

Review

Lymph node lymphangiogenesis: a new concept for modulating tumor metastasis and inflammatory process

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Summary. The proliferation of lymphatic endothelial cells (LECs) occurs not only in tumor and inflamed tissues, but also in regional draining lymph nodes (LNs). The lymph node lymphangiogenesis (LNLG) has recently emerged as a prominent area in biomedical research, because it is involved in the pathogenesis of several human diseases. The LEC functional features and lymphatic remodeling regulated by lymphangiogenic factors actively promote tumor metastasis and the inflammation process. VEGF-A/VEGFR-2 and VEGF-C/-D/VEGFR-3 have been implicated as the prime mediators in inflammation- or tumor-induced LNLG. This knowledge may provide a foundation for further understanding of specific modification in the gene expression, cell migration, and differentiation of LECs and other cells in lymphatic-associated diseases. Importantly, it should be taken into consideration that inflammation and lymphangiogenesis are strongly linked in the formation and metastasis of cancer when designing therapeutic strategies.

Key words:

Lymphatic endothelial cells, Lymph node, Lymphangiogenesis, Tumor, Inflammation, VEGF-A/-C/-D

Introduction

The lymphatic system is involved in the physiological processes of immune surveillance and tissue fluid homeostasis, and the pathological processes, including tumor cell metastasis, wound healing, and chronic inflammation. Over the past few decades, there has been exciting progress in understanding the molecular mechanisms that regulate lymphatic formation and function. Lymphatic endothelial cells (LECs) respond to a changing microenvironment of dynamic

lymphatic beds during inflammation, tumorigenesis and detrimental factors to lymphatics, e.g., infection, trauma, surgery and radiation (Ji, 2005). Lymphangiogenesis, a complex process of new lymphatic budding, sprouting, and remodeling, is regulated by multiple growth factors, cytokines and chemokines. The vascular endothelial growth factor-C (VEGF-C) plays a crucial role in the establishment of the first lymphatics during embryonic development, and in the mediation of tumor metastasis and inflammation processes. A correlation between VEGF-C and VEGF-D expression, tumor lymphangiogenesis and the formation of metastases in regional lymph nodes (LNs) has been documented in a range of animal and human tumors, including malignant melanoma and lung, breast, thyroid, uterus, prostate and colorectal carcinomas (Stacker et al., 2002; Alitalo et al., 2005; Ji, 2006a). Moreover, the up-regulated expression of VEGF-C/-D is sufficient to induce a molecular link between lymphangiogenesis and leukocyte recruitment to sites of inflammation. The contribution of inflammatory cells to lymphangiogenesis has also been shown in various lymphatic-associated disorders, e.g., wound healing and transplants (Ji, 2007). The lymphangiogenic stimulators, VEGF-C/-D are secreted from tumor cells and peritumoral stromal cells in certain cancers. Inflammation may induce the expression of VEGF-C/-D/VEGFR-3 in dendritic cells (DCs) and macrophages, possibly through the intermediary of pro-inflammatory cytokines, and adenoviral delivery of either VEGF-C or VEGF-D can evoke lymphangiogenesis (Hamrah et al., 2003; Baluk et al., 2005). Although there is insufficient evidence to explain the mechanism of tumor cell dissemination and inflammation process, the possibility has been raised that VEGF-C/-D modulate the number and size of newly formed lymphatics or alter the functional properties of pre-existing lymphatics as the extracellular microenvironment changes (Ji, 2006a; Ribatti et al., 2007).

LNs and lymphatics are a structural and functional entity. Although substantial progress has been made in assessing LN function and identifying lymphatic sinus,

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the process of tumor- or inflammation-induced lymph node lymphangiogenesis (LNLG) has only recently been described (Table 1) and remains largely unexplored. Lymphatic sinus remodeling or dilatation in tumor-draining LNs was demonstrated at premetastatic stages in melanoma and nasopharyngeal carcinoma models, and in human breast cancer (Qian et al., 2006; Harrell et al., 2007). The induction of sentinel LNLG was suggested to be driven by VEGF-A and VEGF-C in transgenic mouse models (Hirakawa et al., 2005, 2007). Furthermore, prolonged tissue inflammation potentially induced lymphangiogenesis with significantly increased numbers of LECs both in the inflamed tissue and in the tissue-draining LNs in VEGF-A Tg mice (Halin et al., 2007). LNLG mediated by VEGF-C/D/VEGFR-3 and other signaling pathways may provide important options to inhibit the entry of tumor cells into the lymphatics for the prevention of cancer metastasis and tumor-induced inflammation processes. This review will highlight current understanding of the regional draining LNs in their functional features and interaction with the pathological microenvironment, and emphasize the importance of LNLG for modulating tumor metastasis and inflammatory processes.

