Lymphology 23 (1990) 135-139

HISTOLOGICAL FRAMEWORK OF LYMPHATIC VASA VASORUM OF MAJOR ARTERIES: AN EXPERIMENTAL STUDY

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ABSTRACT

We investigated the histological framework of lymphatic vasa vasorum of major arteries in the rabbit and guinea pig combining the "natural filling method" with light and transmission electron microscopy. An absorbing adventitial lymphatic network consisting of large and sparsely distributed vessels with capillary structure occupied a more external arterial wall position than blood capillaries. The latter were smaller, more numerous, densely distributed, and located closer to the arterial lumen at the media-adventitial border. Periarterial lymphatics (with the structure of absorbing lymph vessels) encircled the wall of the major arteries and formed a rich and irregular plexus. The topography and anatomic structure of these absorbing lymph vessels suggest that lymphatic drainage plays a significant role in large arterial wall homeostasis.

It has been hypothesized that lymph vessels of the arterial wall play an important role in the drainage of interstitial fluids and solutes. Some reports describe the effect of experimentally induced lymphostasis in the coronary arteries of the dog (1) and in the aorta of the rat (2), dog (3-5), and human (6). The effects vary from simple edema to loss of smooth muscle cells and elastic fibers in the media and thickening of the intima. Past studies of the architectural arrangement of arterial wall lymph vessels have relied primarily on light microscopy injecting coloring dyes, opaque or plastic substances with direct observation or inspection of corrosion casts (7-9). Unfortunately, each of these methods either fails to delineate lymphatics adequately or leads to observer misinterpretation and therefore they are best replaced by "natural filling" which relies on administered agents that increase lymph production. Unlike injection techniques, the natural filling method allows more precise assessment of the relationships of absorbing lymph vessels to parenchymal and stromal elements of organs, and therefore better comparison between morphology and topography of lymphatic versus blood capillary networks in the same general area particularly when combined with transmission electron microscopy.

MATERIALS AND METHODS

Twelve white New Zealand rabbits weighing 2-3kg and 12 guinea pigs weighing 250-350g of both sexes were ether anesthetized, and given 0.15mg/kg histamine followed by 200u/kg i.v. heparin. Five minutes after histamine administration, the ascending aorta was cannulated and the systemic circulation perfused with saline for 5 min and then Karnovsky fluid for 15 min. From each rabbit or guinea pig, three samples of thoracic aorta, common carotid, brachial, mesenteric, and

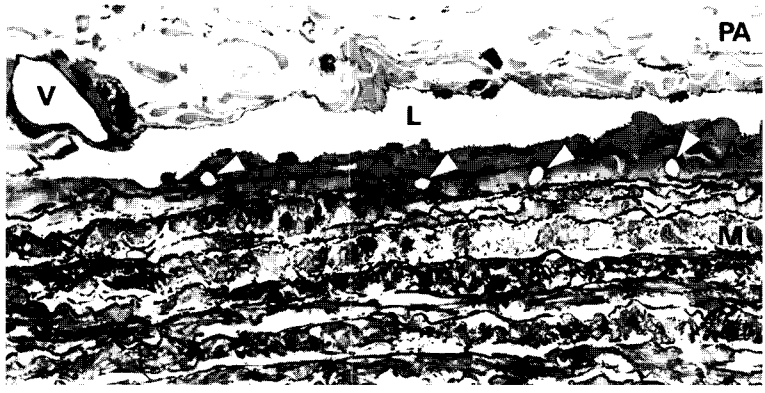

Fig. 1. A large absorbing periadventitial lymphatic in the rabbit thoracic aorta. Four blood capillaries (white arrowheads) are visible at the media-adventitia border. L = lymphatic, V = venule, M = media, A = adventitia, PA = periadventitial connective tissue. Electron microscopy confirmed that (L) was a lymphatic. Original magnification was x160.

