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REVIEW

Lymphatic endothelium in health and disease

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Abstract The lymphatic vascular system has an important role in the maintenance of tissue fluid pressure homeostasis, in the mediation of the afferent immune response via recruitment of antigen-presenting cells toward draining lymph nodes, and in the intestinal absorption of dietary lipids. Substantial progress in our understanding of the development and the molecular mechanisms controlling the lymphatic system has been made during the last few years, based on a recent wave of discoveries of lymphatic endothelial cell-specific markers and growth factors. This has also led to new insights into the role of lymphatic endothelium in a number of diseases, including primary and secondary lymphedemas. The emerging role of lymphatic endothelium in the context of inflammation indicates that therapeutics targeting the lymphatic vasculature might represent a new strategy for anti-inflammatory therapies.

Keywords Lymphangiogenesis · Lymphedema · Inflammation · Development · VEGF-C

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Introduction

To cope with the complex needs of transporting fluids, gases, nutrients, signaling molecules, and cells through tissues, vertebrate organisms have developed two complementary vascular networks, the blood and the lymphatic vasculatures. The physiology of the blood vascular system and the role of angiogenesis (the growth of new blood vessels from pre-existing ones) in disease have been thoroughly studied over the last few decades (Carmeliet 2003). In contrast, substantial progress in our understanding of the development and molecular mechanisms controlling the lymphatic system, first identified almost four centuries ago (Asellius 1627), has only been made during the last few years, based on a recent wave of discoveries of lymphatic endothelial cell (LEC) markers and growth factors. Although the endothelial cells of blood vessels (BECs) and of lymphatic vessels have many features in common, such as their apical-basal polarity and the expression of certain pan-endothelial markers, the unique functional roles of these two vascular networks require extensive specialization.

Physiological functions of the lymphatic vascular system

Tissue fluid homeostasis

The primary role of the lymphatic vascular system is the maintenance of tissue fluid homeostasis by draining excess interstitial fluid (leaking from blood capillaries) and returning it into the blood flow. The lymphatic vascular network is composed of initial lymphatic capillaries and of collecting lymphatic vessels. The initial lymphatic vessels lack a basement membrane and are thin-walled with wide lumina lined by a single layer of overlapping LECs that are

anchored to the extracellular matrix by fibrillin-containing anchoring filaments (Gerli et al. 2000). Under conditions of high interstitial pressure, lymphatic capillaries are pulled open by these anchoring filaments, facilitating the uptake of fluid, macromolecules, and cells. The importance of anchoring filaments and the extracellular matrix for lymphatic drainage has recently been demonstrated in extracellular matrix glycoprotein Emilin1-deficient mice (Danussi et al. 2008). These mice display pronounced lymphatic drainage impairment and increased lymphatic leakage.

Lymph flow is unidirectional from the lymphatic capillaries to larger collecting lymphatics and finally into the thoracic duct, which empties into the inferior vena cava (Cueni and Detmar 2006). The forces that move the lymph through the vessels include smooth muscle contraction along the collecting vessels, respiratory movements, and skeletal muscle action (Bridenbaugh et al. 2003). Collecting lymphatic vessels differ from initial lymphatic capillaries in several aspects, in addition to their larger diameter. They are covered by smooth muscle cells and contain intraluminal valves that prevent the backflow of lymph. The exact mechanisms of fluid uptake by initial lymphatic vessels have remained unclear. However, recent studies indicate that overlapping flaps of oak-leaf-shaped LECs of the initial lymphatics lack intercellular junctions at the tip but are anchored on their sides by discontinuous button-like junctions. These differ from the continuous zipper-like junctions seen in the collecting lymphatics (Baluk et al. 2007).

Immune cell trafficking and immune surveillance

The lymphatic system also plays a major role in immune reactions. Immune cells such as antigen-presenting dendritic cells, memory T lymphocytes, and macrophages and soluble antigens use the lymphatic network to drain from peripheral tissues to regional lymph nodes where the immune responses are initiated (Cavanagh and Von Andrian 2002). There is increasing evidence for an active role of lymphatic endothelium in mediating the entry of antigen-presenting cells and lymphocytes into lymphatic vessels (Johnson et al. 2006; Randolph et al. 2005). Indeed, the interaction of immune cells with lymphatics represents an important step in the regulation of the immune response (Ledgerwood et al. 2008). The molecular mechanisms involved will be discussed in more detail below in the context of lymphatic vessel involvement in inflammation.