Intranodal lymphatic pathways and functional features

LNs function as fluid exchange chambers in which

protein-free fluid is transferred between the blood and lymph compartments in the direction required to establish equilibrium of the hydrostatic and colloid osmotic forces acting across the blood-lymph barrier (Adair and Guyton, 1983). LNs form a major protection against macromolecules and organisms, removing them by phagocytic activity and exposing them to a wide variety of powerful defensive actions carried out by lymphocytes resident within them, or added to the population of defensive cells circulating in the lymph and blood.

Initial lymphatics are blind-ended vessels with typical intercellular junctions, whereas the collecting lymphatics, which propel the lymph forward into LNs, are encircled by a smooth muscle cell layer. Lymphangiogenesis occurs in the developing embryo and postnatal life, however, the distinction between them is not absolute, because both require LEC differentiation to form mature lymphatic networks. In response to lymphangiogenic signals, quiescent endothelial cells that line lymphatic sinuses in the LNs may migrate, proliferate and form a primary channel, and then remodel into a functional lymphatic sinus. LNLG may not be restricted to early embryogenesis, but may also have a physiological role or contribute to lymphatic-associated diseases in adults. LNs are permeated by lymphatic sinuses, through which lymph percolates after its entry from the afferent lymphatics. Afferent lymphatics branch to form the subcapsular sinus from

Table 1. Lymph node lymphangiogenesis in lymphatic-associated disorders of the experimental model and human.

Animal models (animal/cell line et al.)/or Human diseases (tissue/organ et al.)	Molecular mediators	Biological features (cytokine/growth factor and others)	References
E μ -c-myc (C57Bl/6J) transgenic mice / metastatic murine model of Burkitt's lymphoma	VEGF	Increased VEGF production by c-Myc-expressing B lymphocytes induces intranodal lymph circulation and LNLG	Ruddell et al., 2003
K14-GFP-VEGF-A transgenic mice / squamous cell carcinogenesis regimen	VEGF-A	VEGF-A-induced sentinel LNLG precedes tumor metastasis and promotes LNM; Tumor-induced lymphatics maintain LYVE-1 and <i>Prox-1</i> -positive phenotype	Hirakawa et al., 2005
BALB/c mice / human nasopharyngeal carcinoma cell line CNE-2	No data	The extent of lymphatic sinus dilation in sentinel LNs is correlated with the primary tumor weight	Qian et al., 2006
K14-GFP-VEGF-C transgenic mice / squamous cell carcinogenesis regimen	VEGF-C	VEGF-C secreted by primary skin tumors induces LNLG and expansion of intranodal lymphatic sinuses, which may facilitate further LNM	Hirakawa et al., 2007
C57BL/6J mice / B16-F10 murine melanoma cells	VEGF-A	Tumor-induced sentinel LNLG requires B lymphocytes and is accompanied by increased lymph flow	Harrell et al., 2007
Human (N=65) and (N=26) / breast cancer with sentinel LN biopsy or involvement	VEGF-D	Sentinel LNLG is involved in metastasis of nonsentinel axillary LNs; Lymphangiogenesis in LNM is driven by VEGF-D	Van den Eynden et al., 2007a, 2007b
C57BL/6 mice deficient for B lymphocytes (μ MT); C57/BL6OT-II TCR-OVA transgenic mice / immunization with keyhole limpet hemocyanin-complete Freund's adjuvant	VEGF-A	VEGF-A-expressing intranodal B lymphocytes orchestrate LNLG and lymphatic sinus expansion; Inflamed LNLG enhances DC mobilization; VEGFR-2, VEGFR-3 or a combination of both antibodies markedly decrease but do not abrogate inflamed LNLG	Angeli et al., 2006
VEGF-A transgenic mice / delayed-typed hypersensitivity reactions in the ears	VEGF-A	Tissue inflammation specifically induces LNLG but not LN angiogenesis; LNLG is independent of the presence of intranodal B lymphocytes and can be blocked by anti-VEGF-A	Halin et al., 2007

