Lymphology 21 (1988) 93-98

WEIBEL-PALADE BODIES IN ENDOTHELIAL CELLS OF NORMAL THORACIC DUCTS AND DEEP CERVICAL LYMPHATICS IN RABBITS

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ABSTRACT

The endothelial cells of normal thoracic ducts and deep cervical lymphatics were examined by electron microscopy using conventional staining methods and acid-phosphatase and ruthenium red (RR) reactions. The endothelial cells contained rod-shaped, circular and elliptical bodies of moderate density. The shape and structure of all these bodies were the same as those of the Weibel-Palade bodies (WPB) in the endothelial cells of blood vessels. They were usually found near the Golgi complex in groups, and the long axis of the rods paralleled the Golgi saccules. In addition, a peculiar vacuolated rod with a bulge was found adjacent to the WPB. Single coated vacuoles were occasionally located next to the WPB. Acid-phosphatase activity and RR positive material were not seen in the WPB and the vacuoles. Our observations suggest that the WPB have a close relationship, morphological as well as functional, to the Golgi complex in lymphatic endothelial cells.

It is well known that the endothelial cells of blood vessels contain Weibel-Palade bodies (WPB) (1). There have been, however, very few descriptions of WPB in lymphatic endothelial cells, and most of these have been reports of autopsy or biopsy material (2-5). Recently, immunoelectron microscopy has revealed that factor VIII-related antigen (von Willebrand factor) is present in WPB in blood vascular endothelial cells (6,7). Since von Willebrand factor has been confirmed in the endothelium of thoracic ducts and collecting lymphatics *in vitro* and *in vivo* under both normal and pathological conditions (8-12), we examined the endothelial cells of normal thoracic ducts and deep cervical lymphatics in healthy rabbits by electron microscopy and found WPB in these endothelial cells. The present study reports the fine structure and distribution of WPB in such endothelial cells in relation to the cell organellae and other cellular inclusions demonstrated by conventional staining methods, and acid-phosphatase and ruthenium red reactions.

MATERIALS AND METHODS

Thoracic ducts

The thoracic cavities of 29 adult male rabbits were opened under intravenous nembutal anesthesia. Some thoracic ducts (Group 1) were fixed *in situ* with Karnovsky's fixative. Others (Group 2) were fixed by perfusion of the ducts with 0.5% glutaraldehyde-1.5% paraformaldehyde mixture after perfusion with Ringer's solution to wash the vascular bed. Group 1 was used for structural analysis of the thoracic duct endothelium and group 2 for cytochemical demonstration of acid-phosphatase activity. After fixation segments of the thoracic ducts

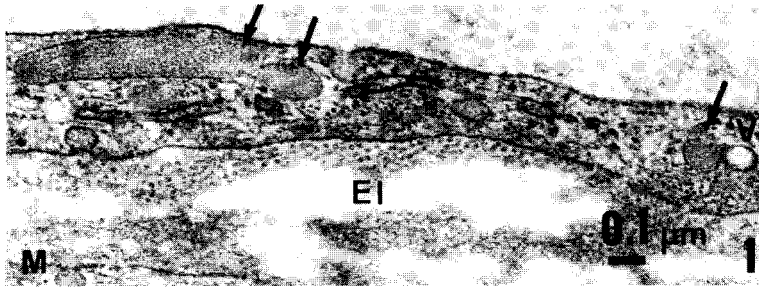

Fig. 1. Endothelial cell of a rabbit's thoracic duct. A rod-shaped and several elliptical Weibel-Palade bodies (WPB) (arrows) are seen in the cell. Tubular structures are faintly visible in the bodies. Note a coated vacuole (V) adjacent to a WPB. El: Elastic fiber; M: Smooth muscle cell. x60,000.

were removed at the 5th to 9th costal level and cut into small blocks and immersed in the same fixative for 2 hours; specimens from Group 1 were postfixed in 1% OsO_4 for 2 hours, dehydrated and embedded in an epoxy resin by conventional methods. Thin sections were stained with uranyl acetate and lead citrate. Acid-phosphatase activity was detected by the lead substrate technique (Gomori) (13) after the tissue blocks from Group 2 had been rinsed in 0.1M cacodylate buffer for 24 hours. The specificity of enzyme staining was checked in control tissue incubated in cacodylate buffer without substrate. The tissue was processed by the methods outlined for the first group, except that the thin sections were stained only with uranyl acetate.

