PRESENCE OF ENDOTHELIAL FENESTRATIONS IN THYMIC CAPILLARIES OF MICE

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INTRODUCTION

The existence of a blood-thymus barrier which prevents passage of antigen into the thymic parenchyma has been postulated (1). The structure of thymic capillaries is, therefore, of considerable interest. It had been suggested that the capillaries in the thymus represented an unusually closed

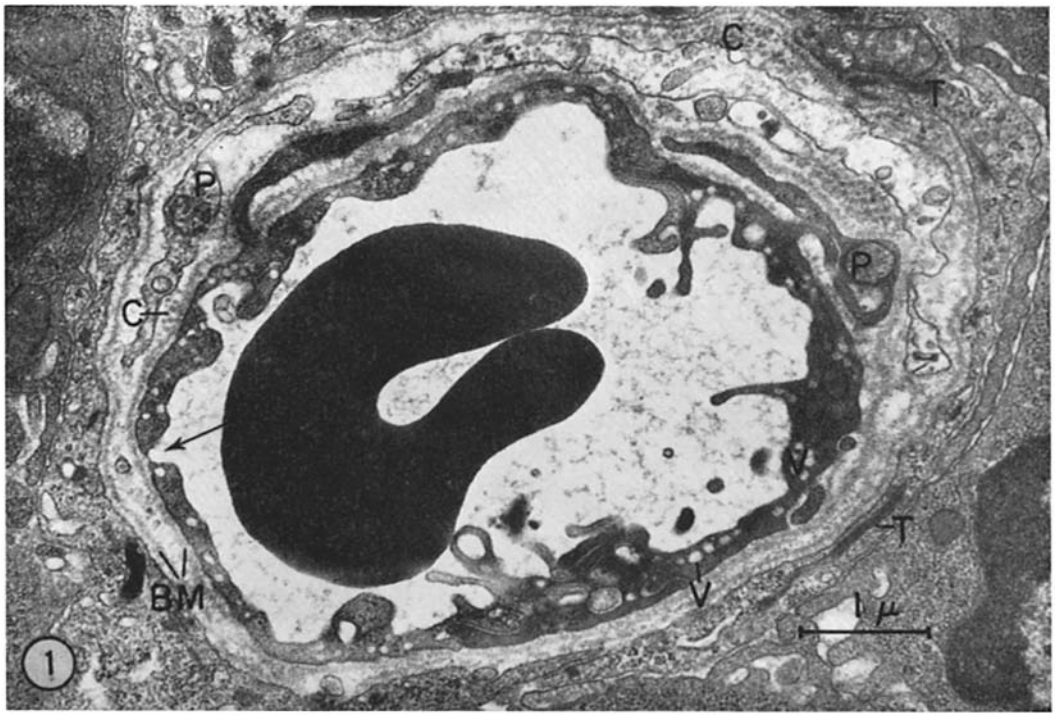

FIGURE 1 Cross-section of a thymic capillary lined by four endothelial cells. An erythrocyte lies in its lumen. One endothelial cell shows a fenestration (arrow). Note the micropinocytic vesicles (*V*) in the endothelial cells and the marginal flaps near cell junctions. One basement membrane (*BM*) encloses the endothelium; another lines the epithelial cells surrounding the capillary. Pericytes (*P*) and collagen fibrils (*C*) are seen between the two basement membranes. Tonofibrils (*T*) are seen in the epithelial cells. × 17,500.

system which formed a barrier to the movement of molecules between the blood vessels and the thymic parenchyma (2, 3). However, in ultrastructural studies of mouse thymus tissue we have observed that the capillaries of the thymuses are structurally no different than those of other organs with respect to the presence of fenestrations in their endothelial cells.

MATERIALS AND METHODS

The thymuses of ICR/Ha Swiss mice were fixed at 4°C for 2 hr in 5% glutaraldehyde buffered with 0.1 M phosphate buffer (pH 7.4) or in the formaldehyde-glutaraldehyde fixative described by Karnovsky (4). In either case, washing in a 0.1 M phosphate buffer (pH 7.4) containing 0.2 M sucrose for 24 hr was followed by postfixation with 2% osmium tetroxide in Millonig's phosphate buffer (5) at pH 7.4.

The tissue was then dehydrated in ethanol and embedded in Epon 812. Sectioning was carried out with a Porter-Blum MT 2 Microtome, and sections were stained with methanolic uranyl acetate (6) and lead citrate (7). Sections were examined in a Hitachi HU 11-A electron microscope at 75 kv.

OBSERVATIONS

The thymuses of 15 mice of various ages were studied. Endothelial fenestrations were observed in the thymuses of two newborn mice, one 12-day-old mouse, one 45-day-old mouse, and two 70-day-old mice.

Capillaries which exhibited fenestrated endothelium were usually of intermediate size and were found both in the capsule and in the thymic parenchyma. The fenestrations were approximately 300–470 A in diameter, and the closing membranes were about 65 A thick.