LNLG: lymph node lymphangiogenesis; LN: lymph node; LNM: lymph node metastasis; DC: dendritic cell

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which numerous radial cortical sinuses lead to the medulla, coalescing as larger medullary sinuses. At the hilum, medullary sinuses are confluent with the efferent lymphatics (Fig. 1). All the lymphatic sinuses are lined by an endothelium that is continuous despite the constant passage of lymphocytes and other cells through the sinus walls in both directions. The lymphatic endothelium hyaluronan receptor (LYVE-1), a molecular marker for LECs is expressed on the endothelium of lymphatic sinuses and some high endothelial venules in LNs (Wrobel et al., 2006), although it is also expressed by a subset of tumor-induced macrophages, liver blood sinusoids, and spleen (Mouta Carreira et al., 2001; Jackson et al., 2001). Improved real-time imaging techniques can assess afferent or efferent lymphatic function and the course of dynamic lymph flow through a node, and even allow the characterization of phenotypes at cellular or subcellular resolution *in vivo* (Saban et al., 2007; Harrell et al., 2007; Hayashi et al., 2007).

Sentinel LN metastasis is the first step in the spreading of cancer in many malignancies. Based on the observations that lymphatic vessel density is correlated with the extent of LN metastasis and/or unfavorable prognosis of certain cancers, the LNLG in the postnatal period is considered to be a major contributor to tumor metastasis (Achen and Stacker, 2006; Ji, 2006a). Sentinel LNs are highly susceptible to modulation by tumor-generated bioactive molecules that downregulate

multiple crucial and interlinked LN functions (Cochran et al., 2006). In the pathological condition, intranodal lymph is exposed to activated macrophages and DCs, which are entangled amongst the reticulin fibers, as well as to the activities of B- and T-lymphocytes adhering to the endothelia. Moreover, the differential combination of several adhesion molecules, along with the cooperation of different sinusoidal cell types, provides a unique, organ-specific environment for metastatic cell arrest and sheltered survival (Martens et al., 2006). In order to metastasize, a tumor cell must possess or acquire the ability to surmount a variety of obstacles that include de-adhesion from the primary tumor, invasion into initial lymphatics, survival in lymph circulation, extravasation and growth in the secondary site (Wong and Hynes, 2007). The metastatic process, including LNLG, is likely governed by a complex series of interactions among tumor cells, immune cells, endothelial cells, and extracellular matrix components, through secretion of a variety of signals, e.g., stromal molecules, chemokines, cytokines, and growth factors (Ji, 2006b; Koyama et al., 2008). The extracellular matrix provides scaffolds for cellular support and presents in all tissues and organs. The lymphatic development in responding to microenvironment changes in the tumor tissue or tumor-induced inflamed tissue may depend upon interactions between LECs and these signaling molecules (Ji, 2006b). However, the intracellular signaling events leading to LEC proliferation, migration and survival are

