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AN IMMUNOELECTRON MICROSCOPIC STUDY OF VON WILLEBRAND FACTOR IN THE THORACIC DUCT ENDOTHELIUM OF RATS

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ABSTRACT

The localization of Von Willebrand factor-like immunoreactivity (VWFI) in the thoracic duct endothelium was investigated by means of preembedding immunoelectron microscopy. In the endothelial cells, VWFI was observed within rod-shaped Weibel-Palade bodies (WPBs) with an occasional tadpole-like appearance. The WPBs occasionally appeared to be associated with Golgi complexes of the endothelial cells. Some cisternae of the rough endoplasmic reticulum located in the perikarya of the endothelial cells also exhibited VWFI. There was a small number of large VWFI vesicles in the periphery of the endothelial cells. These findings provide morphological evidence for the thoracic duct as a site of VWF synthesis and suggest further that VWF is produced in the rough endoplasmic reticulum and transported into the WPBs and large vesicles occasionally via the Golgi apparatus.

Von Willebrand factor (VWF) bound to plasma Factor VIII has been shown to act as a stabilizer of Factor VIII (1-3). VWF liberation from Factor VIII results in activation of the factor, and thus facilitates local coagulation of the blood (1-6). By light- and electron microscopic immunohistochemistry, VWF has been localized to vascular endothelium (7-10) in agreement with a previous *in vitro* study (11). Several light microscopic immunohistochemical studies have demonstrated that VWF is also present in lymphatic endothelium (12,13). Although an electron microscopic study has suggested that the Weibel-Palade body is a site of VWF storage and/or production in the endothelium of collecting lymphatic vessels (14,15), the precise subcellular location of VWF in lymphatic endothelium still remains to be determined. The present study was designed to investigate the distribution of VWF-like immunoreactivity (VWFI) in the endothelium of the rat thoracic duct by preembedding immunoelectron microscopy.

MATERIALS AND METHODS

Seven Wistar male rats weighing 200g were housed at constant temperature (20°C) in a 12 hour on, 12 hour off light-dark cycle, and given foot and water ad libitum. Each animal was deeply anesthetized with pentobarbital and perfused transcardially with 100ml of saline, followed by 200ml of 4% paraformaldehyde-0.05% glutaraldehyde-0.2% picric acid in 0.1M phosphate buffer (PB) (pH 7.4) (16). Following perfusion, the thoracic duct was quickly removed and cut into tissue blocks. The tissue blocks were sectioned at 60μm with a Dosaka microslicer. The sections were washed five times with phosphate buffered saline (PBS) (10 min for each washing) and processed for VWF immunohistochemistry using an antiserum against VWF (DAKOPATTS). The sections were

