学位論文内容の要旨
（Summary of dissertation）

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Histochemical analysis of a hyaluronan receptor LYVE-1 in the reticulo-endothelial system
（細網内皮系におけるヒアルロン酸受容体 LYVE-1 の組織化学的解析）

【Background and Objectives】As a lymphatic vessel endothelial hyaluronan receptor, LYVE-1 has been identified as a reliable marker that is expressed in the lymphatic endothelium, hepatic sinusoidal endothelium, and some macrophage lineages. However, I found a broader distribution of LYVE-1 in the endothelia of other organs, including the lung, adrenal gland, heart, and adenohypophysis. Most of these belong to the forgotten concept known as the reticulo-endothelial system (RES). The results confirm that LYVE-1 is a key molecule for a reconsideration of the RES concept. Macrophages are important members of RES, some of them being also found to express LYVE-1. However, based on functional characteristics and marker substances, macrophages can be further classified into several different types. Also, it often is difficult to discriminate macrophages from dendritic cells and related cells. Because the entire morphology and location of cells are important indexes for macrophages/dendritic cells and their subtypes, I use whole mount preparations of the mesentery instead of tissue sections.

【Materials and Methods】Adult ddY and C57BL/6 mice were used in this study. For conventional immunohistochemistry, cryostat sections from tissues fixed in 4% paraformaldehyde and whole mount preparations of the mesentery were prepared and incubated with LYVE-1 and F4/80 antibodies. Silver-intensified immuno-gold method was performed for electron microscopy. To verify the uptake of exogenous particles, LPS-treated and control mice were injected directly with fluorescent latex beads, 20 and 100 nm in diameter. In an in situ hybridization analysis, the sections were hybridized in a hybridization buffer containing ^{33}P-labeled or digoxigenin-labeled oligonucleotide probes.

【Results】 Immunohistochemically, LYVE-1 was found not only in hepatic endothelial cells and splenic sinus endothelial cells, but also in vascular endothelial cells in the lung, the medulla and deep cortex of the adrenal gland, and the atrium of heart. At the same regions, the LYVE-1-immunoreactive endothelial cells were associated topographically with dense distributions of macrophages. Some of sinus endothelial cells in the bone marrow were also positive for LYVE-1. Immuno-gold particles showing ultrastructural existence of LYVE-1 were localized along the entire length of the plasma membrane in all cell types. LPS elevated the mRNA expression of LYVE-1, especially in the liver, increasing 4 times after an exposure to LPS. When animals were stimulated by LPS for 24 h, the capillary endothelium in the liver and lung vigorously captured particles of 20 nm in size while the endothelium under normal conditions did not. I then examined the activity for degrading hyaluronan by detecting hyaluronidases in tissues. Because of the most intense expression of hyaluronidase mRNA in the medulla of the lymph node, we compared the detailed expression pattern with that of LYVE-1 mRNA in the same region. I found an intense expression of hyaluronidase mRNA in macrophages instead of LYVE-1-expressing reticular cells. However, both types of cells directly contacted each other.

Observation of whole mount preparations of the mesentery revealed the entire shape and distribution of LYVE-1-immunoreactive cells (LYVE-1$^+$ cells), dendritic in shape. There are two types of LYVE-1$^+$ cells which segregated and possessed their own territory of distribution. In the observations of cryostat

sections, both types of LYVE-1$^+$ cells appeared to be wholly embedded in membranous tissues of the mesentery and weakly or moderately positive for F4/80. Immunohistochemistry using the F4/80 antibody detected typical round macrophages with uneven distribution and these macrophages were absolutely negative in reaction for LYVE-1. When fluorescent latex particles of 20 nm in diameter were injected into the peritoneal cavity, only F4/80$^+$ macrophages vigorously ingested the latex particles at 3 h after the injection; no LYVE-1$^+$ cells contained latex particles in their cytoplasm, although a moderate uptake of latex particles by LYVE-1$^+$ cells was recognized at 1 or 2 days after the injection. In addition to the mesentery, the capsules and interstitium of the kidney, thymus, and lymph node contained LYVE-1$^+$ F4/80$^+$ cells. In the contrast, other F4/80$^+$ macrophages such as microglia, hepatic sinusoidal macrophages (Kupffer cells), alveolar macrophages, macrophages in the lymph node medulla, ovarian corpus luteum, and splenic red pulp were all negative for LYVE-1.

【Discussion】The RES was a historical concept which characterized its higher activity to eliminate circulating exogenous substances and particles such as endotoxins and bacteria. Recent studies have presented evidences to confirm that the uptake ability of RES is stronger than that of macrophages. However, there are no available markers specific for all members of RES, which resurrect the concept of the RES. The present study showed that most of LYVE-1-expressing cells largely belong to the RES, including vascular endothelial cells in the lung, medulla and deep cortex of the adrenal gland, and the heart, suggesting that LYVE-1 is a novel useful maker for RES. LYVE-1 was initially regarded a key receptor responsible for the uptake and transport of hyaluronan in the lymph circulation. However, subsequent studies using knock-out mice have revealed that the expression of LYVE-1 in the lymphatic vessels is dispensable for both the hyaluronan metabolism and leukocyte trafficking. The present study showed that LPS stimulation elevated the expression of LYVE-1 and the uptake ability of latex beads in the RES. LYVE-1-expressing cells may be involved in uptake and degradation of hyaluronan and related exogenous substances under the inflammation conditions more than the normal conditions. Because of the lack of hyaluronidase activities and frequent contact with macrophages, the LYVE-1-expressing RES cells may capture circulating hyaluronate and process it with relation to adjacent macrophages with intense hyaluronidase activities.

Based on the immunoreactivities to the LYVE-1, there are two types of macrophages. In the connective tissues such as the capsules and interstitium, the macrophages are positive to the LYVE-1 antibody. In the contrast, the macrophages in the parenchyma of organs are absolutely free from LYVE-1 immunoreactivity. These LYVE-1-negative macrophages are not embedded in the connective tissue and may lack an intimate relation to the extracellular matrix. It is likely that LYVE-1 of macrophages plays a role in the formation of scaffolds for cell adhesion to the extracellular matrix. In the mesentery, there are two types of macrophage-like cells. One is round-shaped LYVE-1$^-$ macrophages; another one is LYVE-1$^+$ macrophage-like cells with dendritic processes, which are weakly positive for F4/80. The F4/80 immunoreactivity of LYVE-1$^+$ cells increased in intensity by LPS stimulation together with a dynamic morphologic change, indicating that they quickly respond to inflammatory events. Taken together, cellular detection of LYVE-1 may give insights to the research field of macrophages and related cells.

【Conclusion】 LYVE-1 turns out to be a novel marker of RES and involved in the uptake of hyaluronan. LYVE-1 may play a role in cell adhesion and migration of macrophagic cells within connective tissues rich in hyaluronan, and a change in LYVE-1 expression becomes a reliable sign of activated conditions in inflammation. By using LYVE-1 antibody, I found novel types of LYVE-1-immunoreactive macrophage-like cells in the mesentery.