Deep cervical lymphatics

The deep cervical lymphatics of 15 adult male rabbits were exposed under nembutal anesthesia and fixed *in situ* with Karnovsky's fixative. Distal sections of the lymphatics were excised and cut into small blocks. Two thirds of the tissue blocks were processed by the conventional methods used in the first group of thoracic ducts. The remaining blocks were exposed to ruthenium red (RR) in both the adelehyde and OsO_4 fixation stages by the method reported by Luft (14). The tissues were rinsed with buffer, dehydrated as usual through an ethanol series, and embedded in Epon. The thin sections were examined after brief staining with uranyl acetate and lead citrate.

RESULTS

Low magnification micrographs showed that the endothelial cells of both the thoracic ducts and the deep cervical lymphatics contained rod-shaped, circular and elliptical bodies of moderate density, usually in groups. These were located in the perinuclear region, often near the Golgi complex. Sometimes one or two bodies were found in the periphery of the cytoplasm, apart from the groups. They were all bound by a limiting membrane *(Fig. 1)*. High magnification micrographs revealed that the rods contained 6 to 8 straight tubules embedded in the matrix which was of a moderate, sometimes relatively high density. The tubules were regularly spaced and paralleled the long axis of the rods. The rods paralleled flattened Golgi saccules *(Fig. 2)*. They usually measured about 1.5µm x 0.1µm. In the elliptical bodies, similar short tubules were detectable *(Fig. 3)*. The circular bodies contained numerous densely-packed vesicles which were tightly bound by a limiting membrane *(Figs. 3, 4)*. Each vesicle had a distinct wall and was separated from the others by a layer of electron-dense matrix. The diameter of these circular bodies was similar to or a little larger

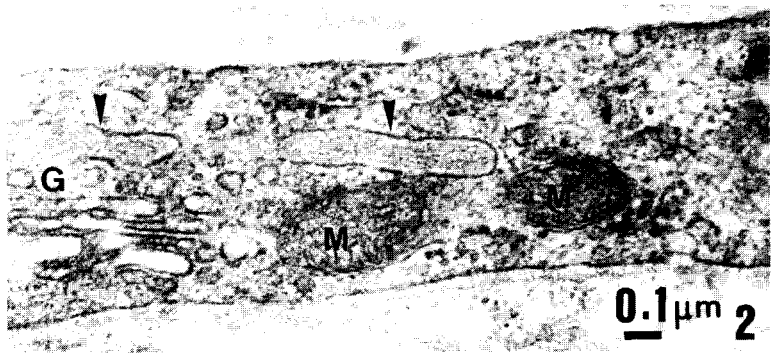

Fig. 2. Endothelial cell of a thoracic duct. Two rod-shaped WPB (arrow heads) with parallel arrangement of internal tubules are adjacent to the Golgi complex (G). M: Mitochondria. x65,000.

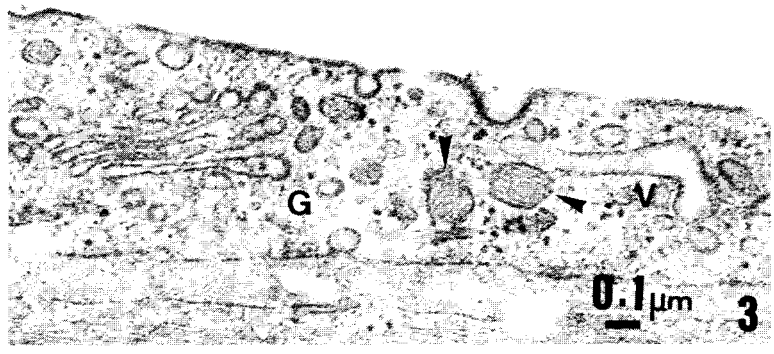

Fig. 3. WPB in Golgi zone (G) in an endothelial cell from a thoracic duct. Small membrane-bound tubules are detectable in a moderately dense matrix of the bodies (arrow heads). A rod-shaped vacuolated body showing a bulge (V) is adjacent to a WPB. The boundary membrane is coated with fine, radially oriented bristling filaments. x60,000.

