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The Morphology and Ultrastructure of Mesenteric Lymphatic Vessels in Mice

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Abstract **Original Research Article**

Objective: To investigate the morphological ultrastructure of mesenteric lymphatic vessels in mice. Methods: Fourteen male Kunming mice (6–8 weeks old, 18–22 g) were subjected to a 24-hour fasting period with unrestricted access to water, followed by a high-fat/cholesterol diet (comprising egg yolk and fat) 2 hours prior to sample collection. Mesenteric lymphatic vessels were harvested under microscopy for subsequent histological and transmission electron microscopy (TEM) analyses. ***Results***: The mesenteric lymphatic vessels were characterized by small diameters, thin walls composed of three distinct layers, and the presence of multiple intraluminal valves. The ultrastructure of the intima and adventitia was observed to be similar. The intima consisted of a single layer of endothelial cells with discontinuous basement membranes, and no smooth muscle cells were detected in the media. The adventitia was composed of connective tissue, including collagen fibers and fibroblasts. ***Conclusions***: This study provides a detailed delineation of the ultrastructure of murine mesenteric lymphatic vessels, contributing to the morphological understanding of this system.

Keywords: Mouse, Mesenteric Lymphatic Vessels, Mesenteric Lymph Node, Morphology, Ultrastrcture.

INTRODUCTION

The lymphatic system, a vital component of the circulatory system, plays a crucial role in both health and disease [1–3]. Although historically underexplored until the mid-19th century, advancements in modern imaging and molecular techniques have significantly enhanced our understanding of its structure and function. Intestinal lymphatic vessels are physiologically indispensable for processes such as fat absorption, immune surveillance, and peripheral lipid transport. Clinically, they are implicated in conditions like mesenteric lymphadenitis, tumor metastasis, and chylous ascites [4–6]. Tumor metastasis prompts lymphatic remodeling and increased permeability, with intratumoral or peritumoral lymphatic density being associated with malignancy and patient prognosis [7–13]. Nonetheless, current research, which predominantly relies on immunofluorescence and immunohistochemistry (IHC), is insufficient in revealing the complete ultrastructural details of lymphatic vessels beyond capillaries. Recent in vivo imaging techniques, such as indocyanine green (ICG) and magnetic resonance imaging (MRI), are restricted to visualizing luminal structures and lack the resolution necessary for detecting full-thickness anatomical changes [14]. Consequently, further ultrastructural investigations are necessary.

In mesenteric lymphatic research, while large animal models are predominantly utilized, mice present distinct advantages in terms of genetic manipulability and cost-efficiency. Nevertheless, the absence of standardized protocols for labeling murine mesenteric lymphatics poses a significant barrier to comprehensive morphological characterization. Previous studies in rats have successfully visualized mesenteric lymphatic vessels through a "post-fasting high-fat feeding" method. In contrast, distinguishing murine mesenteric lymphatics from vasculature using transmission electron microscopy (TEM) is challenging due to their smaller size and thinner smooth muscle layers. To overcome this limitation, we arranged three mesenteric lymphatic vessels of the mice in parallel into a bundle and ligated them at the middle and two ends for detailed ultrastructural examination. Our research endeavors to elucidate the ultrastructure of lymphatics and establish a morphological framework for investigating interactions between tumors and lymphatic systems.

MATERIALS AND METHODS

All procedures complied with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care Committee of Xuzhou Medical University.

1. Animals and Preparation: Fourteen male Kunming mice (6–8 weeks, 18–22 g) were fasted for 24 h (water ad libitum) and fed egg yolk and fat 2 h before sampling.
2. Sample Collection: Mice were anesthetized with 10% chloral hydrate (0.35 mL/100 g, intraperitoneal). Mesenteric lymphatic vessels were dissected, fixed in 4% paraformaldehyde (histology) or 4% paraformaldehyde+2% glutaraldehyde (TEM), and then, the mesenteric lymphatic vessels of the three segments of mice taken from the dissection were arranged into a bundle and ligated at the middle and two ends respectively.
3. HE Staining: Paraffin sections were dewaxed, rehydrated, stained with hematoxylin-eosin (HE), and imaged.
4. IHC Staining: Sections underwent antigen retrieval (citrate buffer, pH 6.0), endogenous peroxidase blockade (3% H_2O_2), and blocking (5% BSA). Primary antibody (LYVE-1, 1:400, Abcam) was applied overnight, followed by HRP-conjugated secondary antibody (1:50, Hongda Biotech) and DAB visualization.
5. TEM: Fixed samples were postfixed in 1% osmium tetroxide, dehydrated in acetone, embedded in resin, sectioned, and stained with uranyl acetate/lead citrate.