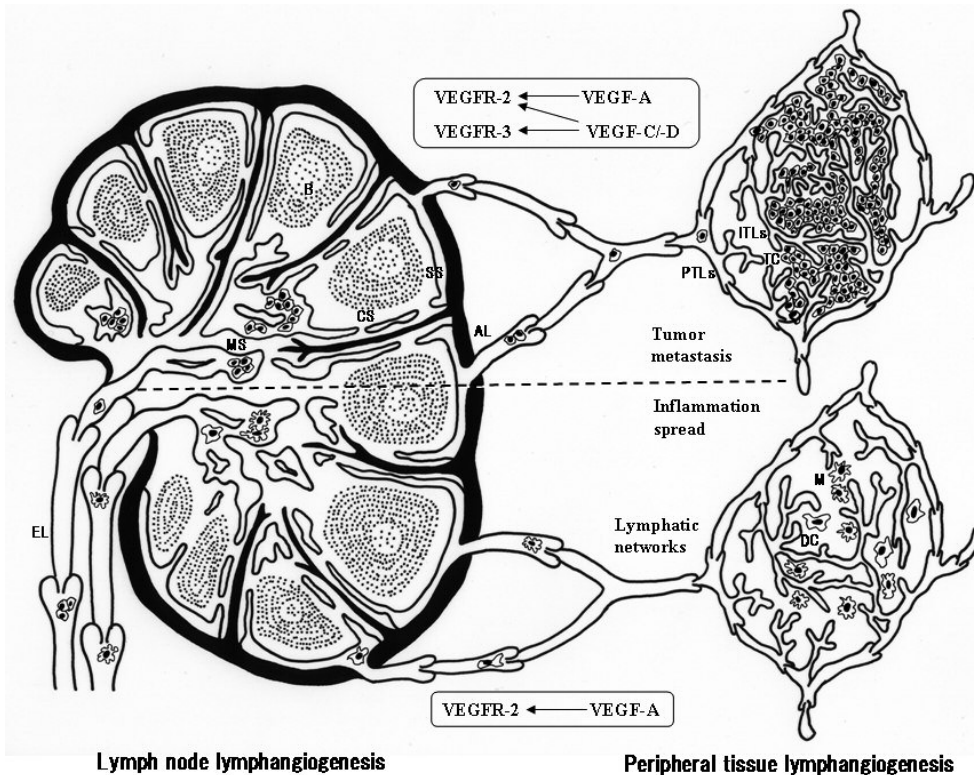


Fig. 1. Lymph node lymphangiogenesis is shown in the process of tumor metastasis and inflammation. VEGF-A/VEGFR-2 and VEGF-C/D/VEGFR-3 are important molecular mediators in lymphatic formation and remodeling. AL: afferent lymphatics; SS: subcapsular sinuses; CS: cortical sinuses; MS: medullary sinuses; EL: efferent lymphatics; B: B lymphocytes; TC: tumor cells; DC: dendritic cells; M: macrophages; PTL: peritumoral lymphatics; ITL: intratumoral lymphatics.

not fully understood now. The peritumoral or intratumoral lymphangiogenesis increases the lymphatic area within or close to the tumor tissues and increases contact between tumor cells and LECs. This, in turn, is thought to facilitate entry of tumor cells into the lymphatic network and thereby promote metastasis (Ji et al., 2007). Tumor-induced LNLG surely provides an ideal site for cancer cell growth and a route for further metastasis. The fact that lymphatic formation involves several common pathways, e.g., VEGF-A/VEGFR-2 and VEGF-C/-D/VEGFR-3, suggests that a variety of signaling molecules contain a compensatory mechanism to biologically secure lymphangiogenesis. For the same reason, effective anti-lymphatic effects may require multiple inhibitions, targeting several components within these pathways (Sundlisaeter et al., 2007). Detailed knowledge about LNLG, especially its molecular determinants, will be instrumental in designing therapeutic approaches for treatment of lymphatic dysfunction in cancer and inflammation.

LNLG is intimately linked to the processes of inflammation

The lymphatic network dramatically expands in LNs of immunized mice, as evidenced by LYVE-1 immunofluorescent staining. Lymphatics are not just passively dilated but actively proliferate with enhanced labeling of the proliferation marker Ki-67 (Halin and Detmar, 2006). Chronic inflammation actively induces LNLG, which is controlled remotely by lymphangiogenic factors, especially the key trigger VEGF-A produced at the site of inflammation. During the inflammatory response, tissue macrophages and activated keratinocytes can produce high levels of VEGF-A (Ray and Fox, 2007; Chen et al., 2008). In the $\text{E}\mu\text{-c-myc}$ mice after immunization with bacterial antigens and complete Freund adjuvant, B cell accumulation within draining LNs is found to be associated with increased lymphangiogenic factor VEGF-A production and LNLG (Angeli et al., 2006). Immunofluorescent analysis has revealed that VEGF-A expression colocalizes with B cell follicles in activated LNs, and B cells can be stimulated to secrete enhanced levels of VEGF-A *in vitro*, suggesting an involvement of the B cell-derived mediator in LNLG and DC recruitment (Halin and Detmar, 2006). Uniquely, follicular activated B lymphocytes can orchestrate expansion of the lymphatic sinuses within the immunized LNs and the upstream site of inflammation. The lymphangiogenic response within inflamed LNs may lead to increased antigen drainage and migration of antigen presenting cells from the periphery to the draining LNs, thereby boosting the immune response. The lymphatic expansion is also regulated by the pattern of genes expressed by DCs themselves, and by signals within the surrounding peripheral environment (Angeli et al., 2006; Halin and Detmar, 2006). A possibility has been speculated that activated B cells in immunized LNs may be one of the latent variables to drive