Dietary fat absorption by intestinal lymphatics

Within the small intestine, specialized lymphatic vessels in the villi, called lacteals, absorb dietary lipids and the fat-soluble vitamins A, D, E, and K. Mucosal enterocytes assemble chylomicrons, viz., lipoprotein complexes of 100–2000 nm in diameter, that are exocytosed into the lamina propria where they come into contact with the lacteals (Blomhoff et al. 1990). Chylomicrons constitute up to 15% of the lymph volume after ingestion of a fat-containing meal. Little is known concerning the modifications of chylomicrons permitting their uptake by lacteal vessels (Van Dyck et al. 2007) or regarding the mechanisms of lipid uptake into lymphatics. However, recent studies have indicated the presence of a link between fat metabolism and lymphatic function in the intestine, based on studies in mice with a deficiency of fasting-induced adipose factor (Fiaf, also known as angiopoietin-like protein-4; Backhed et al. 2007) or of the lymphatic-specific transcription factor Prox1. Prox1 heterozygous mice die within the first 2–3 days after birth on all except one genetic background (Wigle and Oliver 1999). The surviving mice show adult onset obesity with fat deposits in the tissue surrounding the leaky lymphatics (Harvey et al. 2005). Prox1 heterozygous mice also have high levels of circulating insulin and leptin. Additional evidence for a relationship between lymphatic vessels and adipose tissue come from the observation of ectopic adipose tissue growth in edematous regions of individuals with chronic lymphedema (Brorson et al. 2006), and lymph fluid has been reported directly to promote adipocyte differentiation in vitro (Harvey et al. 2005). Studies into the mechanisms of lipid absorption by intestinal lymphatics are needed and might lead to a better understanding of malabsorption syndromes/vitamin deficiencies and of the mechanisms of intestinal lymphatic drug transport (Nordskog et al. 2001).

Development of the lymphatic vasculature

At the beginning of the last century, two opposing models for the embryonic development of the lymphatic system were proposed. Based upon results obtained by ink injection experiments, Florence Sabin proposed that isolated primitive lymph sacs originated from endothelial cells that bud from the cardinal vein during early development (Sabin 1902). The two jugular lymph sacs were thought to develop at the junction of the subclavian and anterior cardinal veins. According to this model, the peripheral lymphatic system originates from the primary lymph sacs and spreads by endothelial sprouting into the surrounding tissues and organs, where local capillaries are formed (Gray 1985; Sabin 1902). An alternative model proposed that lymphatic vessels developed independently from mesenchymal precursor cells (but not from embryonic veins), and that connections with veins were only established later on during development (Huntington and McClure 1910).

At present, most experimental evidence supports Sabin's model of embryonic lymphatic development. A number of molecular genetics studies have shown that LECs do indeed sprout from the veins in the jugular area, and studies in Prox1 deficient mice have provided insight into the molecular players involved in this process (Wigle et al. 2002; Wigle and Oliver 1999). Recently, a *Cre/loxP*-based lineage-tracing study of mice expressing *Cre* recombinase under the control of *Tie2*, *Runx1*, or *Prox1* promoter elements has provided strong evidence that the mammalian lymphatic system has a venous origin only (Srinivasan et al. 2007). However, circulating bone-marrow-derived cells have been found, at a small percentage of lymphatic vessels affected, to participate in experimental corneal lymphangiogenesis and in chronic renal transplant rejection (Kerjaschki et al. 2006; Religa et al. 2005). The relevance of these observations for normal lymphatic development is unknown.

Molecular control of lymphatic vascular development

The homeodomain transcription factor Prox1 is the earliest protein known to be expressed in a polarized fashion in the cardinal vein (Wigle and Oliver 1999). A number of studies have revealed that Prox1 is a master gene controlling lymphatic-specific differentiation (Hong et al. 2002; Petrova et al. 2002; Wigle et al. 2002). In mice, Prox1-positive cells appear around embryonic day 9.5 (E9.5) in the cardinal vein and then bud off and migrate to form the first lymph sacs (Fig. 1). Prox1 null embryos die at approximately E14.5 and completely lack a lymphatic vasculature without defects of the blood vasculature (Wigle and Oliver 1999). Prox1 is at present considered to be the most specific lineage marker of the lymphatic endothelium (Wigle et al. 2002). Little is known about the signals upstream of Prox1 that lead to lymphatic commitment of blood vascular endothelium. In contrast, a number of Prox1 target genes have been identified. Overexpression of Prox1 in cultured vascular endothelial cells leads to a lymphatic reprogramming of these cells with upregulation of LEC markers and suppression of certain BEC markers (Hong et al. 2002; Petrova et al. 2002). Prox1 also increases endothelial cell motility and migration (Mishima et al. 2007; Dadras et al. 2008 (in press). LYVE-1, a homolog of the hyaluronan receptor CD44 (Banerji et al. 1999), is the first marker of lymphatic competence and is expressed in the cardinal vein at E8.5 (Oliver 2004). In adults, LYVE-1 is expressed not