than the short axis of the rods. The shape and structure of all these bodies were the same as those of the WPB in the endothelial cells of blood vessels reported by Weibel-Palade (1). Some sections showed that the structure of some rods was indistinct near their ends, but the ends themselves were distinct and sometimes contiguous with the elliptical tubule-containing bodies. These features suggest that the rods have curved endings. The tubule-containing bodies were seen near both the luminal and abluminal plasma membrane, but they were more prominent on the luminal side. They were occasionally located just beneath the luminal plasma membrane, sometimes next to the vesicles and caveolae *(Fig. 5)*. A peculiar rod-shaped body was found immediately adjacent to the elliptical bodies with a lumen which was vacuolated and quite lucent, especially at the dilated curved end, where a bulge was present *(Fig. 3)*. The boundary membrane of the vacuolated rod was coated with fine radially oriented short bristling filaments. The inner features were similar to those of the condensing vacuoles of the Golgi complex. The other end was occupied by a fine granular matrix, which might be part of the tangentially sectioned coating. The tubule-containing elliptical bodies were often located adjacent to the single coated vacuoles *(Fig. 1)*. The tubule-containing bodies and coated vacuolated bodies mentioned above

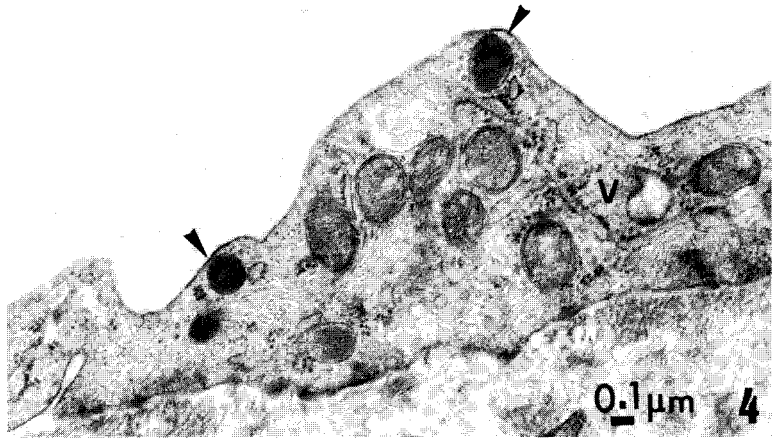

Fig. 4. Endothelial cell of a deep cervical lymphatic. Two WPB are seen just beneath the luminal plasma membrane. They contain numerous densely packed vesicles, presumably tubules in cross-section. Intracellular filaments are well developed. Note a coated vacuole (V). x35,000.

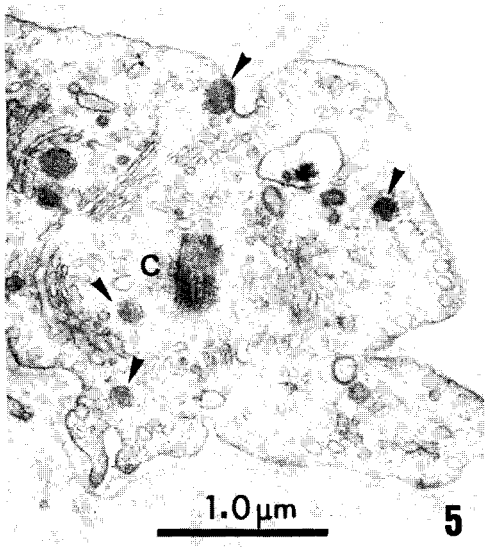

Fig. 5. Several WPB (arrow heads) in the Golgi zone of an endothelial cell of a deep cervical lymphatic. One body is located immediately adjacent to both the luminal plasma membrane and a vesicle. C: Centriole. x34,000.

were acid-phosphatase negative *(Fig. 6)*. Electron dense deposits were occasionally seen in the inclusions 0.2 to 0.5µm in diameter. They were considered to be lysosomes. The extracellular filamentous structures, caveolae of the luminal plasma membrane and vesicles in the luminal portion of the cytoplasm showed an intensely positive RR reaction, but the WPB remained unstained even though they were located next to the RR positive vesicles *(Fig. 7)*.