lymphangiogenesis within inflamed tissues. However, a recent study using VEGF-A transgenic mice has demonstrated that inflammation-induced LNLG is independent of the presence of intranodal B lymphocytes. The tissue lysates from the inflamed LNs have shown an elevated level of VEGF-A protein, rather than mRNA (Halin et al., 2007), indicating that VEGF-A produced at the inflamed tissues is subsequently transported to the draining LNs through afferent lymphatics. Administration of a neutralizing monoclonal antibody against VEGF-A may potentially block the inflammation process and LEC proliferation in the affected tissues or activated LNs.

VEGF-A may induce VEGF-C expression either by increasing the recruitment of VEGF-C-producing inflammatory cells, or by inducing VEGF-C expression in endothelial cells (Cursiefen et al., 2004). The indirect mechanism appears to play a major role in non-malignant models of injury and at sites normally devoid of VEGF-C. In VEGF-C-producing tumors, the above mechanism appears to be supplemented by a direct action on pre-existing lymphatics through activation of VEGFR-2 (Hirakawa et al., 2005; Roberts et al., 2006). VEGFR-2, the major signaling receptor for VEGF-A, has been shown to be expressed in LECs and to promote LEC proliferation *in vitro* (Dixelius et al., 2003; Hong et al., 2004). However, VEGF-A may not be the sole factor inducing LNLG, because blockage of VEGFR-2 signaling only partially inhibits LNLG in response to bacterial immunization. In view of the impediment of LNLG by inhibition of VEGFR-3 signaling, VEGF-C or VEGF-D is also considered to promote expansion of intranodal lymphatic sinuses (Angeli et al., 2006), and therefore the combined treatment with antibodies blocking VEGFR-2 and VEGFR-3 will greatly reduce lymphatic sinuses, cellularity and DC migration to the activated LNs, although the coordination between VEGF-A and VEGF-C/-D remains to be proved in tissue-draining LNs.

Recent studies have revealed a close relationship between lymphangiogenesis and immunity. The inflammatory cells such as macrophages may produce lymphangiogenic factors and physically contribute to lymphatic formation (Cursiefen et al., 2004; Maruyama et al., 2007). To explore biological significance of LECs in chronic inflammation, our recent study has shown that the lymphatic sinuses in the LNs draining nonobese diabetic pancreas increase in numbers and are greatly enlarged in the medullary area (Fig. 2, unpublished data). The LNLG in diabetes is an interesting finding because the autoimmune insulinitic process is well known to be mediated by T-lymphocytes. As the insulinitis progresses, T-lymphocytes and DCs are recruited around or within pancreatic lymphatics in the nonobese diabetic mice. Intercellular junctions became loose and wide enough to allow the entry of DCs and lymphocytes into the lymphatic wall (Qu et al., 2005). The lymphatic remodeling in peripheral tissues enhances DC migration after immunization, which is supposed to depend on the presence of intranodal B cells. Migration of antigen-

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presenting DCs through lymphatics to draining LNs represents a key step in the initiation of an adaptive immune response (Halin and Detmar, 2006). In this regard, it will be important to determine whether anti-lymphangiogenic agents can alleviate certain inflammatory disorders, or whether stimulation of the lymphatic system can increase the potency of immune responses during the vaccination (Angeli et al., 2006). Although the biologic role of lymphangiogenesis in inflamed tissues or draining LNs needs further investigation, it is perceivable that in these tissues, an increase in lymphatic vessel density and surface area will improve tissue fluid homeostasis and alleviate edema formation. Therefore, it may be important to answer the following questions in future study. 1) What is the role of intranodal lymph flow in the regulation of function and morphogenesis of the lymphatic sinus?; 2) What are the phenotype and molecular differences in the