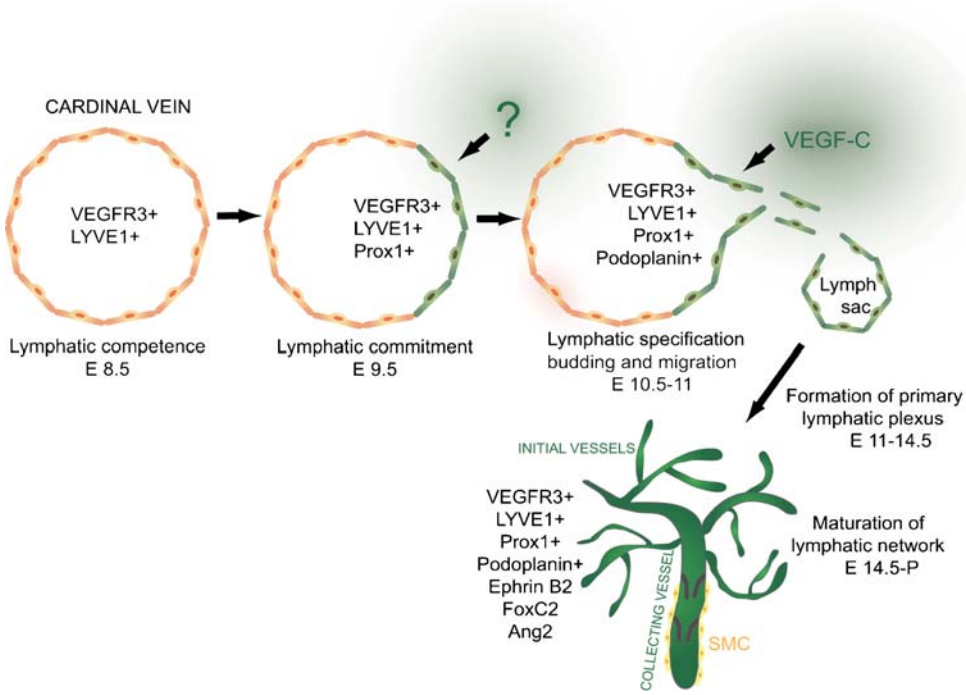

Fig. 1 Current model of the stepwise embryonic development of the mammalian lymphatic system. During early vascular development, endothelial cells of the embryonic cardinal vein express the two lymphatic markers LYVE1 and vascular endothelial growth factor receptor-3 (*LYVE1*+ and *VEGFR-3*+; stage of lymphatic competence). Stimulation by an as yet unidentified mesenchymal signal induces expression of the transcription factor Prox1 in a subset of endothelial cells at the lateral side of the cardinal vein (*Prox1*+; stage of lymphatic commitment). These cells bud off from the vein and migrate into the surrounding tissue to form primitive lymph sacs. During this process, they adopt the expression of additional lymphatic lineage markers. The formation of a mature lymphatic network involves the function of additional genes, viz., podoplanin, ephrin B2, neuropilin-2, and FoxC2, and continues throughout the first few postnatal days. The mature lymphatic network is composed of initial lymphatic vessels and of collecting vessels covered with smooth muscle cells (*SMC*)

only by lymphatic endothelium, but also by liver sinusoids, some lung blood vessels, high endothelial venules, and activated tissue macrophages (Jackson 2003). Recently, LYVE-1 has been reported to be downregulated upon incubation of cultured LEC with tumor necrosis factor-alpha and also in an in vivo inflammation model (Johnson et al. 2007). However, the potential function of LYVE-1 function remains unclear since LYVE-1 deficient mice do not exhibit any abnormal phenotype, including lymphatic development and immune cell trafficking (Gale et al. 2007).

The vascular endothelial growth factor receptor-3 (VEGFR-3, also known as Flt4) is a specific receptor for the lymphangiogenic factors vascular endothelial growth factor-C (VEGF-C) and VEGF-D (Tammela et al. 2005a). Even before the onset of lymphatic vascular differentiation, VEGFR-3 is highly expressed by blood vascular endothelial cells, but its expression becomes gradually restricted to lymphatic endothelial cells after mid-gestation (Kaipainen et al. 1995). In agreement with its early expression in the blood vasculature, VEGFR-3 deletion in mice causes cardiovascular failure and embryonic death before the emergence of the lymphatic vasculature (Dumont et al. 1998). However, VEGFR-3 mutations have been identified as a cause for the cutaneous lymphedema in *Chy* mice, demonstrating the essential role of VEGFR-3 in lymphatic development and function (Karkkainen et al. 2001). In the adult, VEGFR-3 expression has also been reported in proliferating blood vessels during tumor angiogenesis (Laakkonen et al. 2007; Paavonen et al. 2000), indicating that VEGFR-3 might also be a mediator of angiogenesis under these conditions. During embryonic development, its ligand VEGF-C is produced by vascular smooth muscle cells and mesenchymal cells in areas adjacent to the sites of initial sprouting of LECs from veins (Kukk et al. 1996). The importance of VEGF-C/VEGFR-3 signaling in lymphatic development has recently been demonstrated in embryos homozygous for the VEGF-C deletion. These mice lack a lymphatic vasculature because of a lack of sprouting of lymphatically differentiated endothelial cells (Karkkainen et al. 2004). Whereas VEGF-C $^{-/-}$ mice die at around E15, VEGF-C $^{+/-}$ mice survive into adulthood but display severe lymphatic hypoplasia (Karkkainen et al. 2004). Although VEGF-D is able to rescue the sprouting in VEGF-C $^{-/-}$ embryos, VEGF-D deficient mice do not exhibit a lymphatic phenotype, indicating that VEGF-D is dispensable for the development of the lymphatic system (Baldwin et al. 2005). In culture, both VEGF-C and VEGF-D promote the proliferation, migration, and survival of lymphatic endothelial cells via signaling through VEGFR-3 (Makinen et al. 2001).