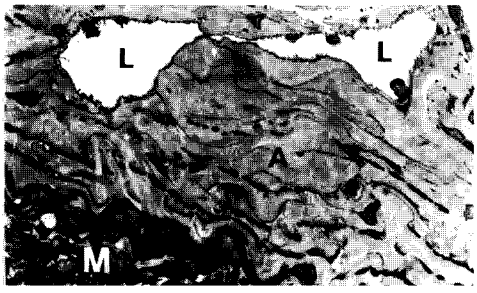

Fig. 2. A lymphatic capillary inside the adventitia. L = lymphatic, A = adventitia, M = media. Original magnification x250.

femoral artery were taken. Coronary and pulmonary artery samples were taken after perfusion began and immediately immersion-fixed. Technically, the coronary arteries and pulmonary arteries were not perfused (as the ascending aorta was cannulated and only the systemic circulation was infused). Samples were further immersed in the same fixative for 3h, postfixed in OsO_4 for 2h, dehydrated in alcohol and embedded in Epon 812. Semithin sections were stained with 1% toluidine blue and observed under a Zeiss Axioplan light microscope. All vasa vasorum observed under light microscopy were checked by electron microscopy to distinguish accurately blood from lymphatic vessels. Ultrathin sections, stained with uranyl acetate and lead citrate, were observed in a Zeiss EM09 transmission electron microscope. Other samples of the same arteries were fixed for 6h in Karnovsky fluid, embedded in methacrylate, stained with hematoxylin-eosin and observed by light microscopy. The latter samples were used to provide a global view of the presence and location of the arterial wall vasa vasorum, and therefore permit the histotopography of the vasa vasorum to be defined using semithin sections.

RESULTS

Vasa vasorum positively identified as lymph capillaries by transmission electron microscopy exhibited a larger lumen than blood capillaries and an extremely fine and irregular endothelial profile under light microscopy. Many lymph vessels were observed in large caliber arteries, but very few were detectable in smaller caliber ones. Lymphatics consistently occupied a periadventitial or adventitial position *(Figs. 1,2)* and were never observed in the arterial media or intima.

Under light microscopy, adventitial lymphatics *(Fig. 2)* were usually small but nonetheless were larger than blood capillaries. Of course, histamine pretreatment with pressure perfusion may have altered the natural state of both blood and lymph

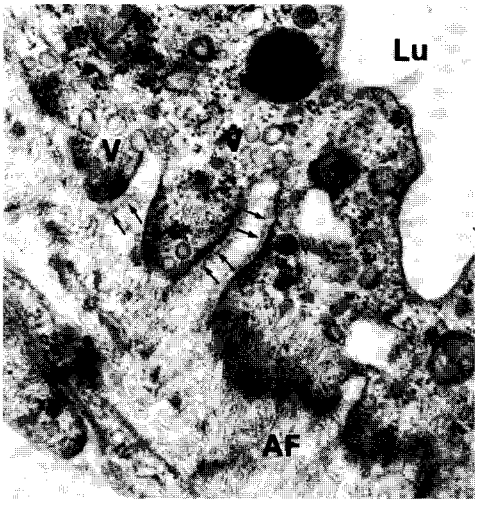

Fig. 3. Endothelial cell of a lymph capillary in rabbit carotid artery. Lu=lumen, V=pinocytotic vesicles, AF=anchoring filaments. The basement membrane (arrows) is discontinuous. Original magnification x30,000.

vessel size. Blood capillaries, which were both more numerous and smaller, uniformly occupied an inner position close to the media-adventitial border *(Fig. 1)*. The natural filling induced by histamine made possible the localization of adventitial lymphatics that otherwise would not show up in the connective tissue environment because of their extremely thin wall and tendency to collapse. Lymphatics were also present in the loose connective tissue of the periadventitial area *(Fig. 1)*. These lymphatic vessels were relatively wide, mainly laminar, richly interconnecting, and encircling the arterial wall as a sort of wrapping network. As blood vessels were rarely encountered in this area, the lymphatic periarterial plexus was distinct.

Morphometric analysis of the rabbit thoracic aortic wall showed a prevalence of blood vessels (56%) over lymphatic adventitial (19%) and periadventitial (25%) vessels. Blood vessel area was 30±5μ (mean±SE) compared with 131±36μ of the adventitial and 1683±376μ of periadventitial lymphatics.

Transmission electron microscopy confirmed the characteristics of absorbing lymphatics: the vascular wall of adventitial lymphatics consisted of a single layer of very thin endothelial cells with nuclei bulging into the lumen; the endothelial profile was uniformly irregular due to folds and luminal digitations, the basement membrane was discontinuous and absent for long stretches; there were pinocytotic vesicles and conspicuous anchoring filaments *(Fig. 3)*. The vessel wall of periarterial lymphatics did not appear to be more complex than that of the absorbing lymphatics of the adventitia and showed the same ultrastructural features *(Fig. 4,5)*.