peripheral and intranodal activated LECs?; 3) What is the interaction between angiogenesis and lymphangiogenesis in abnormal LNs? 4) Whether the LNLG is functional or whether the expansion of lymphatic sinuses in the regional LNs is controlled by lymphangiogenic factors released in inflamed-draining tissues or by intranodal mediators; 5) Whether the lymphatic expansion regresses both in the LNs and in the peripheral tissues once the inflammatory stimulus is cleared; 6) Whether LEC proliferation persists over time to allow more efficient DC migration and more rapid initiation of an immune response in the case of a subsequent exposure to antigen (Halin and Detmar, 2006); and 7) How are LECs activated in LNLG via the potential mediation of B lymphocytes and DCs? Substantially, the increased knowledge about progressive organization of cell infiltrates into LNLG is expected to contribute to early control of chronic inflammatory

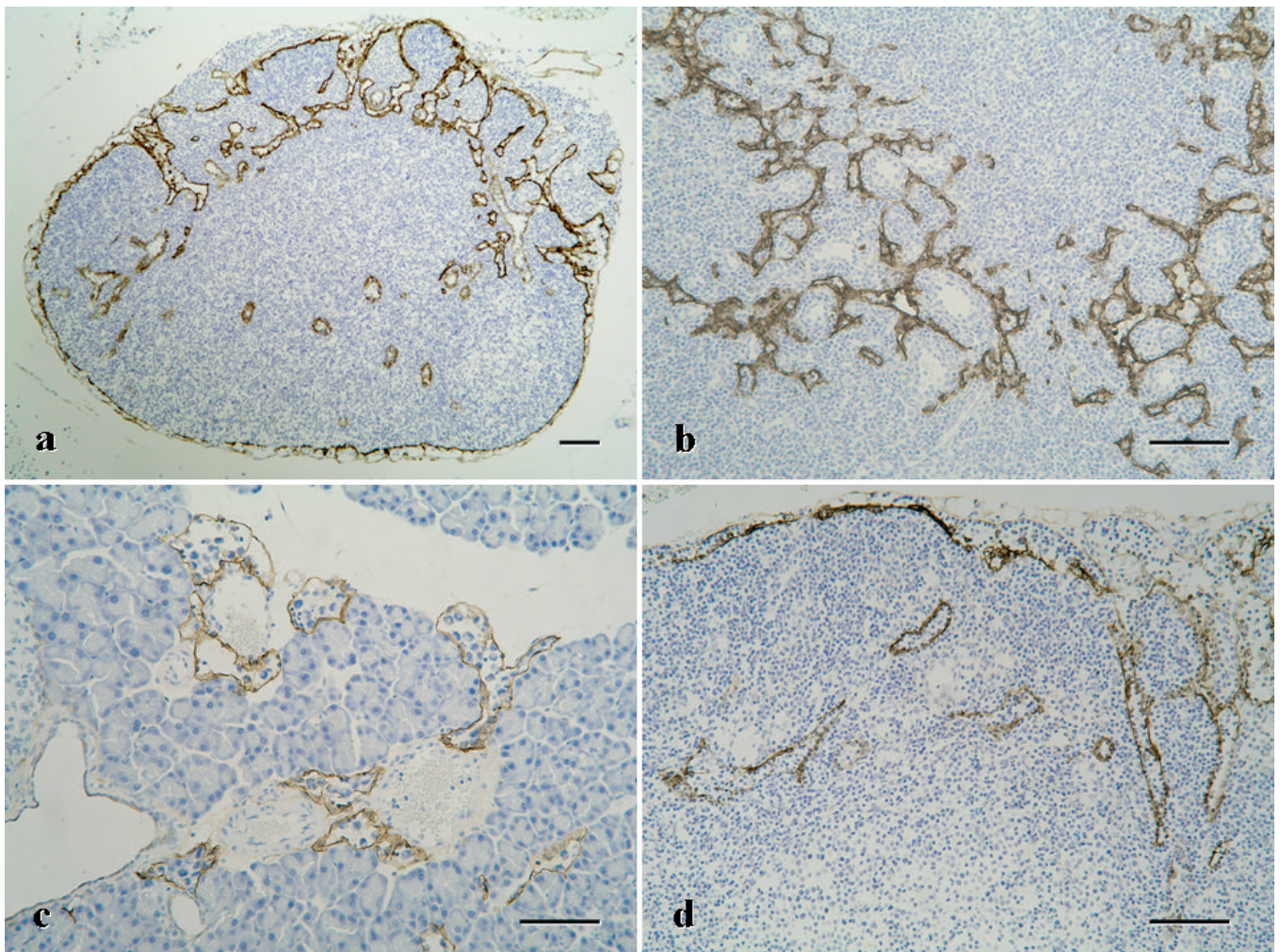


Fig. 2. Lymph node lymphangiogenesis in the autoimmune insulinitic development of nonobese diabetic mice (**a, b**) and the hybridoma-induced tumor metastasis of the mouse model (**c, d**). **a, b, d**: LYVE-1-expressing accumulated lymphatic sinuses are shown in the subcapsular, cortical and medullary areas. **c**: Increased lymphatics filled with metastatic tumor cells are expressed with podoplanin in the pancreas. Bars: 100 μ m

lymphatic insufficiency.

Lymphangiogenesis in the tumor-draining LNs may actively promote lymphatic metastasis

Although the pattern of metastatic spread may vary in cancers, the initial step usually involves dissemination to regional LNs. Sentinel LNLG, an important pathological feature in some cancers, may actively contribute to tumor metastasis, whereas the extent of sentinel LN involvement is an independent predictive factor for cancer survival. The tumor-induced LNLG usually precedes metastasis and leads to increased tumor spread to distal LNs and beyond to distant organs. The pre-metastatic lymphatic dilation or remodeling in tumor-draining LNs has recently been described in spontaneous metastatic models of melanoma, nasopharyngeal carcinoma, murine developing lymphomas, transgenic mouse models of chemically induced skin cancer, and in human breast cancer and melanoma (Ruddell et al., 2003; Dadras et al., 2005; Qian et al., 2006; Harrell et al., 2007; Hirakawa et al., 2005, 2007; see Table 1), suggesting that tumor-secreted factors are also strong mediators for local LNLG. In human