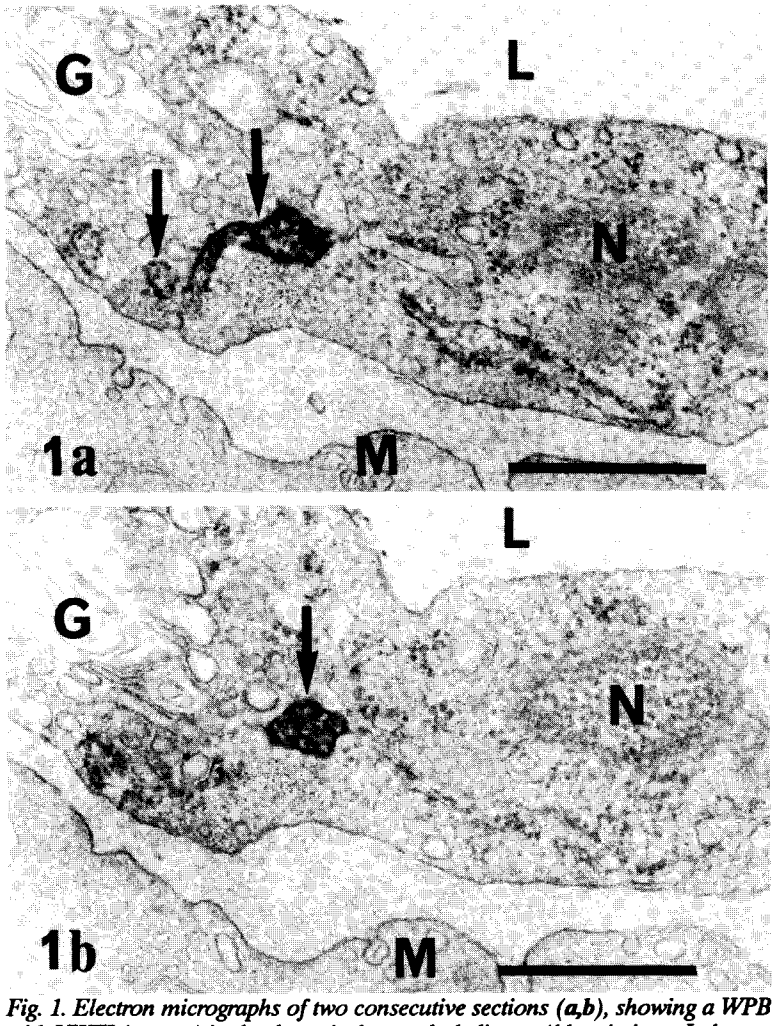

*Fig. 1. Electron micrographs of two consecutive sections (**a,b**), showing a WPB with VWFI (arrows) in the thoracic duct endothelium. Abbreviations: L, lumen of a thoracic duct; G, Golgi complex; N, nucleus of endothelial cell; M, smooth muscle cell. Bar=0.5μm; x60000.*

postfixed with 1% osmium tetroxide in 0.1M PB (pH 7.4), dehydrated in a graded series of ethanol while being stained with 1% uranyl acetate in 70% ethanol, transferred to propylene oxide and embedded in an epoxy resin. Ultrathin sections were made with a Porter-Blum MT2-B ultramicrotome and observed in Hitachi H-300 and Hitachi H-800 electron microscopes.

Control sections were first incubated with the antiserum absorbed with an excess of VWF, and then they were processed as described above.

RESULTS

In the thoracic duct endothelium, VWF-like immunoreactivity (VWFI) was observed with rod-shaped bodies with an occasional tadpole-like appearance *(Fig. 1a)*. The head of the rod-shaped bodies, approximately 0.15μm in diameter, was continuous with a thinner curved trunk that bulged slightly at the tail *(Fig. 1a)*. The rod-shaped bodies, when sectioned transversely or obliquely, showed circular or elliptical VWFI structures of varying size *(Fig. 1b)*. These bodies containing

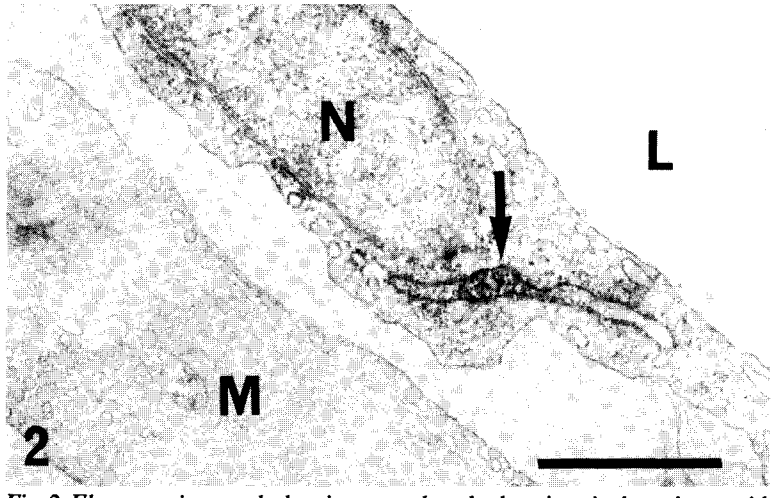

Fig. 2. Electron micrograph showing a rough endoplasmic reticulum cistern with VWFI (arrow) in the thoracic duct endothelium. Abbreviations: L, lumen of a thoracic duct; N, nucleus of endothelial cell; M, smooth muscle cell. Bar = 1μm; x28000.