When LECs further migrate to form the first capillary networks, they begin to express the mucin-type transmembrane glycoprotein podoplanin (Schacht et al. 2003). Podoplanin is strongly expressed by lymphatic endothelium but not by blood vascular endothelium in vivo and in vitro (Breiteneder-Geleff et al. 1999; Hirakawa et al. 2003). Podoplanin null mice are characterized by lymphedema, abnormal patterning of the lymphatic vessel network, and impaired lymphatic transport (Schacht et al. 2003), but the detailed molecular functions of podoplanin remain unknown. A number of additional molecules appear to have a role during lymphatic development. Adrenomedullin, known as a potent vasodilator, has been recently found to play a role in lymphatic development based on studies in several knockout mouse models (Fritz-Six et al. 2008). Deficiency of adrenomedullin or of its receptors RAMP2 or calcitonin receptor-like receptor lead to pronounced edema associated with hypoplastic jugular lymph sacs and death at mid-gestation. However, whether impaired adrenomedullin signaling in blood vascular endothelium, associated with enhanced vascular permeability, contributes to the edema phenotype remains unclear (Ichikawa-Shindo et al. 2008).

Molecules involved in the maturation of the lymphatic vascular network

The formation of a mature lymphatic network continues throughout the first few postnatal days, and mechanisms for keeping the blood and lymphatic vascular compartments apart are essential to maintain the proper function of both networks. Circulating endothelial progenitor cells have been found to express the tyrosine kinase SYK and its substrate adaptor molecule SLP-76. Deficiency in SYK or SLP-76 results in arterio-venous shunting and connections between blood vessels and blood-filled lymphatic vessels that show mosaic expression of the lymphatic marker LYVE-1 (Abtahian et al. 2003; Sebzda et al. 2006). These findings have been interpreted to demonstrate a role of circulating progenitor cells for the maintenance of vessel integrity (Abtahian et al. 2003; Sebzda et al. 2006). However, a recent report indicates that the lymphatic-specific glycoprotein podoplanin mediates platelet aggregation via activation of C-type lectin-like receptor 2 (CLEC-2) expressed on platelets, and that this effect involves the activation of SYK and SLP-76 (Christou et al. 2008; Suzuki-Inoue et al. 2007). Thus, interactions between podoplanin and platelet-expressed CLEC-2 might represent a mechanism for preventing leaks between blood and lymphatic vessels.

Fasting-induced adipose factor (Fiaf/Angptl4) also appears to be involved in vascular separation since Fiaf-deficient mice show dilated and blood-filled intestinal lymphatics after birth, associated with reduced expression of the lymphatic marker Prox1 (Backhed et al. 2007). The underlying mechanisms contributing to this phenotype remain to be elucidated. Similarly, blood-filled lymphatic

vessels have been found in embryos deficient in adaptor proteins of the Spred/Sprouty family, Spred-1 and Spred-2 (Taniguchi et al. 2007). Spreds are known as negative regulators of growth factor and cytokine-induced ERK activation and might potentially be involved in the negative regulation of VEGFR-3 signaling.

Besides vascular separation, additional molecular and cellular mechanisms are involved in promoting lymphatic vessel maturation. These include the differentiation of lymphatic capillaries and the collecting lymphatic network, the formation of valves, and the coverage of collecting lymphatic vessels and ducts by smooth muscle cells. Maintenance of VEGF-C signaling seems to be important for all of these processes (Karpanen et al. 2006b). An additional molecule that interacts with VEGF-C and VEGF-D, viz., neuropilin-2 (Nrp-2), has been shown to be essential for lymphatic vessel development and maturation, since Nrp-2 knockout mice fail to develop small-diameter lymphatic vessels (Yuan et al. 2002). Nrps are non-kinase type I transmembrane proteins that have an important role in axon guidance within the nervous system. Whereas Nrp-1 is mainly expressed by arterial endothelial cells, Nrp-2 expression is restricted to veins and lymphatics (Hong et al. 2002; Karkkainen et al. 2001; Yuan et al. 2002). Nrp-2 serves as a co-receptor for VEGF-C and may enhance signaling via VEGFR-3 (Karpanen et al. 2006a). Integrins are also involved in postnatal lymphatic maturation, as evidenced by the development of chylothorax resulting from lymphatic fluid accumulation in the pleural cavity in alpha9 integrin-deficient mice (Huang et al. 2000).

Angiopoietin signaling is necessary for normal blood vessel development as has been shown in a number of genetic mouse models (Dumont et al. 1994; Maisonpierre et al. 1997; Suri et al. 1996). Angiopoietin-1 (Ang1) is an activating ligand for the endothelial-specific Tie-2 receptor tyrosine kinase, and paracrine Ang1-mediated activation of Tie-2 acts as a regulator of vessel maturation and vascular quiescence. Ang2 deficient mice are characterized by impaired patterning of the lymphatic vascular network and recruitment of smooth muscle cells during development. Interestingly, these phenotypes (but not the blood vascular abnormalities) can be rescued by Ang1, suggesting that Ang2 might act as context-dependent agonist of Tie-2 during lymphangiogenesis, whereas it plays an antagonist role in angiogenesis (Gale et al. 2002). Recent studies indicate that Ang1 promotes lymphangiogenesis in mice, as demonstrated by the lymphatic hyperplasia observed after the adenoviral or transgenic delivery of Ang1 (Tammela et al. 2005b). The endothelial-restricted expression of Tie-2 has enabled the use of Tie-2 Cre mice for endothelial-specific gene ablation studies (Forde et al. 2002).

