COMPARISON OF FINE STRUCTURE OF LYMPHATICS AND BLOOD VESSELS IN NORMAL CONDITIONS AND DURING EMBRYONIC DEVELOPMENT AND REGENERATION

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It is generally accepted that the major differences in the fine structures of lymphatics and blood vessels are in the basal lamina, intercellular junctions and pericytes. The existence of Weibel-Palade bodies (WPB) (1) in the lymphatic endothelium is considered to be a timely topic in investigations of the microcirculation. In the present study, the fine structures of lymphatics were compared with those of blood vessels during embryonic development and regeneration after experimental obstruction of lymphatics (2). High endothelial venules (HEV) were also observed in relation to lymphocyte migration in lymph nodes during embryonic development (3). The immune cells in the lumina of the HEV were compared with those of the lymphatic capillaries in bronchus- and gut-associated lymphoid tissues (BALT, GALT) by immunoelectron microscopy (4,5). Marked quantitative differences were noted in the structural components of the endothelial cells in a survey of numerous electron micrographs of lymphatics in various tissues and organs. Patent junctions, lysosomes, and microfilaments were compared among the lymphatics.

MATERIALS AND METHODS

Lymphatics and blood vessels of the cervical region and cervical lymph nodes were examined in 36 rabbits, ranging in age from the 18th fetal day to the 10th postnatal month. The deep cervical lymphatics of 35 rabbits were ligated and their distal sections were examined at various intervals from 3 hours to 27 weeks after the ligation. Thin sections stained conventionally and with ruthenium red (RR) (6) were observed by electron microscopy. Adult lymphatics in the lung, heart, liver, intestines, ovary, uterus, oviduct, skin, and walls of serous cavities were stained conventionally and with acid-phosphatase reactions and examined by electron microscopy. Liver, skin, and ovarian lymphatics were compared with each other and with their accompanying blood vessels, by a morphometric method. Adult specific pathogen-free (SPF) Wistar rats were used for the immunoelectron microscopic studies of the lymphocyte subpopulations in the GALT and BALT with an immunoperoxidase technique. The following antisera were used: mouse-anti rat T cell, Ia (Selalab), IgG, IgA and IgM (all from Serotec).
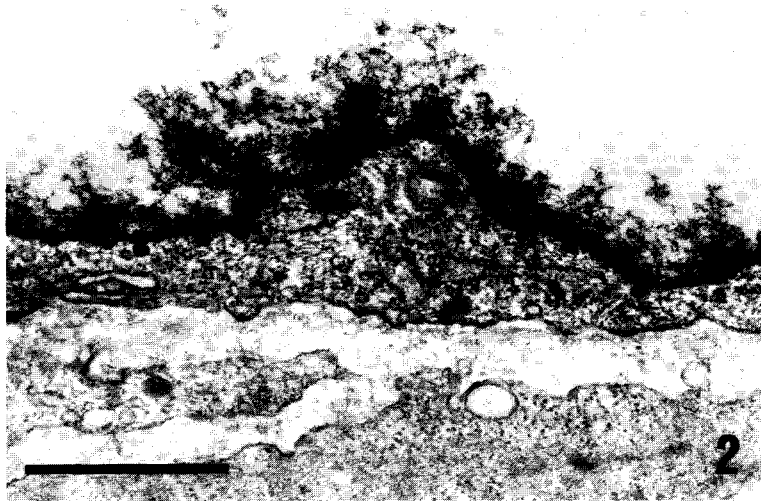
RESULTS

Comparison of lymphatics and blood vessels during embryonic development and regeneration

