

Ultrastructural features of high endothelial venules; a special form of multivesicular body or an endocrine granule?

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High Endothelial venules represent essential counterparts of lymphocyte recruitment. Ultrastructural features of the endothelial cells of these specialized vessels are of special interest. We observed and presented previously undefined organelles in the cytoplasm of rat high endothelial cells resembling multivesicular bodies or secretory granules, using zinc-iodide osmium tetroxide fixation/staining technique at electron microscopic level. [Turk J Med Res 1996; 14(1):5-9]

Key Words: High endothelial venules, Lymph node, Ultrastructure, Multivesicular bodies, Secretory granules

Leukocyte recruitment is essential for regulating immune functions and many inflammatory processes. During lymphocyte recirculation, high endothelial venules (HEVs) represent a primary site for lymphocyte migration to lymphoid organs and to connective tissues under certain inflammatory conditions. These structurally specialized vessels were first identified by Thome 1898 (1), named by Schulze 1925 (2), and recognized to be a selective site for lymphocyte migration by Gbwans 1959 (3). Though sometimes used as synonymously, HEVs are distinguished from the latter, by their histological features such as being rich in lymphocytes in their lumen, with the presence of migrating lymphocytes in their walls and especially being lined by taller (cuboidal) endothelial cells. Development and ultrastructure of these vessels is previously studied and well documented in several species (4-16). Today it is generally accepted that certain adhesion molecules regulate lymphocyte and other leukocytes migration through endothelium under certain circumstances by E-P- and L- Selectins, ICAM-1, ICAM-2, LFA-1, Mac-1, p150/95, VCAM-1, Cadherins, PECAM-1 and several MECA antibodies (17-24). In the recent years, experiments carried out using monoclonal antibodies to several adhesion molecules have answered some of the questions about this corn-

plex processes (25-29). Some of these studies were directed to HEVs (30-33).

During our examination the postnatal development of rat lymph nodes using Zinc Iodide-Osmium tetroxide (ZIO) technique with special regard to stromal elements (reticular cells and vasculature) we observed a previously undefined organelle in the endothelium of the HEVs and some capillaries which we believed to be representing a structure having a special function in these cells and regarding the importance of HEVs in lymphocyte migration we preferred to present this ultrastructural finding.

MATERIALS AND METHODS

Twenty-five adult Wistar rats at varying ages, weighing 150-200g were used to obtain tissue samples. Animals were sacrificed by decapitation under ether anesthesia and samples of lymph nodes were quickly removed. A group of specimens were immersed in the ZIO solution used by Niebauer et al. (1969) (34) and kept in the dark for 24 h in this solution at room temperature for fixation and staining as described previously (35). Then tissue samples were processed according to the routine electron microscopic embedding procedures, semi-thin (1 µm thick) and thin (70 nm thick) sections were cut; examined and photographed at light (Olymps BH2) and electron microscopes (Zeiss EM9-S2). Some other tissue samples were processed according to the routine electron microscopic procedures (prefixed with 1% glutaraldehyde in Sorenson buffer and postfixed with 2% osmium tetroxide) and examined serving as controls.

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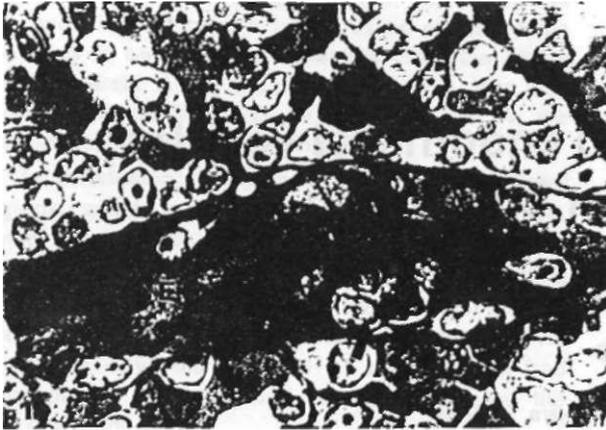


Figure 1. Semi-thin sections from the paracortex of a week old rat lymph node. A HEV with ZIO positive tall endothelial cells (E) and migrating lymphocytes in tis wall are seen (-»)Methylene blue-azur II, x40.

