer of cytoplasmic extensions of epithelial reticular cells. In the space a basement membrane is also visible close to the epithelial cells. The epithelial cells are easily distinguished by their characteristic features that are previously reported. In some
sections the capillaries are seen surrounded by a continuous layer of epithelial reticular cells (Fig. 2). However, when the capillaries are examined along the course for a longer distance, the pericapillary connective tissue spaces are found not to be lined by a continuous layer of epithelial cells. As seen in Figs. 3 and 4, the lining epithelial layer is discontinuous with

Fig. 5. A medullary vein (VEIN) is cut transversely. Some of endothelial cells possess an extremely dense cytoplasm. The vein is surrounded by a perivascular space which contains lymphocytes. The space is lined by a layer of epithelial reticular cells (ERC). ×7,200
frequent interruption. At sites where epithelial sheath is interrupted, a basement membrane just on the sheath also disappear abruptly. The pericapillary spaces are in direct continuity with the meshes of the epithelial reticulum of the cortical parenchyma and often contain lymphocytes and macrophages in variable numbers. Macrophages, though generally few in number, are more frequent in the spaces than in the parenchymal meshes.

**Venules and veins in the medulla:** As described above, the blood vessels which are commonly seen in the medulla are venules and veins. Light microscopy reveals that the veins in the medulla are surrounded by a considerably wide space which is encircled by PAS-positive fibers and often contains varying numbers of lymphocytes. The space has been considered to be a passageway of thymic lymphocytes which proliferate in the cortex and migrate into the medulla. In electron microscopy the venules and veins are essentially the same in structure. The endothelial cells are usually flattened in shape without any fenestration. Their cytological details are generally similar to those of the capillary endothelial cells. It is of interest that the endothelial cells lining venules or veins often vary in electron density of the cytoplasm. As seen in Fig. 5, some of the endothelial cells have an extremely electron dense cytoplasm. Venous vessels in some other tissues also are found to possess occasional endothelial cells with electron dense cytoplasm, but the significance of occurrence of such electron dense endothelial cells is obscure. Just outside the endothelial cells a basement membrane is visible in the perivascular space. In the space there are smooth muscle cells and adventitial cells.

![Fig. 6. The relationship of the perivascular space of a medullary vessel (V, lower left) to the surrounding medullary tissue (right) is shown. At the intercellular gap of epithelial reticular cells (ERC), lymphocytes (L) are seen. Arrows indicate basement membranes. ×8,200](image)
With increase of the size of the vessels there occurs an increase of the number of smooth muscle cells. The muscle cells are seen, on close inspection, to be also enclosed with basement membranes. The perivascular spaces contain amorphous substance of low density and varying amounts of delicate collagen fibrils. As seen in light microscopy, varying numbers of lymphocytes are contained in the spaces (Fig. 5). The perivascular spaces are bordered by a discontinuous layer of epithelial reticular cells. The epithelial cells have a basement membrane on the surface. At gaps where the epithelial cell layer is interrupted, the spaces communicate with the meshes of the medullary epithelial framework, and lymphocytes are often seen occluding the gaps (Fig. 6).

Arterioles in the vicinity of the cortico-medullary boundary: In the deep cortex and peripheral medulla there are sometimes arterioles (Fig. 7). Their walls have usual coats; endothelium, smooth muscle cells and adventitial cells. Basement membranes are seen just outside the endothelium and around the smooth muscle cells.

![Fig. 7. An arteriole (A) in the peripheral medulla is cut transversely. ERC epithelial reticular cells. X7,400](image)