The fine structures of the endothelial cells of the blood vessels could be distinguished on the 18th fetal day. The endothelial cells were surrounded by a
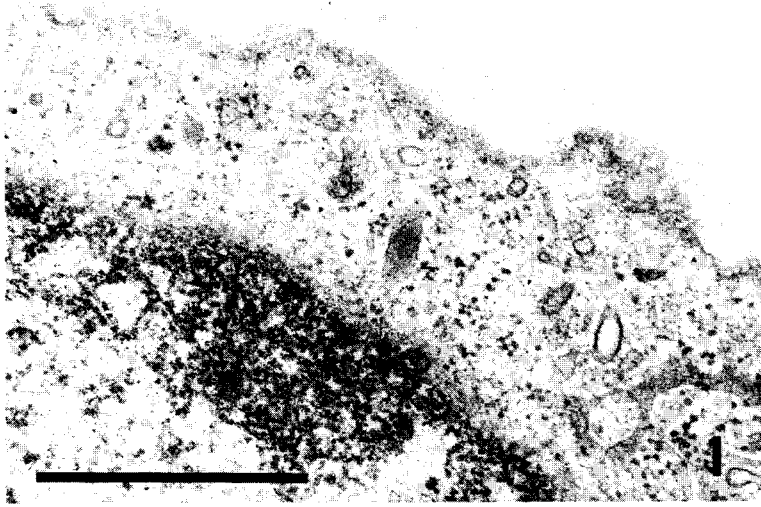
Fig. 1. Weibel Palade bodies in an endothelial cell of a rabbit lymphatic on the 18th fetal day (x40,000).

Fig. 2. An endothelial cell of a ligated lymphatic in an adult rabbit showing red ruthenium positive material on the luminal side (x30,000).

faint but continuous fuzzy coat. No such coat was detected on the vessels, where the endothelial cells were thinner, and the lumina were irregular in shape. There were, however, no definite structural features in the small vessels that could be positively identified as lymphatics at this stage. The large lymphatics, identified as cervical lymphatics, sometimes showed small aggreegations of intracellular filaments on the abluminal plasma membrane of the endothelial cells. WPB were found in the endothelial cells of the cervical lymphatics and blood vessels at this stage *(Fig. 1)*. They were usually located in the Golgi zone. Numerous free and poly-ribosomes were prominent in both vessels. Fungiform projections occasionally extended from the cell bodies into lucent mesenchymal area. The latter appeared more frequently in the small blood vessels. The fuzzy coat gradually

became a continuous basal lamina in the blood vessels on the 20th fetal day. The endothelial cells were partly surrounded by projections of the mesenchymal cells located nearby, which decreased in thickness and became pericytes. Such features were never observed in the lymphatics. At this stage, it was difficult to distinguish between the lymphatics and blood vessels by the type of intercellular junctions. Anchoring filaments appeared at a later stage.

The regeneration of lymphatics was preceded by an invasion of blood vessels into the subendothelial area of the ligated lymphatics. Newly formed lymphatic capillaries were already present one week after the ligation. But at this stage, the newly formed blood capillaries displayed some similarities to lymphatic capillaries, and differentiating them was somewhat difficult. Some showed an irregular-shaped lumen, and the adjacent endothelial cells were loosely apposed. In such cases, the continuous basal lamina and the presence of pericytes, although poorly developed, were the main features by which blood capillaries could be distinguished from lymphatic capillaries. WPB were clearly visible in the endothelial cells of both capillaries. In contrast to the vessels during embryonic development, free and poly-ribosomes were not prominent in these endothelial cells. After four weeks, both capillaries displayed their typical structural features. The characteristic features of the lymphatic capillaries were the discontinuous basal lamina and the absence of pericytes and small cytoplasmic projections on the abluminal side, where the intracellular microfilaments aggregated. Anchoring filaments were faintly and occasionally detectable at this stage. It is noteworthy that elastic fibers of both amorphous and microfibrillar components were clearly seen just beneath the endothelial cells. The RR reaction showed that, under normal conditions, the luminal side of the lymphatic endothelium was stained intensely, while the abluminal side was stained slightly and dye penetrated into the intercellular junctions from the lumi-

nal side. In blood vessels, on the contrary, RR positive material was clearly seen on the abluminal side, and the material penetrated into the intercellular junctions from the abluminal side, when the endothelium was not split open. After the ligation, the amount of RR positive material began to increase on the luminal side of the ligated lymphatics. It reached its maximum after one to two weeks *(Fig. 2)*, but appeared normal again after four weeks corresponding to the regeneration of the lymphatics. The blood vessels near the ligated lymphatics showed a marked increase of RR positive material in the endothelial cell-connective tissue interface, while the luminal side and flocculent material within the lumen remained RR negative *(Fig. 3)*.

