

LYVE-1 EXPRESSION ON HIGH ENDOTHELIAL VENULES (HEVs) OF LYMPH NODES

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ABSTRACT

LYVE-1 (lymphatic endothelium hyaluronan receptor) has been identified as a powerful marker for lymphatic endothelium. Apart from lymphatic endothelium, LYVE-1 is expressed in normal liver blood sinusoids, spleen endothelium and activated tissue macrophages. LYVE-1 has not been detected in blood vascular endothelium with the exception of blood vessels in the lung. High endothelial venules (HEVs) belong to the vascular compartment of lymph nodes. They are the major site of entry for circulating lymphocytes into the node. HEVs are characterized by cuboidal endothelial cells, the existence of discontinuous junctions between these endothelial cells, and the presence of large numbers of lymphocytes within their walls. 40 paraffin-embedded lymph node biopsy specimens from newly diagnosed patients with non-Hodgkin lymphoma were evaluated as well as 10 lymph node biopsy specimens from adult patients with reactive lymphadenitis, and 10 normal, non-metastatic lymph nodes obtained from adult patients during cancer surgery served as controls. Samples were fixed in 10% buffered formalin, paraffin embedded, and stained with hematoxylin and eosin for histopathological evaluation. Sections were also evaluated with mouse monoclonal antibodies against LYVE-1

and CD34, and expression of both LYVE-1 and CD34 was demonstrated in HEVs.

LYVE-1 expression was also found on the endothelial cells of the lymphatic sinus and in reticular cells in the lymph nodes.

LYVE-1 (lymphatic endothelium hyaluronan receptor) has been identified as a powerful marker for lymphatic endothelium. LYVE-1 is a 322-amino acid type-I integral membrane glycoprotein, which is 41% similar to the CD44 hyaluronan receptor. LYVE-1 expression is primarily restricted to the endothelial surface of lymphatic vessels. Apart from lymphatic endothelium, LYVE-1 is expressed in normal liver blood sinusoids, spleen endothelium and activated tissue macrophages (1,2). Until now, LYVE-1 has not been detected in any other blood vascular non-sinusoidal endothelia with the exception of blood vessels in the lung. Expression of LYVE-1 is observed in sites where uptake and degradation of hyaluronan occurs (2).

High endothelial venules (HEVs) belong to the blood vascular compartment of lymph nodes. They are the major site of entry for circulating lymphocytes into the node and are characterized by cuboidal endothelial cells, the existence of discontinuous junctions between these endothelial cells, and the presence of large numbers of lymphocytes within their walls (3,4).

MATERIAL AND METHODS

Lymph nodes were obtained during diagnostic and therapeutic surgical lymphadenectomies at the Lower Silesia Centre of Oncology in Wrocław. The study was approved by the Ethical Committee of the Wrocław Medical University.

Forty paraffin-embedded lymph node specimens from newly diagnosed patients with non-Hodgkin lymphoma were evaluated (23 aggressive and 17 indolent lymphomas). In addition, 10 lymph node specimens from adult patients with reactive lymphadenitis and 10 normal, unaffected lymph nodes were used as controls.

Samples were fixed in 10% buffered formalin, paraffin embedded and stained with hematoxylin and eosin for histopathological evaluation. Lymph node sections were stained immunohistochemically for LYVE-1 and CD34 as follows. Sections were deparaffinized, dehydrated, and pre-treated with Target Retrieval Solution (DakoCytomation) at 95°C for 20 min. Following a wash in Tris-buffered saline (TBS), they were treated with 3% H₂O₂ for 10 min, washed in distilled H₂O (10 min) and PBS (5 min), and incubated with mouse monoclonal antibodies against LYVE-1 (RELIATech GmbH, Germany; diluted 1:200) or CD34 (Class II, Clone QBEnd 10, DakoCytomation, Denmark; diluted 1:50) for 60 min at room temperature. For all slides, a wash in TBS was followed by treatment with peroxidase-labeled polymer conjugated to goat anti-rabbit or anti-mouse immunoglobulins (Envision+kit; DakoCytomation, Denmark) for 30 min at room temperature. Immunostaining was visualized with diaminobenzidine tetrahydrochloride (DAB) and then counterstained with hematoxylin. In each case, the negative control was included with Primary Negative Control (DakoCytomation, Denmark).

RESULTS

We have demonstrated expression of

both LYVE-1 and CD34 in HEVs in normal and pathological lymph nodes (*Fig. 1A,1B; Fig. 2A,2B*). LYVE-1 expression was seen also on the endothelial cells of the lymphatic sinus and in reticular cells in the lymph nodes (data not shown). For comparison, we found LYVE-1 and CD34 was expressed on the endothelial surface of the hepatic sinusoids (*Fig. 3A,3B*) as described previously by Carreira et al (2).