Ephrin/Eph signaling systems are involved in both angiogenic and lymphangiogenic processes. EphB2 knockout mice show defects in LEC sprouting, lymphatic patterning, and valve-formation and also have the ectopic coverage of skin lymphatic capillaries by smooth muscle cells (Makinen et al. 2005). The forkhead transcription factor FOXC2 is highly expressed in the developing lymphatic vasculature and in the luminal valves of adult lymphatic vessels. In Foxc2 deficient mice, the lymphatic vessels fail to form valves and lymphatic capillaries acquire ectopic coverage by smooth muscle cells and components of the basal lamina (Petrova et al. 2004). Overall, studies of lymphatic development have led to the identification of a number of novel markers for distinguishing lymphatic and blood vessels in murine and human tissues (Table 1).

Additional lymphatic growth factors

Hepatocyte growth factor (HGF) has recently been identified as a potent lymphangiogenic factor (Kajiya et al. 2005). HGF promotes LEC proliferation, migration, and tube formation via its receptor HGF-R (Fig. 2). The promigratory effects of HGF are partially mediated by the alpha9 integrin that is specifically expressed by LECs (Huang et al. 2000). HGF also promotes lymphangiogenesis in several organs in vivo, as demonstrated in HGF transgenic mice (Kajiya et al. 2005). Fibroblast growth factor-2 (FGF-2) was one of the earliest angiogenic factors identified and its role in angiogenesis has been well documented (Auguste et al. 2003). FGF-2 also promotes lymphangiogenesis by induction of VEGF-C secretion by blood vascular endothelium and perivascular cells in the mouse cornea assay (Kubo et al. 2002). Prox1 has been found to upregulate FGF receptor-3, which binds FGF-2 and mediates lymphangiogenesis independently of VEGFR-3 activation (Shin et al. 2006). Platelet-derived growth factor-BB (Cao et al. 2004) and insulin-like growth factors 1 and 2 (Bjorndahl et al. 2005) have been recently identified as additional factors with lymphangiogenic activity in vitro and in vivo. In consideration of the number of lymphangiogenic factors identified and the substantial cross talk with diverse receptors (Fig. 2), some of these factors may exhibit complex additive or synergistic effects on lymphatic growth and function.

Lymphatic involvement in disease

The distinct role of lymphatic vessels in various diseases has, for a long time, been obscured by the lack of lymphatic-endothelium-specific markers, growth factors, and receptors. The recent discovery of lymphatic markers such as podoplanin, Prox1, and LYVE-1 has made it possible to distinguish lymphatic vessels from blood vessels in situ and to isolate LECs for gene expression

Table 1 Markers for lymphatic (*LEC*) and blood vascular endothelial (*BEC*) cells

Marker	Molecular function	LEC	BEC	Refrences
Prox1	Transcription factor	++	–	Wigle and Oliver 1999
Podoplanin	Transmembrane glycoprotein	++	–	Schacht et al. 2003
CD31	Adhesion molecule	+	++	Albelda et al. 1991
CD34	Adhesion molecule	–(+)[a]	++	Young et al. 1995
CD44	Hyaluronan receptor	–	+	Kriehuber et al. 2001
Lyve1	Hyaluronan receptor	++	–	Jackson 2003
Vegfr3	Tyrosine kinase receptor	++	–(+)[b]	Kaipainen et al. 1995
Vegfr1	Tyrosine kinase receptor	–	+	Hirakawa et al. 2003
Vegfr2	Tyrosine kinase receptor	+	++	Quinn et al. 1993
Vegf-C	Growth factor	–	+	Hirakawa et al. 2003
Nrp-1	Semaphorin and growth factor receptor	–	+	Hong et al. 2002
Nrp-2	Semaphorin and growth factor receptor	+	–(+)[c]	Yuan et al. 2002
SLC/CCL21	CC-chemokine	+	–	Gunn et al. 1998
CCL20/MIP-3α	CC-chemokine	+ (++)[d]	– (++)[d]	Hirakawa et al. 2003
Endoglin/CD105	Low-affinity receptor for TGF-ß	–	++	Hirakawa et al. 2003
Meca-32	Unknown	–	++ [e]	Penn et al. 1993
Desmoplakin	Anchoring protein of adherens junctions	+	–	Ebata et al. 2001
VE-cadherin	Adhesion molecule	+	++	Baluk et al. 2007
PAL-E	Antibody recognizing Nrp1	–	++[f]	Jaalouk et al. 2007
Interleukin-8	CXC-chemokine	–	+	Petrova et al. 2002
Collagen IV	Basement membrane molecule	–(+)[g]	++	Hirakawa et al. 2003
Collagen XVIII	Basement membrane molecule	–(+)[g]	++	Hirakawa et al. 2003; Petrova et al. 2002
Integrin alpha9	Adhesion molecule, VEGFR-3 coreceptor	+	–	Huang et al. 2000; Petrova et al. 2002
Macrophage mannose receptor (MRC1)	L-selectin receptor	+	–	Irjala et al. 2001