These results indicate that the differences between lymphatics and blood vessels appear in the endothelial cell-connective tissue interface at an early stage of both embryonic development and regeneration, and WPB were already found at that stage in both vessels. Previously, we found WPB in the endothelial cells of thoracic ducts and deep cervical lymphatics in normal adult rabbits. Recent immunoelectron microscopic studies (7) have revealed that Factor VIII antigen is located in WPB. It is significant that these structures appear at a very early stage not only in blood vessels but also in lymphatics in both embryonic development and regeneration, suggesting that WPB probably play an important role in the lymphatic system.

Comparison of special types of blood vessels and lymphatics and cellular migration

HEV were clearly distinguished from other vessels on the 20th fetal day. They were characterized by their voluminous endothelial cells and extremely narrow lumina. On the 22nd fetal day, the endothelial cells were increased in number and thickness, and showed a well-developed Golgi complex *(Fig. 4)*. An especially well-developed Golgi complex was noted on the 28th fetal day, and the
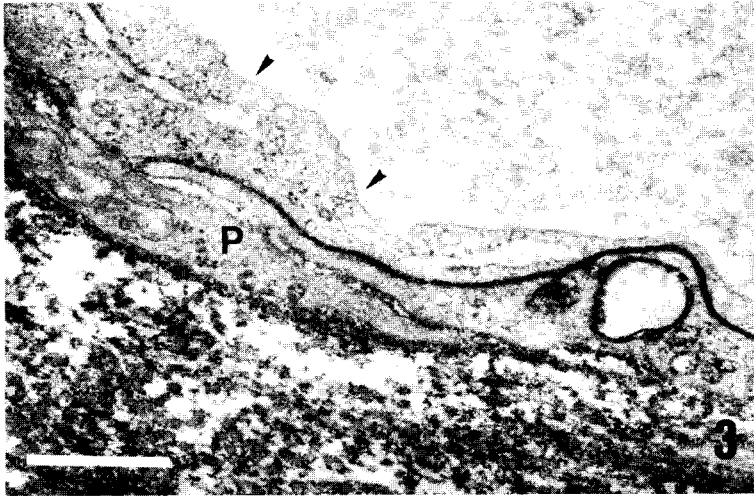
Fig. 3. Two endothelial cells of a blood vessel located near the ligated lymphatic. RR positive material is seen on the abluminal side, but not on the luminal side (arrow heads). The intercellular slit between the adjacent endothelial cells is stained with RR. P: Pericyte (x21,000).
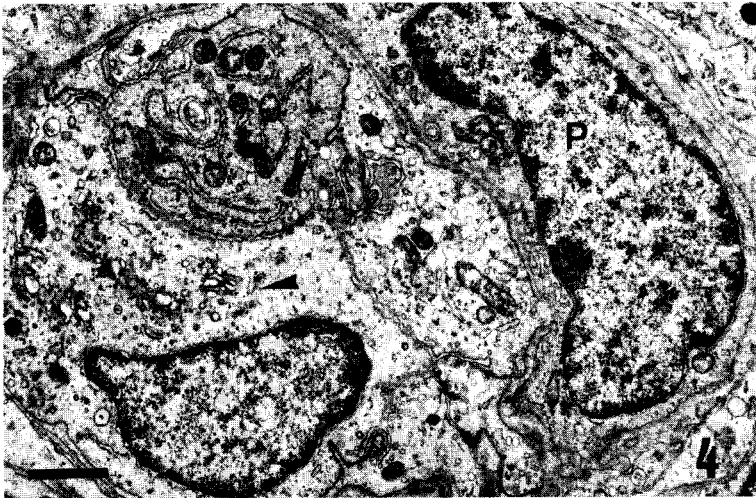
Fig. 4. HEV in a rabbit lymph node on the 22nd fetal day showing voluminous endothelial cells, extremely narrow lumen and high developed Golgi complex (arrow head). P:Pericyte (x12,000).

migration of lymphocytes into the endothelium was first observed at this stage. These results indicate that HEV are embryologically destined for a special type of blood vessel. The function of the Golgi complex of endothelial cells may involve selective capture of circulating lymphocytes. In the BALT and GALT in adult SPF Wistar rats, almost all types of immunoreactive cells were seen in both HEV and lymphatic capillaries (Figs. 5,6). Among them, however, lymphocytes with T cell antigen were the most numerous. The relative proportions of cell types varied in the BALT and GALT (4,5).