RESULTS

Fully developed typical HEVs were observed in the lymph nodes of the 4 weeks old rats. These vessels were easily distinguished by their tall endothelium which were usually ZIO positive and numerous migrating lymphocytes in their walls (Figure 1).

At electron microscopic level HEVs were observed to be lined by cuboidal endothelial cells which are rather rich in organelles. Surrounding these endothelial cells which rest on their basal lamina, there are several thin processes of ensheathing cells (Figure 2). Some of the endothelial cells appeared to be darker due to ZIO reaction while others did not. In addition to normal composition of organelles such as numerous granular endoplasmic reticulum cisternae, mitochondria, few lysosomes and Golgi complex. Some of these endothelial cells were observed to be containing special membrane bounded organelles resembling multivesicular bodies (MVBs) (Figure 2). These membrane bounded organelles contain varying size of ZIO positive droplets (Figure 3-inset).

At higher magnification these structures were seen to be containing droplets of varying shapes as well, though most of them were rounded (Figure 4a-b). Such structures were also observed in the endothelial lining of small capillaries or probably developing HEVs which were surrounded by huge pericytes (Figure 5).

Similar ultrastructural findings were determined in the routinely processed samples except in the identification of the above described membrane bounded organelles.

DISCUSSION

Having a special function for lymphocyte traffic, HEVs show distinct structural features and some functional

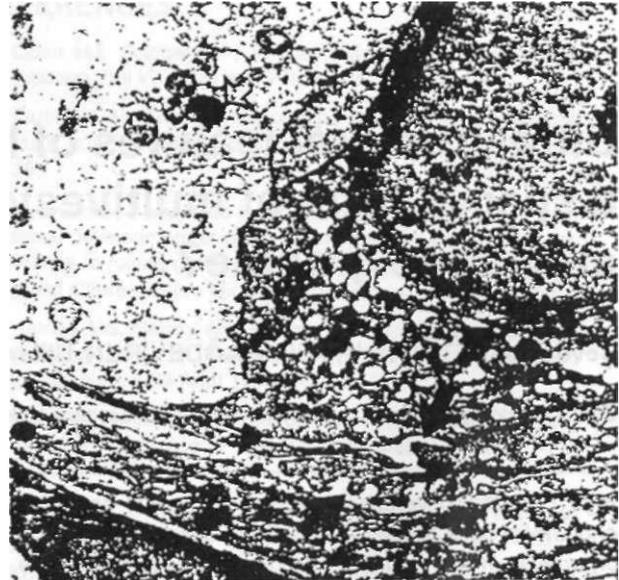


Figure 2. An electron micrograph of the basal portion of a HEV. A dark and a light endothelial cell with a MVB-like structure (=>) in its cytoplasm and several thin processes of ensheathing cells (-») were seen each surrounded by their basal lamina (>). Lead citrate-uranyl acetate, x2200.

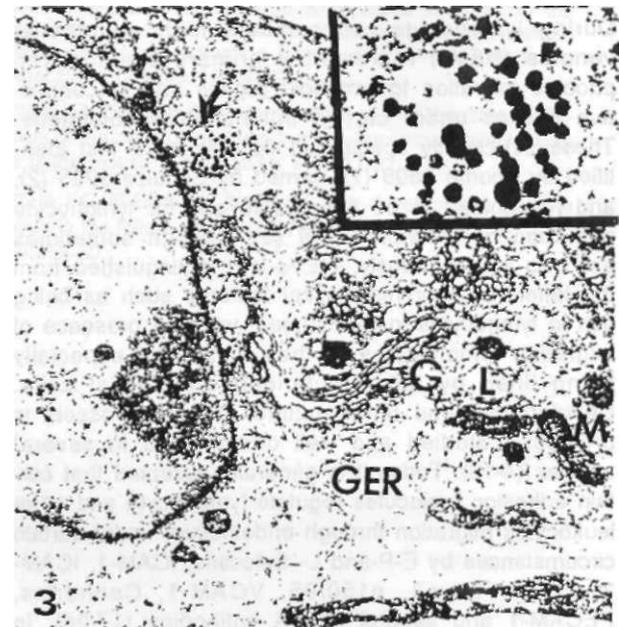


Figure 3. A section through a high endothelial cell lining HEV. In the organelle rich cytoplasm of the light endothelial cell presence of a MBV-like structure near the nucleus is seen (=>). Golgi complex (G), Mitochondrion (M), Granular endoplasmic reticulum (GER), Lysosome (L). Lead citrate-uranyl acetate, x8500.

