Lymphatic neoangiogenesis in human neoplasia and transplantation as experiments of nature

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Introduction

A plexus of lymphatic vessels guides interstitial fluid, passenger leukocytes, and tumor cells towards regional lymph nodes. Microvascular endothelial cells of lymph channels (LECs) are difficult to distinguish from those of blood vessels (BECs) because both express a similar set of markers, such as CD31, CD34, podocalyxin, von Willebrand factor (vWF), etc. Analysis of the specific properties of LECs was hampered so far by lack of tools to isolate LECs. Recently, the 38 kD mucoprotein podoplanin was found to be expressed by microvascular LECs but not BECs in vivo. Here we isolated for the first time podoplanin$^+$ LECs and podoplanin$^-$ BECs from dermal cell suspensions by multicolor flow cytometry. Both EC types were propagated and stably expressed VE-cadherin, CD31, and vWF. Molecules selectively displayed by LECs in vivo, i.e., podoplanin, the hyaluronate receptor LYVE-1, and the vascular endothelial cell growth factor (VEGF)-C receptor, Flt-4/VEGFR-3, were strongly expressed by expanded LECs, but not BECs. Conversely, BECs but not LECs expressed VEGF-C. LECs as well as BECs formed junctional contacts with similar molecular composition and ultrastructural features. Nevertheless, the two EC types assembled in vascular tubes in a strictly homotypic fashion. This EC specialization extends to the secretion of biologically relevant chemotactic factors: LECs, but not BECs, constitutively secrete the CCR7 ligand SLC/CCL21 at their basal side, while both subsets, upon activation, release MIP-3a/CCL20 apically. These results demonstrate that LECs and BECs constitute stable and specialized EC lineages equipped with the potential to navigate leukocytes and, perhaps also, tumor cells into and out of the tissues.

Lymphatic Neoangiogenesis in Human Cancer.

Formation of lymphatic metastasis is the initial step of generalized spreading of tumor cells, and predicts poor clinical prognosis. Lymphatic vessels generally arise within the peritumoral stroma, although the lymphangiopoetic vascular endothelial growth factors (VEGF)–C and -D are secreted in apparently small amounts by tumor cells. In a carefully selected collective of human cervical cancers (stage pT1b1) we demonstrate by quantitative immunohistochemistry and in situ hybridization that density of lymphatic microvessels is significantly increased in peritumoral stroma, and that a subset of stromal cells express large amounts of VEGF-C and -D. The density of cells producing these vascular growth factors correlates with peritumoral inflammatory stroma reaction, lymphatic microvessel density, peritumoral carcinomatous lymphangiosis, and with the frequency of lymph node metastasis. The VEGF-C and -D producing stroma cells were identified in situ as a subset of activated tumor associated macrophages (TAMs) by expression of a panel of macrophage specific markers, including CD68, CD23, CD14 and others, in double-immunofluorescence confocal microscopy. These group of TAMs also expressed the VEGF-C and -D specific tyrosine kinase receptor flt-4. As TAMs are derived from monocytes in the circulation, a search in peripheral blood for candidate precursors of flt-4 expressing TAMs revealed a subfraction of flt-4 expressing, CD14-positive monocytes, that, however, failed to express VEGF-C and -D. Only after in vitro incubation with TNFα, LPS or VEGF-D these naive monocytes started to synthesise VEGF-C de novo. In conclusion VEGF-C expressing TAMs play a novel role in peritumoral lymphangiogenesis, and subsequent dissemination in human cancer.

Lymphatic Neoangiogenesis in Human Renal Transplants.

Renal transplant rejection is caused by a lymphocyte rich inflammatory infiltrate that attacks cortical tubules and endothelial cells. Immunosupressive therapy reduces the number of infiltrating cells, however, their exit routes are not known. Here we demonstrate by immunohistochemistry on human renal transplant biopsies, a > 100 fold increase of lymphatic vessel density over normal kidneys in grafts with nodular mononuclear infiltrates, using antibodies to the lymphatic endothelial marker protein podoplanin. Nodular infiltrates are constantly associated with newly formed, Ki-67 expressing lymphatic vessels and contain the entire repertoire of T- and B-lymphocytes to provide specific cellular and humoral alloantigenic immune responses, including Ki-67$^+$ CD4$^+$ and CD8$^+$ T-lymphocytes, S100$^+$ dendritic cells, as well as Ki-67$^+$CD20$^+$ B-lymphocytes and l- and k-chain-expressing plasmacytoid cells. Numerous chemokine receptor CCR7$^+$ cells within the nodular infiltrates are apparently attracted by secondary lymphatic chemokine (SLC/CCL21) that is produced and released by lymphatic endothelial cells in a complex with podoplanin. Thus, lymphatic neoangiogenesis contributes not only to the export of the rejection infiltrate, but is also involved in the maintainance of a potentially detrimental alloreactive immune response in renal transplants, and provides a novel therapeutic target.

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A Novel Role for Podoplanin in Lymphangiogenesis.

In order to analyze podoplanin function in the development of the lymphatic vasculature, we generated podoplanin$^{-/-}$ mice in 129S/v : Swiss background.

The lack of podoplanin resulted in loss of about 30 % of the embryos. Within the first week, approximately 55% of born podoplanin$^{-/-}$ mice died, but 20% of born mice survived till adulthood. With respect to the lymphatic vasculature, young podoplanin$^{-/-}$ mice showed enlarged and tortuous lymphatic vessels, while the lymphatic network was impaired. Additionally, throughout the small intestine a network of dilated, tortuous and leaky lymphatic vessels resulting in the chylous ascitis and hyperemic Peyer´s patches were found. Lymphatic fluid transport, as revealed by intradermal injection of Chicago sky blue dye, was impaired in the knockouts. These data indicate that formation of the lymphatic vessel system is erroneous in lymphoid and non-lymphoid tissues in podoplanin$^{-/-}$ mice. The most impressive alkteration of the lymphatic vessels in Podoplanin -/- mice, however, was their lack of separtion from the blood vasculature during their entire life span. We have demonstrated that this separation defect is due to the platelet aggregating effect of podoplanin on LEC surfaces. Thus, platelets that come in contact with LECs in the embryonic lymphatic saccules aggregate and degranulate, and induce constriction of the blood vascular-lymphatic anastomosis (10).

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