DISCUSSION

Small lymphatic vessels are characterized histologically by the lack of erythrocytes in the lumen and lack of a basement membrane. Recently, several immunohistochemical markers specific for lymphatic endothelial cells have been identified. LYVE-1, a CD44 homologue, is a hyaluronic acid receptor predominantly expressed on lymphatic endothelium. Hyaluronan (HA) is an extracellular matrix glycosaminoglycan with proinflammatory, angiogenic and leukocyte migratory functions. The lymphatic system is thought to play an important role in the metabolism of hyaluronic acid, although its role is not fully understood. LYVE-1's physiological function might be in transporting HA across the lymphatic vessel wall (1,2,5). Jackson suggests that LYVE-1 may shuttle across the lymphatic endothelium and transport HA from tissue to lymph by transcytosis (6,7). Akashima demonstrated that antibodies against LYVE-1 did not react with the endothelial cells of small blood vessels, and LYVE-1 was not detected on endothelial surface of vWF positive cells (1). In the lymph nodes, LYVE-1 expression was found on the endothelial cells of the lymphatic sinus and in reticular cells (6).

HEVs are venous vessels situated in the inner cortex of lymph nodes. The endothelium of HEVs is composed of cuboidal to columnar cells with swollen bodies. The vascular wall of HEVs is infiltrated by numerous leukocytes, and HEVs are the major pathways used by

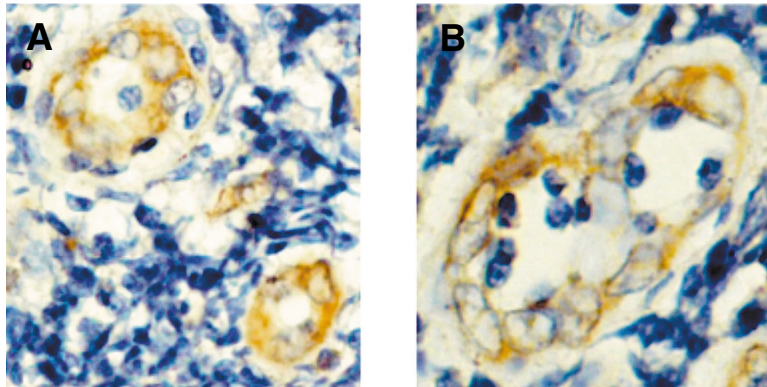


Fig. 1. Expression of LYVE-1 in endothelial cells of high endothelial venules (HEVs) in lymph node cortex. A – magnification x400; B - magnification x600. Background staining with hematoxylin.

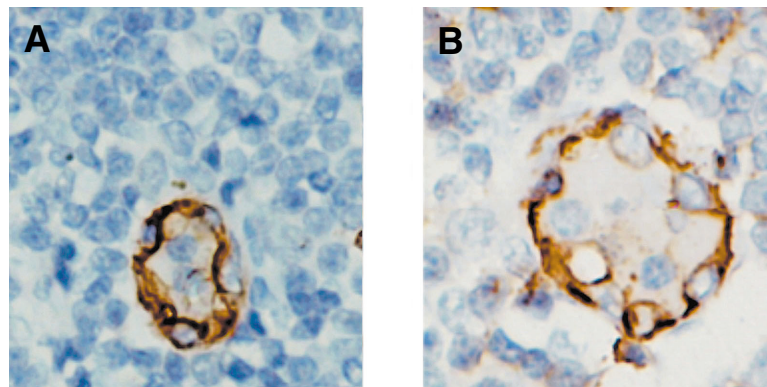


Fig. 2. Expression of CD34 in endothelial cells of high endothelial venules (HEVs) in lymph node cortex. A – magnification x400; B - magnification x600. Background staining with hematoxylin.

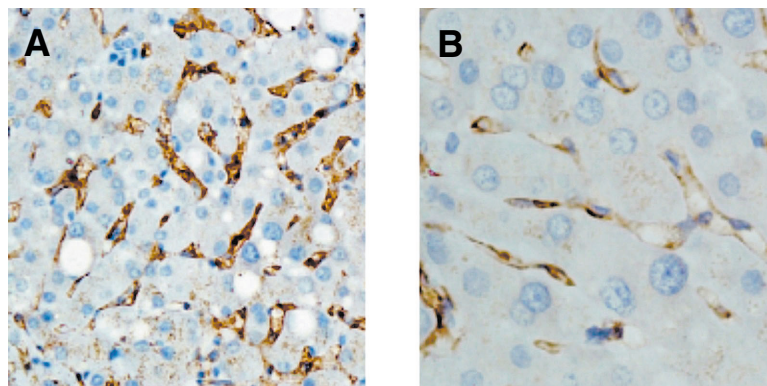


Fig. 3. Expression of LYVE-1 (A) and CD34 (B) on the endothelial surface of hepatic sinusoid. A – magnification x40; B - magnification x200. Background staining with hematoxylin.

lymphocytes to enter the lymph nodes (4,8,9). Presence of LYVE-1 receptor on the HEVs endothelium supports the hypothesis that LYVE-1 is involved in cell adhesion promoting lymphocyte migration into lymph nodes.

Our study demonstrates for the first time the expression of LYVE-1 on the endothelium of high endothelial venules (HEV).

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