assumptions were derived from these structural findings. With the development of monoclonal antibody techniques and immunohistochemistry, researchers

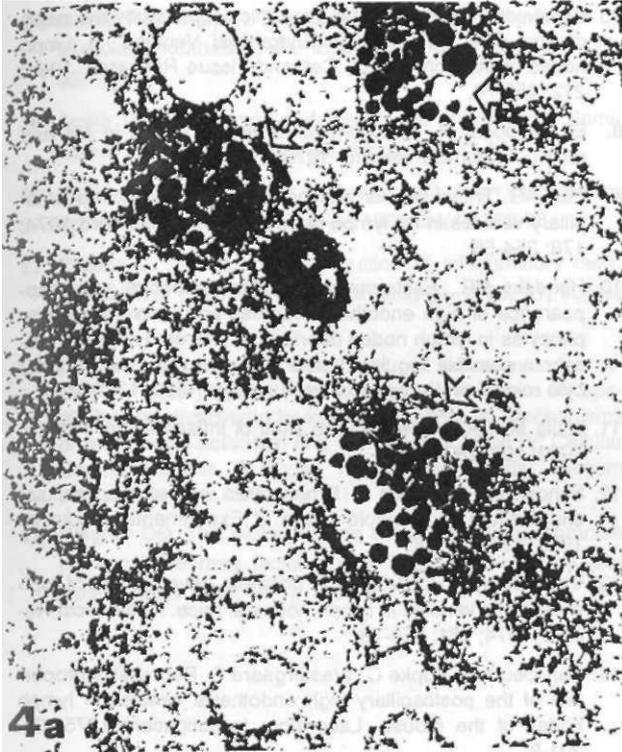


Figure 4a. High magnification of the cytoplasm of an endothelial cell is seen. Three MBV-like structures (⇒) containing varying sizes and shapes of ZIO positive droplets are distinguished.

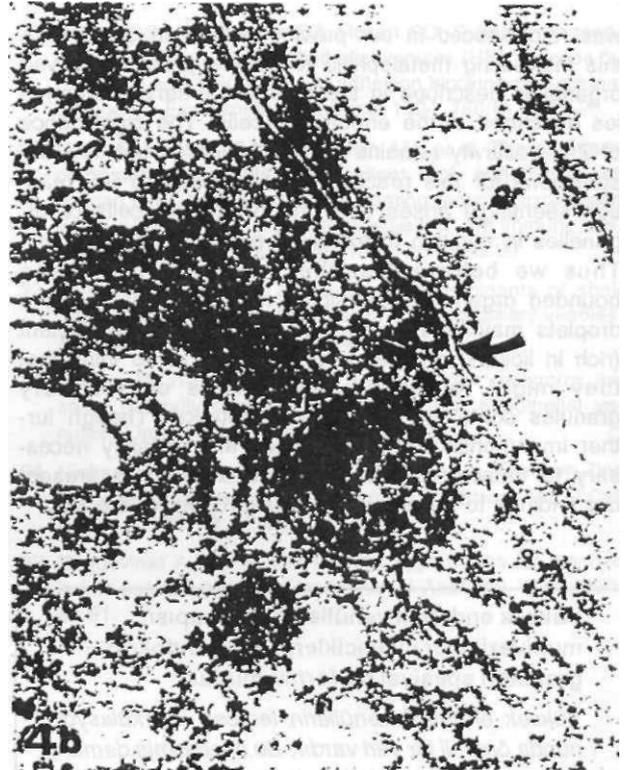


Figure 4b. A similar structure (⇒) at the border (→) of adjacent endothelial cell. Lead citrate-uranyl acetate, x7800.