RESULTS

In a gross examination, 2–3 mesenteric lymphatic vessels were observed accompanying the mesenteric arteries and veins (Fig. 1), converging into the mesenteric lymph nodes (*). Immunohistochemistry (IHC) analysis confirmed the presence of LYVE-1-positive endothelial cells (Fig. 2D–F). Hematoxylin and eosin (HE)-stained cross-sections revealed irregularly shaped lumens lined by flattened endothelial cells with spindle-shaped nuclei, and the absence of smooth muscle on the wall or erythrocytes in the lumen was noted (Fig. 2A–C). The lymphatic vessels exhibited smaller lumens, thinner walls, and incomplete muscular layers in comparison to the adjacent vasculature. Transmission electron microscopy (TEM) demonstrated luminal diameters of approximately 30 μm (Fig. 3A), endothelial cells with discontinuous basement membranes, and various intercellular junctions, including tight, adherens, and desmosomal junctions (Fig. 3A–D). Additionally, plasmalemmal vesicles, mitochondria, rough endoplasmic reticulum, and lysosomes were identified within the endothelial cytoplasm (Fig. 3B, D). Valves were also present within the lumen (Fig. 3C).

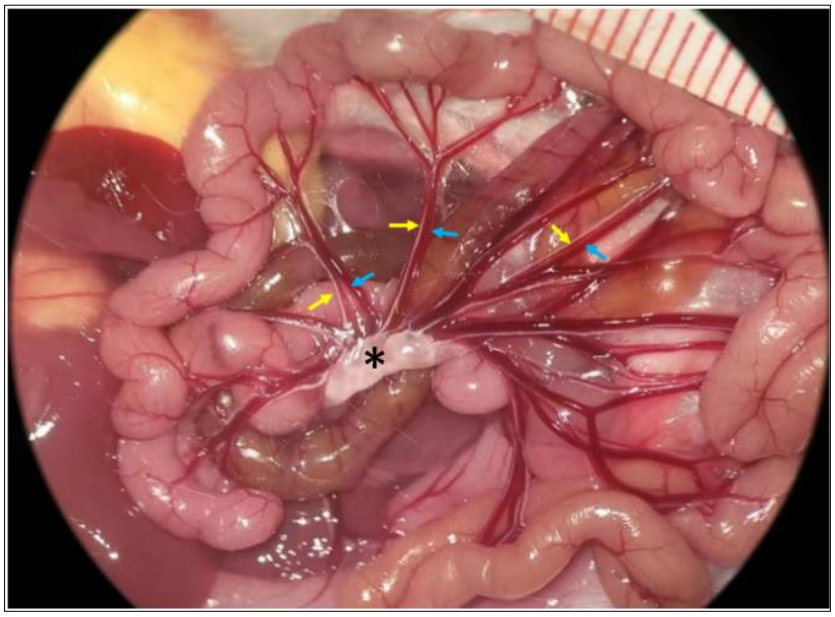

Figure 1: **Gross morphology of murine mesenteric lymphatics**

*=mesenteric lymph nodes. The yellow arrows point to lymphatic vessels, and the blue arrows point to blood vessels that accompany the lymphatic vessels.