[a] CD34 expression has also been found on the LECs
[b] VEGFR-3 expression has been observed in tumor-associated blood vessels
[c] Neuropilin-2 is also expressed in veins
[d] Both LECs and BECs express CCL20 after activation
[e] Meca-32 is an exclusive mouse antigen
[f] PAL-E recognizes Nrp1. PAL-E labels only human vasculature
[g] Initial lymphatics lack or have an incomplete basement membrane

and proteomic profiling studies and for functional in vitro tests (Hirakawa et al. 2003; Kriehuber et al. 2001; Roesli et al. 2008). These studies, together with numerous new genetic mouse models, have provided new insights into the contribution of lymphatic endothelium to various diseases. We will focus, in our review, on lymphatic vascular involvement in lymphedema and in inflammatory diseases, since the role of lymphatic vessels in cancer metastasis has

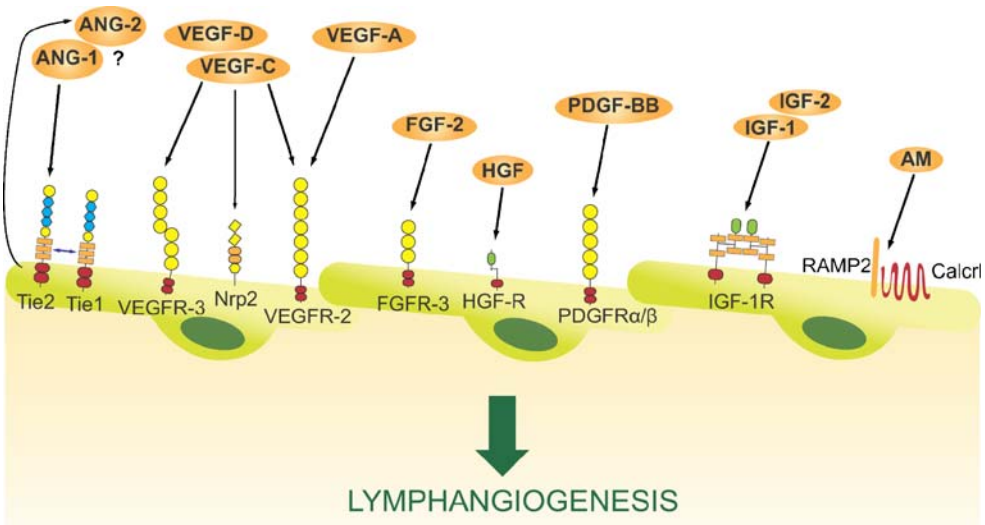

Fig. 2 Representation of lymphangiogenic growth factors and their receptors expressed by lymphatic endothelium. Several vascular endothelial growth factors (*VEGF-A*, *VEGF-C*, *VEGF-D*) promote lymphangiogenesis by activation of VEGF receptors-2 and -3 (*VEGFR-2*, *VEGFR-3*) and neuropilin-2 (*Nrp2*). Additional lymphatic growth factors include angiopoietin-1 (*ANG-1*), hepatocyte growth factor (*HGF*), fibroblast growth factor-2 (*FGF-2*), insulin-like growth factors (*IGF-1*, *IGF-2*), platelet-derived growth factor-BB (*PDGF-BB*), and adrenomedullin (*AM*)

been comprehensively covered in recent reviews (Tobler and Detmar 2006; Wissmann and Detmar 2006).

Lymphedema

Impaired formation or function of the lymphatic network results in disfiguring and occasionally life-threatening swelling of the limbs, called lymphedema (Szuba and Rockson 1997). Lymphedema can arise as a hereditary primary disorder or as a secondary disorder. It is accompanied by fibrosis and susceptibility to infections and inflammation (Tabibiazar et al. 2006). Currently, there is no cure for lymphedema, and available treatments consist predominantly of remedial massage and restrictive bandaging.

Mutations that compromise the development or function of lymphatic vessels and that lead to lymphedema have been identified in several human genes. Inactivating mutations in the kinase domain of the VEGFR-3 gene have been found in congenital lymphedema (Milroy's disease) with autosomal dominant inheritance (Brice et al. 2005; Butler et al. 2007; Karkkainen et al. 2000). These mutations inhibit phosphorylation of the receptor and prevent its downstream signaling. VEGFR-3 mutations have been detected in patients with family history of lymphedema, although *de novo* mutations have also been reported (Ghalamkarpour et al. 2006). Because of a naturally occurring mutation in VEGFR-3, a similar phenotype is seen in the *Chy* mouse (Karkkainen et al. 2001). In addition, recent studies have identified mutations in the FOXC2 gene as being responsible for lymphedema-distichiasis syndrome (Fang et al. 2000; Mangion et al. 1999; Sholto-Douglas-Vernon et al. 2005). The underlying mechanisms have been elucidated in FOXC2 deficient mice (Petrova et al. 2004). Mutations of the SOX18 gene, a SRY-related transcription factor, cause autosomal recessive and dominant forms of hypotrichosis lymphedema telangiectasia syndrome (Irrthum et al. 2003). The link to the SOX18 gene has been established by the phenotypic homology to "ragged" mice in which the phenotype is caused by four different premature truncations in SOX18 (Pennisi et al. 2000). In humans, mutations in the DNA-binding domain of SOX18 have been found in the recessive form of the disease, whereas in the dominant form, a heterozygous nonsense mutation has been found in the transactivation domain (Irrthum et al. 2003). The expression of SOX18 is regulated by VEGFR-3 activation and is an early marker of lymphatic differentiation; however, its detailed function remains currently unclear (Cermenati et al. 2008).