The fenestrations were usually located some distance from cell junctions. They occurred in regions of attenuated cytoplasm of the endothelial cells which were 500–1900 A thick. The cytoplasm

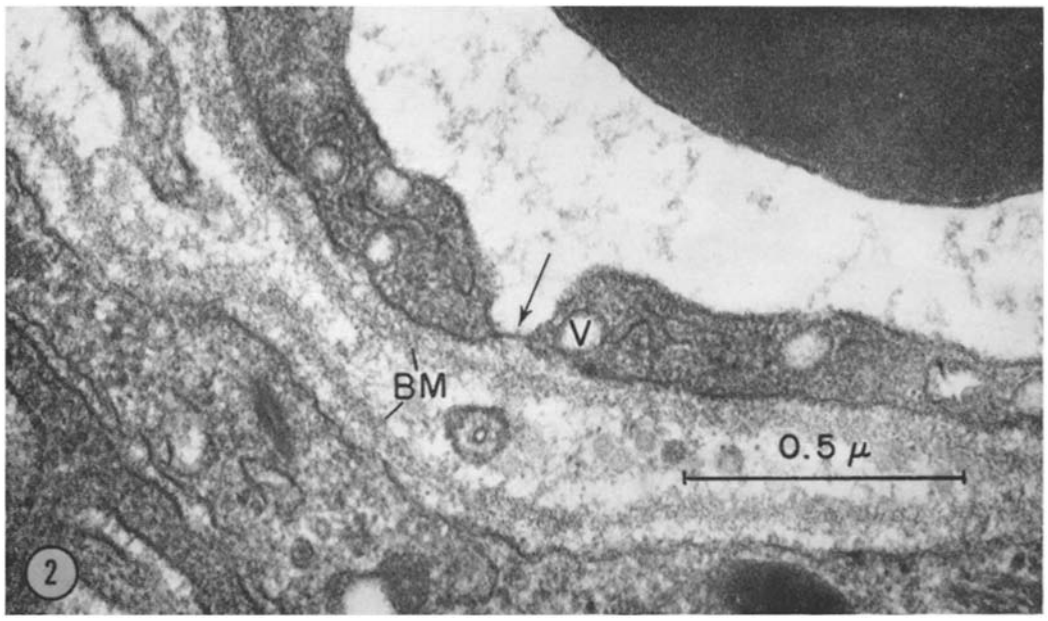

FIGURE 2 Portion of the same area containing the fenestration. The erythrocyte is seen in the upper right corner. The fenestration (arrow) is closed by a thin membrane. Micropinocytic vesicles (*V*) are seen in the endothelial cell. The two basement membranes (*BM*) shown in Fig. 1 are in close proximity with each other in this region. × 75,000.

in these regions was usually free of organelles. The basement membrane which lined the outer margin of the endothelium sometimes filled the indentation made by the fenestration up to the closing membrane.

Fig. 1 is a low magnification electron micrograph showing a capillary containing an erythrocyte. The capillary is lined with an endothelium made up of four endothelial cells, one of which shows a typical fenestration (arrow). The endothelium is surrounded by a basement membrane, pericytes, and collagen fibrils. The entire capillary is surrounded by thymic reticular epithelium cells, containing tonofibrils. The epithelial cells are separated from the perivascular connective tissue of the capillary by a basement membrane.

Fig. 2 is a higher magnification of the region of the capillary endothelium containing the fenestration. The diameter of the fenestration (arrow) is 470 A and the thickness of the closing membrane is 65 A. The cytoplasm of the endothelial cell near the fenestration is approximately 1900 A thick. The basement membrane surrounding the capillary endothelium and another basement membrane which separates the thymic reticular epithelium cell from the connective tissue of the capillary are in close proximity to each other in this region.

DISCUSSION

Marshall and White (1) observed that the cells of the thymuses were incapable of producing antibodies under normal conditions, but were able to do so when stimulated by direct inoculation of antigen intrathymically. It was inferred from this and vital dye experiments that a blood-thymus barrier existed which prevented the passage of antigens from the blood to the thymic parenchyma.

Clark (2) reported that thymic capillaries were not unusual, except for a somewhat thicker endothelium containing fewer endothelial vesicles. The "hemato-thymic barrier" was attributed to a continuous layer of epithelial cells and basement membrane between the lymphoid cells and the connective tissue.

Weiss (3) reported that the fine vessels in the cortex of mouse thymuses appear "unusually competent." He pointed out that their endo-

thelium forms a complete cellular lining without apertures.

In contrast, our observations on the thymic capillaries of mice of various ages reveal the presence of fenestrations in the endothelium. These fenestrations have been described for the capillaries of a variety of tissues and are thought by some to have a function in capillary exchange of water and solutes (8). This has not been definitely established. Rhodin (8) suggested that the fenestrations permit free passage of water and solutes of limited molecular size. Luft (9), however, cautioned that if the fenestration is composed of the classical triple-layered membrane consisting of a lipid bimolecular leaflet core with two protein or mucopolysaccharide outer layers, this structure should inhibit water movement to a great extent. He suggested that the closing membrane of the fenestration is formed by the fusion of only the external of the three layers of both the luminar and basal cell membranes and is, therefore, lacking in the hydrophobic lipid layer. If this is correct, the fenestrations may be highly permeable to water and small molecules in solution. Farquhar and coworkers (10) found that ferritin would pass through endothelial fenestrations in glomerular capillaries. Pappas and Tennyson (11) observed that fenestrations of the capillary endothelium in the choroid plexus and in the ciliary body of the eye were impervious to colloids such as thorium dioxide, colloidal gold, and saccharated iron oxide.

Kouvalainen and Gitlin (12) have recently questioned the existence of a blood-thymus barrier. They found that isotope-labeled albumins, when injected intravenously, passed readily into the extravascular spaces of the thymus. Their data, coupled with the morphological observations presented here, suggest a reconsideration of the concept of a stringent vascular-parenchymal barrier in the thymus. If thymic capillaries are fenestrated, as demonstrated in this report, and if, as suggested in the literature, these fenestrated capillaries are permeable, then the blood-thymus barrier, if it exists, must lie beyond the capillary endothelium.

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