Fig. 5. Part of a HEV and a lymphatic capillary (L) in the GALT of a SPF rat. Cells with T antigen (arrow heads) are seen in both vessels (x2,000).
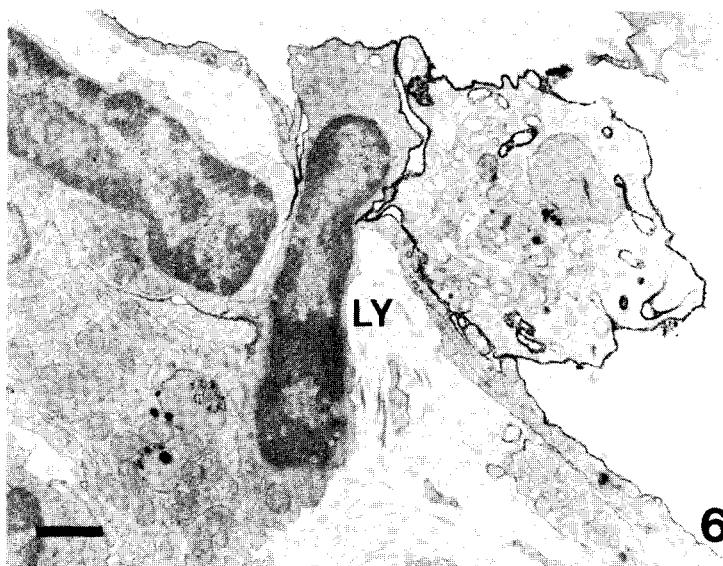
Fig. 6. Part of a lymphatic capillary in the BALT of a SPF rat. A lymphocyte (LY), and a cell with Ia-antigen are seen passing between the endothelial cells (x9,000).

A striking difference between blood and lymphatic vessels is the presence in the former and absence in the latter of fenestrated endothelium. Lymphatic endothelium never contains fenestrae, and patent junctions are rarely seen in experimental animals under normal conditions, although most junctions are loosely apposed. It was, therefore, characteristic that lymphatic capillaries in the ovary and uterus had large patent junctions during a definite period of the estrous cycle (8).

Table 1
Numerical Densities of Acidphosphatase Active Lysosomes in
Endothelium of Lymphatic and Blood Vessels (lysosomes/μm^2)

organs	lymphatic vessels	blood vessels
liver	0.28 ± 0.12	0.05 ± 0.08
ovary	0.17 ± 0.08	0.00 ± 0.01

Numerical Densities of Phagosomes and Lysosomes in Endothelium
of Lymphatic Vessels (phagosomes and lysosomes/μm^2)

organs	lymphatic vessels
liver	0.18 ± 0.14
ovary	0.13 ± 0.09
skin	0.02 ± 0.01

uranyl/lead preparation

The increasing absorptive activity of the lymphatic capillaries corresponded to an increasing number of blood capillary fenestrae controlled hormonally. It was also notable that many macrophages appeared in the edema fluid and entered the lumina of the lymphatics through the patent junctions.

Qualitative differences in the fine structural components of endothelial cells among the lymphatics

The occurrence of lysosomes in lymphatic endothelium varied considerably in different tissues and organs. In a survey of numerous electron micrographs of lymphatics in various tissues and organs, lysosomes were most frequently observed in the liver (9) and ovary, but seldom found in skin lymphatics under normal conditions. The morphometric data clearly demonstrated that lysosomes were more abundant in the lymphatics than in the accompanying blood vessels of the liver and ovary *(Table 1)*. Lysosomes and phagosomes were rare in skin lymphatics, with no differences between blood and lymphatic vessels. Marked quantitative differences were also

noted in the intracellular filaments of the endothelial cells between skin and liver lymphatics. The former contained numerous intracellular filaments of various diameters. In the latter, large coated and non-coated vesicles were prominent and intracellular filaments were few and fine. The liver and skin lymphatics showed the most distinct contrast in fine structural features. These differences suggest a variety of functional mechanisms in the lymphatics for absorbing tissue fluids and propelling lymph in the organs.

CONCLUSION

The major differences between lymphatics and blood vessels are at the endothelial cell-connective tissue interface, while the existence of WPB is similar in both. It is significant that there are marked quantitative differences of structural components in the endothelial cells among the lymphatics in different tissues and organs. High endothelial venules in lymph nodes are embryologically destined for a special type of blood vessel, and the function of the Golgi complex in the endothelial cells may play an important role in the migration of lymphocytes.

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