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LYMPHOGRAPHIA

LYMPHATIC VESSELS IN HUMAN SURAL NERVE: IMMUNOHISTOCHEMICAL DETECTION BY D2-40

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ABSTRACT

Lymphatics were detected in the epineurium of the human sural nerve by D-240 immunostaining and confirmed by ultrastructural examination.

Keywords: human sural nerve, epineural lymphatics, D2-40, lymphatic markers, lymphatic endothelium, macrophages, peripheral neuropathy

Investigation of lymphatic vessels has been greatly enhanced by the use of specific markers for lymphatic endothelium such as LYVE-1, podoplanin, prox-1 (1) and D2-40 (2) compared to previous characterizations which relied exclusively on ultrastructural criteria (3). Classically, lymphatic vessels are present in most parts of the body with the exception of central nervous system, bone marrow, cartilage, and cornea. However, recent reports about lymphatic localization in peripheral nerves (4) and other experimental studies (5) suggest that lymphatics are actively involved in the process of peripheral nerve degeneration possibly by contributing to elimination of post-phagocytic endoneurial macrophages. Subsequently, we investigated the presence of lymph vessels in human sural nerve by immunohistochemistry and transmission electron microscopy. Biopsy specimens were surgically excised for diagnostic purposes in cases of inflammatory and non inflammatory neuropathies including 2 cases of peripheral nerve vasculitis, 4 cases of chronic demyelinating inflammatory neuropathy (CIDP), and 3 cases of axonal non-inflammatory neuropathies. Two specimens with minimal histological changes, defined as myelinated fibers density at the lower limits of normal range, were utilized as morphologically normal controls. Samples were routinely processed for light microscopy histology and immunohistology and for transmission electron microscopy. D2-40 localization was carried out by immunoperoxidase detection on 7 µm thick sections, either from formalin fixed, paraffin embedded, or snap frozen blocks. Lymphatics were detected in epineurium in both axonal and demyelinating neuropathies, as well as in nerves with minimal pathological changes. Larger lymphatics were preferentially located adjacent to blood vessels, and smaller lymph vessels were scattered in the epineurial connective tissue *(Figs.1,2)*. No lymphatics were located in endoneurium. Perineurial cells also were D2-40 positive, whereas no localization was observed in blood vessel endothelium. Ultrastructural examination *(Fig. 2)* displayed the typical features of absorbing lymphatic vessels such as a discontinuous basal membrane, anchoring filaments, absence of pericytes and numerous pinocytotic vesicles. No significant morphological

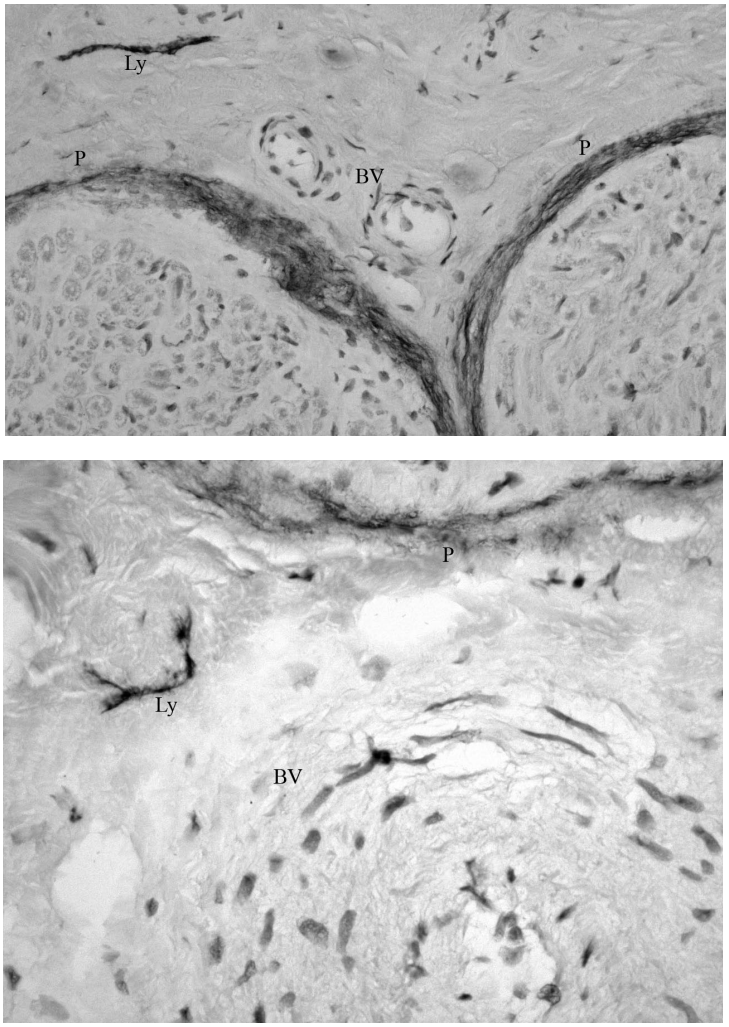

Fig. 1 Light microscopy displaying D2-40 positively stained lymphatic vessels (Ly) in epineurium. The walls of adjacent blood vessels (BV) show no D2-40 positivity. Perineurial sheath (P) is also positive. (Top: original magnification x200; bottom: original magnification x400).

changes of lymphatic endothelium were observed in association with active vasculitis of epineurial blood vessels. CD68 immunohistochemistry assessed that increased density of endoneurial macrophages was a feature consistently associated with axonal loss and myelin remodeling. In fact, resident and hematogenous recruited endoneurial macrophages are engaged in phagocytosis of myelin debris, both in inflammatory pathology (6,7) and in pure wallerian degeneration (8,9). As a macrophage-induced lymphangiogenesis via VEGF-C/D production is recognized in experimental models (10), our results suggest that this topographic immunohistochemical detection of epineurial lymphatic vessels will be a powerful tool for further investigations on the role of lymphatics in nerve pathophysiology.

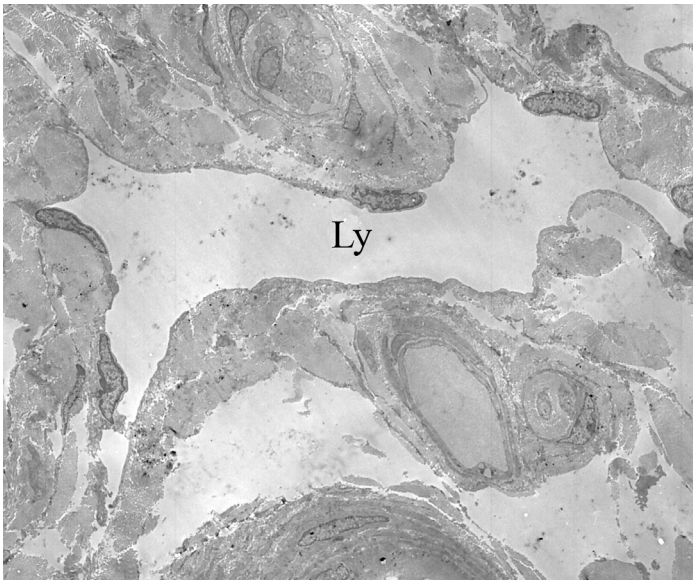

Fig. 2. Transmission electron microscopic image displaying a typical absorbing lymphatic vessel (Ly) in epineurium. The profile of the endothelium is irregular and tortuous with nuclei bulging into the lumen. (Original magnification x1200).

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