have studied the adhesion molecules present on these cells and migrating lymphocytes. Many investigators ascribe special functions to the endothelial cells including those of the HEVs, for the regulation of this process such as expressing certain adhesion molecules or antigen presentation etc. Due to their ultrastructural appearances these cells can easily be considered as examples of metabolically active cell serving probably for this unique function. The presence of previously unmentioned organelle within this endothelium leads us to think that this observation may reflect a special function of the HEV lining cells morphologically. These organelles resemble MVBs but completely different cells from them in their content of ZIO positive droplets. In several other ultrastructural studies on HEVs and also in our examinations using conventional electron microscopic techniques, presence of such an organelle is not observed and/ or mentioned. In the classical histology textbooks MVBs were described as membrane bounded organelles containing many vesicles probably arising from of this organelle or a special type of secretory granule whose content should be determined immunohistochemically. As far as we know there is no specific monoclonal antibody for MVBs, thus this confirmation should be kept in mind. The ZIO technique used in the present study

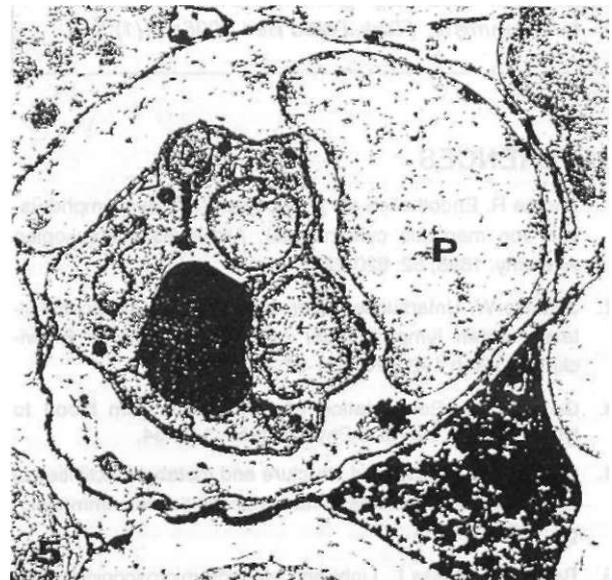


Figure 5. A section through paracortex of a rat lymph node a capillary or a developing HEV with an erythrocyte in its lumen surrounded by an huge pericyte (P) is seen, similar organelles in the cytoplasm of the some (⇒). Lead citrate-uranyl acetate, X2000.

was reintroduced in our previous report in detail (35). this interesting metalophilic technique probably served organelles described in the endothelial served organelles described in the endothelial cells. The significance of ZIO positivity remains to be clarified though few assumptions for this reaction is presented. To us, reaction seemingly arises from the pH of the cells or organelles in addition to expected positivity for the lipids. Thus we believe that the presented membrane bounded organelles containing numerous ZIO positive droplets may be reactive due to their special content (rich in lipids?) or internal pH; leading us to think that they might represent either MVBs or secretory granules containing a special substance. Though further immunohistochemical studies are certainly necessary to answer the question, we decided to present our findings to the interest of researchers of the field.

Yüksek endotelli venüllerin ince yapısı; multiveziküler cisimciklerin veya endokrin granülün spesiyal bir formu mudur?

Yüksek endotelli venüllerin lenfosit resirkülasyonunda önemli bir yeri vardır. Bu özelleşmiş damarların endotei hücrelerinin ince yapısı özel bir ilgi alanını oluşturmaktadır. Bu makalede Zinc-iodide Osmium tetroxide kullanılarak yapılan tıksasyon ve boyama tekniği sonucu, elektron mikroskopu düzeyinde saptanan sıçan yüksek endotelli venüllerinin endotei hücresi sitoplazmalarında daha önce tanımlanmamış organel benzeri multiveziküler cisim veya salgı granüllerine benzer bir yapı tanımlanmıştır. (Turk J Med Res 1996; 14(1):5-9]

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