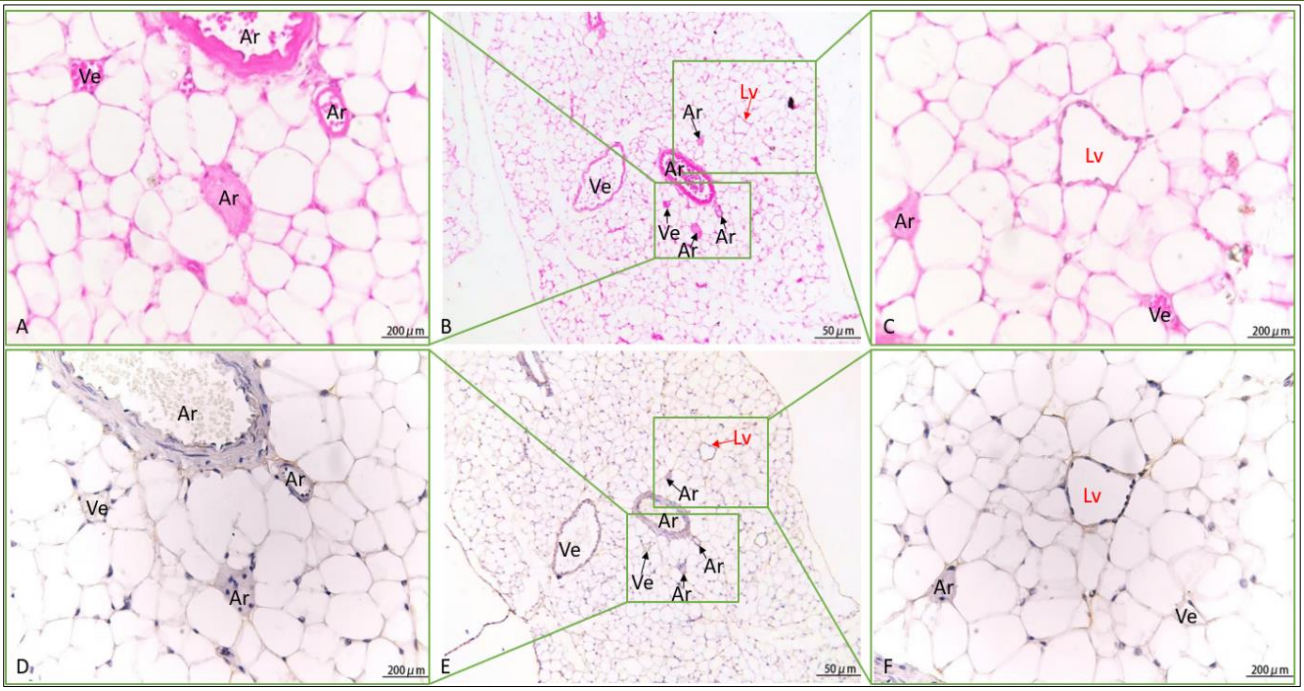

Figure 2: Histological and immunohistochemical characterization of murine mesenteric lymphatic vessels

(A–C) H&E-stained cross-sections showing mesenteric lymphatic vessels (Lv) adjacent to mesenteric arteries (Ar) and veins (Ve), with thin-walled lumens lined by flattened endothelial cells. (D–F) Immunohistochemical staining for LYVE-1 highlights lymphatic endothelial cells with distinct brown-yellow signals. The red arrows point to lymphatic endothelial cells.