A point mutation in the nuclear factor kappa B (NFkB) essential modulator gene NEMO causes a disease with multiple symptoms, referred to as OL-EDA-ID (osteopetrosis, lymphedema, ectodermal dysplasia-anhidrotic, immunodeficiency; Doffinger et al. 2001). This disease indicates the potential importance of the NFkB pathway in lymphatic vascular function. Indeed, NFkB has been found to be constitutively active in lymphatic endothelial cells, although no functional role has as yet been attributed to this activity (Doffinger et al. 2001).

The major cause of secondary lymphedema in developing countries is infection with the mosquito-borne parasites *Wuchereria bancrofti* and *Brugia malayi* (Melrose 2002). This disorder, called lymphatic filariasis and more commonly known as elephantiasis, is a painful and profoundly disfiguring disease characterized by massive damage to the lymphatic vessels leading to permanent disability. Despite its major social and economic impact on affected countries, it is categorized as a neglected tropical disease (Beyrer et al. 2007).

In the industrialized countries, secondary lymphedema is mainly caused by surgical removal of lymph nodes or radiation therapy, particularly in patients with breast cancer. Secondary lymphedema remains a significant clinical problem with, according to some studies, one out of five women developing the condition following treatment for breast cancer (Clark et al. 2005).

The recent identification of growth factors capable of directly inducing the growth of lymphatic vessels, such as VEGF-C and VEGF-D, may provide new strategic approaches for the therapy of lymphedema (Rockson 2005). Adeno-associated virus-mediated delivery of VEGF-C to the skin of *Chy* mice improves the lymphedema phenotype (Karkkainen et al. 2001). Notably, a mutant form of VEGFC, viz., VEGF-C156S, that selectively binds VEGFR-3 but not VEGFR-2 successfully induces the formation of cutaneus lymphatic networks without causing blood vessel leakage, an overt effect observed with wild-type VEGF-C therapy (Saaristo et al. 2002). Therapy with VEGF-C protein has also been successfully applied in a surgical lymphedema model in the rabbit ear (Szuba et al. 2002). Importantly, treatment of lymph node-excised mice with adenovirally delivered human VEGF-C or VEGF-D results in a considerable improvement of the lymphatic draining function, associated with regeneration of mature lymphatic vessels. VEGF-C therapy also greatly improves the outcome of lymph node transplantation, indicating a potential treatment strategy for secondary lymphedema (Tammela et al. 2007).

Inflammation-associated lymphangiogenesis

Inflammation is the normal response of the body to injury or infection but may also develop and persist in the context of autoimmune diseases and during tumor growth. Inflammation has also long been known to induce pronounced changes of the blood vasculature thereby significantly contributing to the clinical symptoms of inflammation: redness, warmth, and

swelling (Pober and Sessa 2007). In addition to angiogenesis, recent studies have detected pronounced lymphangiogenesis in mouse models of chronic airway inflammation, psoriasis, and rheumatoid arthritis (Baluk et al. 2005; Kunstfeld et al. 2004; Paavonen et al. 2002; Zhang et al. 2007). In particular, pronounced lymphatic hyperplasia occurs in human psoriatic skin lesions and also in a VEGF-A transgenic mouse psoriasis model (Kunstfeld et al. 2004). In inflamed tissues, the lymphangiogenic factors VEGF-C and VEGF-A are secreted by immune cells such as macrophages or by resident tissue cells such as fibroblasts and keratinocytes (Ristimaki et al. 1998). Unpublished observations from our laboratory suggest that lymphangiogenesis is also associated with inflammatory bowel disease in a mouse model of interleukin-10 deficiency (Fig. 3). These findings indicate a potential involvement of the lymphatic vascular system in the pathogenesis of human inflammatory bowel syndromes (ulcerative colitis and Crohn's disease) in which lymphatic hyperplasia has scarcely been studied (Pedica et al. 2008).

Recently, we have found that acute ultraviolet B (UVB) irradiation of murine skin results in leaky, functionally impaired lymphatics, and that blockade of VEGFR-3 prolongs UVB-irradiation-induced inflammation (Kajiya et al. 2007). Importantly, studies in mice indicate that UVB-mediated upregulation of VEGF-A induces lymphatic hyperpermeability that can be blocked by systemic application of an anti-VEGF-A antibody (Kajiya et al. 2006). Furthermore, the nitric oxide/soluble guanylate cyclase alpha1beta1/cGMP pathway has been found to modulate lymphatic vessel function in UVB-irradiation-caused inflammation, indicating that blockade of soluble guanylate cyclase might represent a novel therapeutic strategy for inhibiting lymphangiogenesis and inflammation (Kajiya et al. 2008).