breast cancer with a positive sentinel LN biopsy, the sentinel LNLG driven by VEGF-D is associated with the presence of nonsentinel LN metastases, indicating that increased sentinel LNLG is involved in further lymphatic spread of nonsentinel axillary LNs (Van den Eynden et al., 2007a,b). In the orthotopic cutaneous carcinoma, increased numbers of LYVE-1- and *Prox-1*-positive lymphatic networks or sinuses have been found in tumor-associated tissues and in sentinel LNs even before metastasizing (Hirakawa et al., 2005). In our recent investigation of intratumoral and peritumoral lymphatics expressed by podoplanin and LYVE-1 (Ji et al., 2007), the draining LNs of the hybridoma-induced tumor model show lymphatic sinus expansion and LNLG (Fig. 2, unpublished data), especially in the medullary sinuses. Although it was known that regional LNs draining tumor tissues may be enlarged without evidence of metastasis, the extent of lymphatic sinus dilation in the sentinel LNs was found to significantly correlate with the primary tumor weight (Qian et al., 2006). The lymph from primary tumors may induce persistent alteration of lymphatic sinuses in the sentinel LNs. VEGF-A as an inducer of lymphangiogenesis not only strongly promotes multistep carcinogenesis, but also induces active LEC proliferation. VEGF-A is a secreted protein that can travel via the bloodstream to other lymphatic sinus-enriched LNs. Similar to lymphatic formation in the inflammation process, anti-VEGFR-2 antibodies are less effective than anti-VEGFR-3 antibodies in suppressing tumor lymphangiogenesis, suggesting that VEGF-A may regulate LECs by both direct and indirect mechanisms (Whitehurst et al., 2007). In contrast to VEGF-A, VEGF-C does not increase the growth of primary tumors, but instead induces active expansion of lymphatic networks within draining sentinel LNs and

primary tumor lymphangiogenesis in VEGF-C transgenic mice. VEGF-C-induced LNLG has been identified as a novel mediator of tumor metastasis, and may facilitate tumor spread throughout the lymphatic system and to distant organs (Hirakawa et al., 2007). In human, the level of VEGF-C expression corresponding to lymphangiogenesis, served as an indicator of tumor invasiveness and aggressiveness, is also associated with metastatic risk of the primary tumor to sentinel LNs (Dadras et al., 2005). VEGF-A or -C/-D-overexpressing tumor cells still retain strong lymphangiogenic activity after metastasis to sentinel LNs, and may further increase the extent of LNLG.