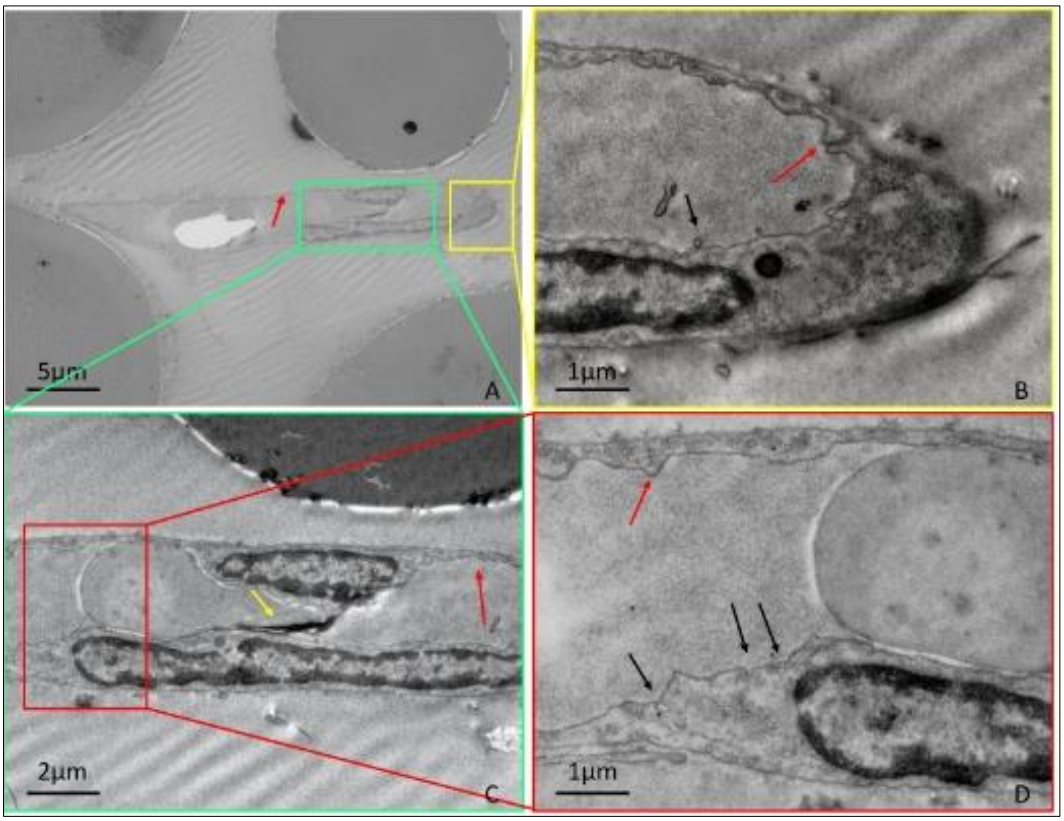

Figure 3: Transmission electron microscopy (TEM) of murine mesenteric lymphatic vessels

(A–D) Ultrastructural features of lymphatic vessel walls. Red arrows: Endothelial cell layer with discontinuous basement membranes and intercellular junctions. Yellow arrows: Intraluminal valve structures. Black arrows: Plasmalemmal vesicles within endothelial cytoplasm.

DISCUSSION

The "lymphatic bundle ligation method" used in this study effectively overcame the technical difficulties caused by the small lymphatic vessel samples in mice. Notably, murine mesenteric lymphatics are devoid of medial smooth muscle cells, a characteristic that distinguishes them from those in humans and other rodents, such as rats [15, 16]. This structural difference highlights the need for caution when extrapolating preclinical findings to human contexts. Unlike vascular endothelia, which possess continuous basement membranes and tight junctions, lymphatic endothelia exhibit discontinuous basement membranes and heterogeneous junctions. These features may facilitate the uptake of interstitial fluid and the infiltration of tumor cells. The presence of open plasmalemmal vesicles suggests a potential role in macromolecular transport, which necessitates further functional validation. A limitation of this study is the reliance on static ultrastructural images, which do not allow for real-time observation of lymphatic dilation during metastasis. Future research should aim to correlate ultrastructural characteristics, such as endothelial microvilli density, with dynamic imaging and genetic models, including junctional protein knockouts, to better understand metastatic mechanisms. This study provides foundational insights into the ultrastructure of murine mesenteric lymphatics, with significant implications for surgical lymphatic preservation and tumor metastasis research.

CONCLUSION

This study, employing an innovative lymphatic bundle ligation technique in conjunction with electron microscopic analysis, systematically elucidates for the first time the ultrastructural features of mouse mesenteric lymphatic vessels. The findings demonstrate that these vessels are characterized by a three-layered thin-walled structure devoid of a smooth muscle layer, with endothelial cells exhibiting a discontinuous basement membrane and open vesicular profiles. These observations provide critical morphological insights into the physiological functions of the lymphatic system and the mechanistic underpinnings of tumor metastasis.

Declaration of Interests: We declare no competing interests.

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