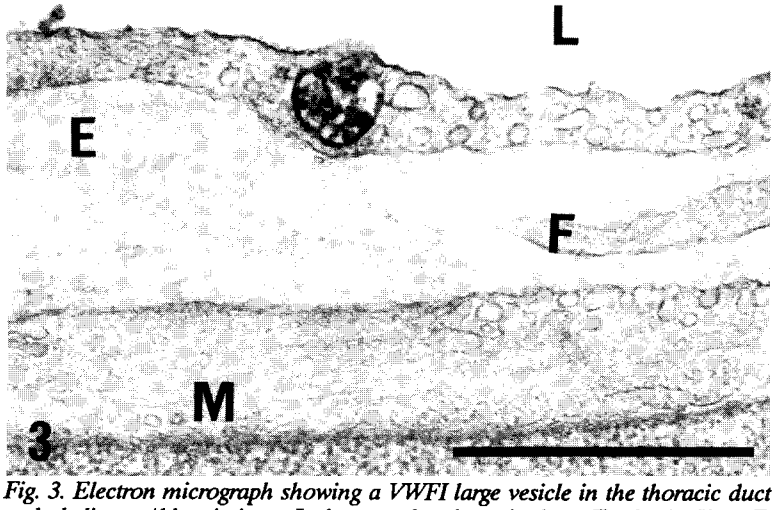

Fig. 3. Electron micrograph showing a VWFI large vesicle in the thoracic duct endothelium. Abbreviations: L, lumen of a thoracic duct; E, elastic fiber; F, fibroblast; M, smooth muscle cell. Bar = 1μm; x46000.

granular immunoreactive products were frequently located in the perikarya of the endothelial cells with occasional apposition to Golgi complexes. They were also seen in areas subjacent to the luminal and abluminal plasmalemma of the endothelium. Although the rod-shaped bodies immunoreactive for VWF seemed to be identical with Weibel-Palade bodies (WPBs) on the basis of their ultrastructural characteristics (15,17), multiple tubular elements within the bodies were rarely found in the electron micrographs (15,17) (see Discussion). Some cisternae of the rough endoplasmic reticulum in the perikarya of the endothelial cells also exhibited VWFI *(Fig. 2)*. There was a small number of large VWFI vesicles 0.3μm in diameter in the periphery of the endothelial cells *(Fig. 3)*. The large vesicles con-

tained granular immunoreactive materials which were unevenly distributed within each vesicle. Other organelles within the thoracic duct endothelium were not stained with the VWF antiserum.

Pretreatment of the antiserum with an excess of the homologous antigen eliminated immunostaining throughout the experiments.

DISCUSSION

A previous electron microscopic study has demonstrated WPBs in the thoracic duct endothelium without providing evidence for the WPBs as a reservoir of VWF (14,15). The present immunoelectron microscopic technique not only showed VWFI in the WPBs but also in the rough endoplasmic reticulum and large vesicles, as suggested earlier by immunoelectron microscopy dealing with the distribution of VWFI in blood vessel walls (8-10). This finding, together with the occasional occurrence of WPBs around Golgi complexes, suggest that VWF is produced in the rough endoplasmic reticulum and transported into the WPBs and large vesicles partly by way of the Golgi complexes (15). Moreover, the granular immunoreactive products in the WPBs and large vesicles further suggest segmental and uneven localization of VWF in these structures.

It is worth noting that the present immunoelectron microscopic staining technique has a notable limitation; it rarely visualizes tubular elements arranged in parallel within the WPBs (15,17). There are two possible explanations for this deficiency. First, because only a small amount of glutaraldehyde, which adversely affects VWF antigenicity when used in greater volume, is added to the fixative, ultrastructural features of the WPBs may be difficult to preserve satisfactorily. Second, intense VWF immunostaining in the WPBs may have obscured the tubular elements. A better fixation method, therefore, that retains both VWF antigenicity and tissue fine structures needs to be developed.

Although the functional significance of lymphatic VWF is beyond the scope of this paper, it is reasonable to speculate, nonetheless, that VWF in the thoracic duct endothelium may be involved in other physiological events apart from blood clotting.

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