Lymphatic vessels also play a key role in the migration of dendritic cells to draining lymph nodes where they initiate the adaptive immune response (Halin and Detmar 2006). Mature inflammatory-signal-activated dendritic cells upregulate the expression of chemokine receptor CCR7. Lymphatic endothelium actively secretes the respective ligand, CCL21, causing dendritic cell chemotaxis toward lymphatic vessels (Bromley et al. 2005; Debes et al. 2005). CCR7 might also mediate the exit of $CD4^+$ memory T cells from tissues to lymphatic vessels (Yuan et al. 2002). Furthermore, inflammatory stimuli have been reported to increase the lymph flow, which promotes dendritic cell migration to lymph nodes (Gunn et al. 1998). There is increasing evidence that inflammation-induced expression of adhesion molecules on lymphatic endothelium might facilitate the exit of dendritic cells and lymphocytes into lymphatic vessels. Incubation of LECs with tumor necrosis factor-alpha upregulates the expression of intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), and E-selectin, and systemic blockade of ICAM-1 and VCAM-1 has been reported to inhibit inflammation-induced lymphatic transmigration in vivo (Johnson et al. 2006). Sphingosine-1-phosphate (S1P) and its receptor $S1P_1$/EDG1 have been implicated in the process of lymphocyte egress from the thymus and lymph nodes (Halin et al. 2005; Matloubian et al. 2004). Recent data indicate that stimulation of $S1P_1$ on T lymphocytes by the $S1P_1$ agonist FTY720 inhibits their migration across the endothelium of afferent lymphatic vessels, leading to retention of T lymphocytes in peripheral tissues (Ledgerwood et al. 2008).

Interestingly, lymphangiogenesis is also observed in the lymph nodes draining the inflamed tissue, indicating a remote control by drainage of lymphangiogenic factors from peripheral tissues, although secretion of lymphangiogenic factors by cells residing within lymph nodes might contribute to this effect (Angeli et al. 2006; Halin et al. 2007).

Overall, the biological role of lymphangiogenesis in the pathogenesis of chronic inflammation needs further clarification. On one hand, activation of lymphatic vessels might

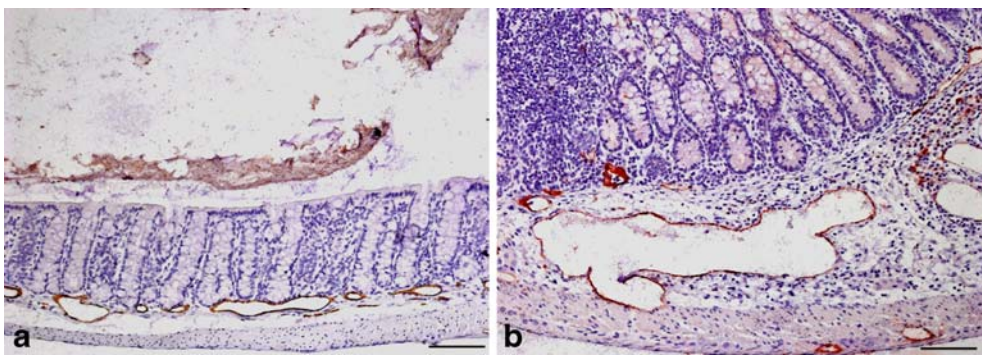

Fig. 3 Lymphangiogenesis in colon inflammation. Whereas wild-type mice show normal lymphatic vasculature in the submucosal area of the colon wall (**a**), C3H/HeJBir.*Il10*$^{-/-}$ mice display dramatically enlarged and tortuous lymphatic vessels accompanying the colon inflammation (**b**). Stained for the lymphatic vessel marker LYVE-1 (*red*); counterstained with hematoxylin. *Bars* 100 μm

promote immune cell trafficking to the draining lymph nodes, thereby supporting the establishment of an inflammatory loop. Indeed, lymphangiogenesis has been proposed to contribute to renal transplant rejection by promoting the export of CCR7-positive inflammatory cells guided by LEC-derived CCL21 (Kerjaschki et al. 2004). Moreover, blockade of VEGFR-3 in the initial stage of inflammation in mouse corneas impairs dendritic cell trafficking to draining lymph nodes, the induction of delayed-type hypersensitivity, and the rejection of corneal transplants (Chen et al. 2004). On the other hand, lymphangiogenesis might be beneficial for the resolution of chronic inflammation since lymphatic vessels drain, and thereby remove, accumulated fluid, immune cells, and inflammatory cytokines from the sites of inflammation. Indeed, inhibition of VEGFR-3 signaling has been found to impair the lymphatic vascular network and to promote mucosal edema in a mouse model of chronic airway inflammation (Baluk et al. 2005). Further experimental studies and observations in human inflammatory conditions indicate that impaired lymphatic drainage leads to the exacerbation of disease (Kajiya et al. 2007; Middel et al. 2006; Ryan 1980). VEGF-A and VEGF-C appear to exert opposing effects in chronic inflammation (Halin et al. 2007; Kajiya et al. 2006, 2007). Whereas VEGF-A might promote inflammation by inducing more leaky and non-functional lymphatic vessels, VEGF-C seems to promote lymphatic flow and the resolution of inflammation. However, more experimental evidence is required to support this concept further. Taken together, the emerging role of lymphatic endothelium in the context of inflammation indicates that anti-inflammatory therapeutics targeting the lymphatic vasculature might represent a new strategy to improve the lives of patients with inflammatory conditions.

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