DISCUSSION

The absorbing lymphatics of the arterial wall adventitia are likely involved in draining intramural tissue fluid and solutes. These lymphatic microvessels are larger, more scattered and more externally located than blood capillaries, which are smaller, more numerous and densely distributed deeper within the vessel wall. These differences in the arterial wall conform to the morphological and histotopographic features in the viscera as described by us previously differentiating the blood capillary and lymphatic absorbing networks (10,11). The lymphatic wrapping plexus observed around large caliber arteries (periarterial lymphatics) also simulates the characteristic structural arrangement of lymphatics around arterial branches in other organs (e.g., liver, lung, kidney, thyroid) previously graphically reconstructed (12-14). Taken together, this consistent anatomic arrangement suggests that the lymphatic vascular system constitutes a major drainage route for the arterial wall interstitium.

Some investigators (15-17) suggest that periarterial lymph vessels facilitate drainage of lipids from the arterial wall and that failure of this drainage function (e.g., lymphostasis) may contribute to atherosclerosis. Others, however (18,19), have not observed any substantial modification in the arterial wall following experimental obstruction of draining lymphatics. This discrepancy may relate to difficulties in producing local lymph stasis by

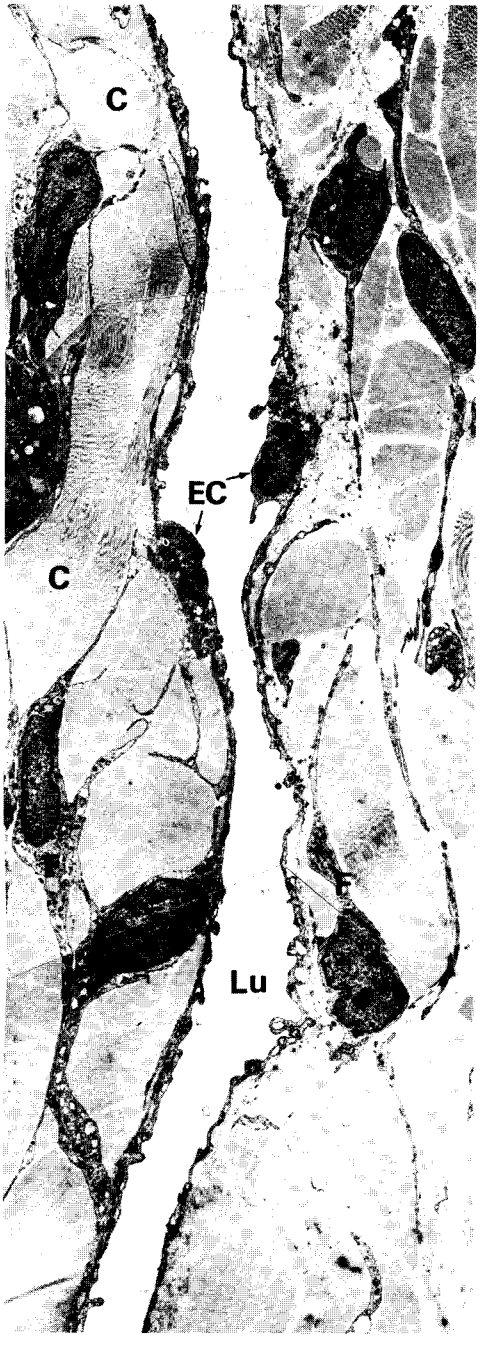

Fig. 4. A segment of an absorbing periadventitial lymphatic encircling the wall of a rabbit carotid artery like a laminar plexus. Lu = lumen, EC = endothelial cells, C = collagen bundles, F = fibroblast. Original magnification x1650.

Fig. 5. Higher magnification of the lymphatic shown in Fig. 4. *The endothelial profile is typical of lymphatic: thin and irregular with many folds and luminal digitations. Original magnification x4500.*

ligature due to the variable morphology of lymph vessel arrangement.

The salient points emerging from this study are not only the description of adventitial lymph vessels which constitute the true lymphatic vasa vasorum of the arterial wall, but also the presence of a vast periadventitial plexus consisting of large caliber absorbing lymphatics involved in the drainage of the periarterial interstitial environment.

ACKNOWLEDGEMENTS

The authors thank Mrs. D. Orazioli and A.M. D'Errico for excellent technical assistance.

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