**Discussion**

Concerning the general vascular pattern of the mouse thymus, the present observations are essentially the same as previously reported (Smith, Thatcher, Kraemer and Holt 1952; Schmidt 1965). The majority of the cortical vessels are capillary and those of the medullary ones are venous. The relationships of the cortical capillaries and medullary veins to the thymic parenchymal tissue have received special consideration for an understanding of the functional structure of the organ.
The thymic cortex, as is well known, is a site of active lymphocytopoiesis. Lymphocytes are closely packed in the meshwork of epithelial reticular cells and undergo very active proliferation. Available information suggests that thymic lymphocytes, unlike those in other lymphoid tissues, are immunologically inactive. A possible explanation for the immunological incompetence of thymic lymphocytes has been given by the concept of a vascular barrier mechanism that prevents the entrance of antigens into the thymic parenchyma from the bloodstream (MARSHALL and WHITE 1961). Morphologically the presence of the barrier was suggested in the light of the electron microscopic finding that the cortical capillary wall is surrounded by a cytoplasmic layer of epithelial reticular cells which separate lymphocytes in the parenchymal meshwork from the vascular wall (CLARK 1963, WEISS 1963, GAUDECKER and HINRICHSEN 1965, KAMEYA and WATANABE 1965). On the other hand, however, CLARK (1964), tracing injected detectable materials in the thymus in light and electron microscopy, examined in detail whether there is a barrier to the penetration of antigen into the parenchyma, and stated that there seems to be no absolute barrier between the parenchyma and blood stream. Most recently, even the entry of lymphoid cells, though to a lesser extent, into the thymic parenchyma is directly confirmed by GALTON and REED (1966), although this has previously been suggested by a possible colonization of lymphoic stem cells. According to the present observations the perivascular spaces around the cortical capillary walls are lined by a cytoplasmic layer of epithelial reticular cells, but the epithelial layer is discontinuous with frequent interruption. Through such interrupted sites of the epithelial lining the pericapillary spaces communicate directly with the intercellular spaces of the cortical meshwork. Thus lymphocytes and macrophages which are contained within the epithelial framework are often found also in the spaces around the capillaries. Morphologically, therefore, no evidence suggests complete isolation of lymphocytes from the blood vessels. In other words, the presence of an absolute vascular barrier such as the blood-brain barrier morphologically cannot be confirmed in the thymus. Explanation for immunological unresponsiveness of thymic lymphocytes should be sought preferably in other grounds. For instance, as suggested by the structural characteristics, the thymus, unlike other lymphoid tissues, may be a particular environment for lymphocytes due to the presence of epithelial reticular cells.

In the thymic medulla, venous vessels are surrounded by a considerably wide space (SMITH and IRELAND 1941). The space around the veins has been considered to serve as a passageway of lymphocytes which are formed in the cortex and migrate into the medulla (SMITH 1955; SAINTE-MARIE and LEBLOND 1958, 1964). The outer boundaries of the spaces, like those of the pericapillary spaces in the cortex, are limited by a layer of epithelial reticular cells. The limiting epithelial layer has frequent intercellular gaps, through which the perivascular spaces are continuous with the intercellular meshes of the medullary tissue. Lymphocytes appear to pass freely between the perivascular spaces and medullary parenchyma through the gaps. The static images, however, are not sufficient to determine the direction in which lymphocytes move. Nevertheless, in the light of the previous finding that in the acutely involuted thymus lymphocytes which are depleted from the cortex and migrate into the medulla are seen accumulated in large numbers in the spaces around the medullary veins (ITO and HOSHINO 1962), it is most likely that lymphocytes pass from the medulla by way of the perivascular spaces. Concerning the further fate of lymphocytes within the perivascular spaces, it is reported, on one hand, that lymphocytes directly reach the blood stream through diapedesis across the vascular walls (SAINTE-MARIE and LEBLOND 1958, 1964). On the other hand, however, it is maintained that diapedesis is not observed in the thymus and that the
lymphatic vessels appear to be a main route for the transfer of lymphocytes (SMITH 1955; KOTANI, SEIKI, YAMASHITA and HORII 1966). In the present observations, no evidence has been obtained suggesting direct entry of lymphocytes into the blood stream across the walls of the veins. However, by studying in limited numbers of sections observed by electron microscopy the possibility of diapedesis of lymphocytes across the venous wall cannot entirely be denied, because it may be rather difficult to encounter lymphocytes just penetrating the vessel wall.

Summary

The vascular pattern of the thymus of the mouse was observed with the light and electron microscope. The present finding of the general vascular pattern was essentially the same as previously reported by SMITH, THATCHER, KRAEMER and HOLT (1952). The thymic vessels in the cortex are, for the most part, capillaries and those in the medulla, venules and veins. In electron microscopy the cortical capillaries possess the same structural features as are generally known for common capillaries. The cortical capillaries are surrounded by pericapillary spaces. The spaces are bordered by a layer of cytoplasmic extensions of epithelial reticular cells. However, since the lining epithelial cell layer is not continuous with frequent interruption, the pericapillary spaces communicate directly with the intercellular meshes of the cortical tissue. Therefore, no morphological evidence suggests complete isolation of lymphocytes from the blood vessels. In the medulla, the veins are also surrounded by a wide space. The space is limited by a layer of epithelial reticular cells, but the epithelial layer often possesses intercellular gaps, by which the perivascular spaces communicate with the intercellular meshes of the medulla. The finding is discussed with regard to transfer of thymic lymphocytes.
References