In B16-F10 melanoma-induced model, the extensive LNLG with B lymphocyte accumulation is accompanied with increased lymph flow through the tumor-draining popliteal LNs as an early response to tumor growth. The tumor-derived immune signaling may promote LN alterations that further actively induce lymphatic metastasis (Harrell et al., 2007). Increased lymph propulsion through the tumor-draining LNs may reduce fluid pressure within initial lymphatics, promoting tumor cells entry into the intratumoral or peritumoral lymphatics. The draining fluid from the primary tumors into the sentinel LNs provides a favorable microenvironment basis for further metastatic spread from the sentinel LNs. Invasion of LNs by tumor cells may result in suppression of LN functions with alterations in immune cell ratio and activity (Shu et al., 2006). B lymphocytes accumulated in the tumor-draining LNs, but not in the primary tumors, are required for the expansion of intranodal lymphatic sinuses, LNLG and increased lymph flow through LNs (Hirakawa et al., 2005; Harrell et al., 2007). DCs are antigen-presenting cells whose function, location, and morphology are highly dependent on their degree of maturation (Germeau et al., 2005). Mature DCs are the most potent antigen-presenting cells capable of priming tumor-specific T cells (Elliott et al., 2007). Exploration of immunologic status of the sentinel LNs, including DC maturation and T-cell responses, is one of the most promising issues in tumor immunology and lymphology. The preservation of the maturation ability of DCs in metastatic sentinel LNs may be crucial for the long-term anti-metastatic host immune response. The interaction between the biology of micrometastasis and immune response is of particular interest for the development of new treatment strategies that combine immunotherapy and targeted therapy (Elliott et al., 2007).

The metastatic dissemination of tumor cells to regional LNs is a common feature of many animal and human cancers. Although great progress has been made in the field of tumor-associated LEC research over the last few years, a number of problems still remain unsolved. How do tumor cells migrate through lymphatic sinus? What are the molecular mechanisms that control the interaction of cancer cells with LECs? Are there organ-specific differences between tumor lymphatics? Are there molecules that are specifically expressed by tumor-activated LECs and may promote

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lymphatic metastasis (Tobler and Detmar, 2006)? It will also be interesting to determine whether tumors metastasize through the pre-existing or newly formed lymphatics and whether LNLG can actively contribute to the recruitment and motility of metastatic cancer cells. Moreover, inflammation is thought to contribute to the development and progression of various cancers. Chronic inflammation can be caused by host injury or infection, autoimmune disease, malignant and benign tumors, or other pathologies, and results in the infiltration of inflammatory cells at specific sites in the body. These so-called tumor-infiltrating lymphocytes include macrophages, T cells, B cells, natural killer cells, neutrophils, and granulocytes (Angelo and Kurzrock, 2007). Lymphangiogenesis induced by inflammation is strongly linked in the formation and metastasis of cancer. Inhibition of metastatic spread may be achieved by restriction of lymphatic growth by using molecules involved in lymphangiogenic signaling (Sundlisaeter et al., 2007). Endostatin, a proteolytic fragment of the vascular and epithelial basement membrane, can regulate the infiltration of VEGF-C-producing mast cells into the tumor tissue, which modulates the terminal differentiation of tumor cells and inflammatory reaction associated with cancer, and leads to decreased lymphangiogenesis and LN metastasis (Brideau et al., 2007). Therefore, anti-VEGF-A/-C/-D treatment can be a novel target for preventing the spread of an early-stage malignancy by inhibition of tumor lymphangiogenesis and/or LNLG.

Conclusion

The newly identified phenomenon of LNLG may represent a key event in facilitating metastatic tumor and inflammation spread throughout the lymphatic system. The molecular players, e.g., VEGF-A/VEGFR-2 and VEGF-C/-D/VEGFR-3 in modulating growth, patterning and differentiation of lymphatic networks will be of great importance in the understanding of LNLG and inhibition of metastatic tumor-cell entry into initial lymphatics. A requirement for B lymphocytes and other immune cells in stimulating LN alterations, resulting in lymphatic sinus expansion and proliferation, might also provide a new target for the development of therapies to control lymphatic-associated diseases.

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