DISCUSSION

The results of this study clearly demonstrate that the endothelial cells of normal rabbit thoracic ducts and deep cervical lymphatics contain tubule-containing rods which are presumably Weibel-Palade bodies. They are usually found near the Golgi complex in groups. The long axis of the rods parallel the Golgi saccules. Sengel and Stoebner (15) report that the innermost cisternae of the Golgi complex contain tubular elements in the endothelial cells of blood vessels and suggest that the tubular inclusions in the cytoplasm are of Golgi origin. Our observations indicate that WPB are closely related to the Golgi complex in lymphatic endothelial cells. Furthermore, a vacuolated rod with a bulge is found adjacent to the WPB. We deduce that single vacuoles occasionally seen next to the WPB may be a cross-section of such a vacuolated rod or vacuoles budding off from such rods. Acid-phosphatase activity is not seen in the WPB or the

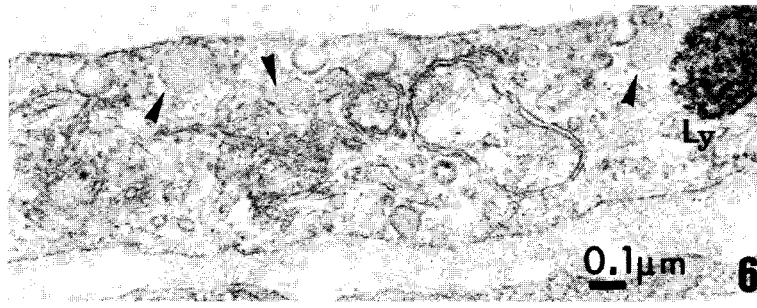

Fig. 6. Acid-phosphatase cytochemistry of a thoracic duct endothelial cell. Reaction product deposits are not seen in the WPB (arrow heads), but are present in the lysosomes (Ly) immediately adjacent to the WPB. x63,000.

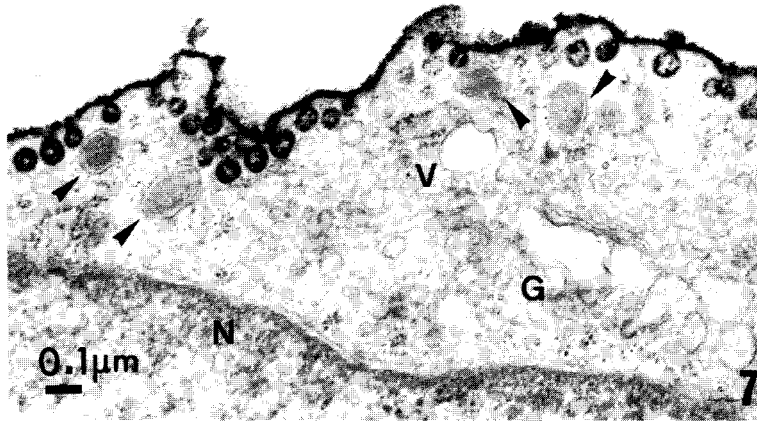

Fig. 7. Endothelial cell of a deep cervical lymphatic exposed to ruthenium red (RR). RR positive material is seen in the luminal extracellular filamentaous structures, caveolae and vesicles, while the WPB (arrow heads) adjacent to the RR positive vesicles remain unstained. G: Golgi complex; V: Coated vacuole; N: Nucleus. x60,000.

vacuoles. The relationship of the WPB and vacuoles is reminiscent of the transformation of Golgi saccules to condensing vacuoles in secretory cells.

Warhol and Sweet (7) confirmed the presence of factor VIII-related antigen (von Willebrand factor) in WPB, cisternae of endoplasmic reticulum and vesicles and vacuoles in the endothelial cells of blood vessels by immunoelectron microscopy. The presence of factor VIII-related antigen has been reported in the endothelial cells of normal thoracic ducts and of mesenteric and lacteal lymphatics *in vitro* and *in vivo* (8,11,12). We assume, therefore, that the WPB in the endothelial cells of normal thoracic ducts and deep cervical lymphatics found in this study contained factor VIII-related antigen, and that the vacuoles may be a morphological representation of a metabolic state of factor VIII-related antigen. The WPB were occasionally located immediately adjacent to the luminal plasma membrane, vesicles and caveolae. However, the WPB were RR negative, while the luminal extracellular filamentous structure, caveolae and vesicles on the luminal side were strongly RR positive. The differences suggest that a different state of factor VIII-related antigen exists within WPB and in the cellular coat. We

conclude that WPB are present in the endothelial cells of both thoracic ducts and deep cervical lymphatics under normal conditions and that they have a close relationship to the Golgi complex. The WPB and vacuoles found in this study may be functionally involved in factor VIII-related antigen and contribute to normal hemostasis.

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