The lymphatic vascular system is crucial for the regulation of tissue fluid homeostasis, immune function, and fat metabolism. Lymphatic dysfunction, either due to gene mutations or secondary to damage to the lymph vessels, may lead to lymphedema, a debilitating condition characterized by chronic tissue edema, impaired immunity, and accumulation of subcutaneous fat.

The lymphatic and blood vascular systems function in concert to regulate the tissue fluid homeostasis of the body. The pumping force of the heart generates a hydrostatic pressure, which pushes fluid out of the semipermeable blood capillaries into the interstitial space. Most of the extravasated interstitial fluid and macromolecules are absorbed back by the lymphatic vessels, whereas some reabsorption may also occur in the venules, depending on the tissue (Figure 1). Increased endothelial permeability, venous obstruction or insufficiency, and lymphatic vessel dysfunction lead to tissue swelling, or edema (Figure 1).

In contrast to the blood vasculature, the lymphatic vascular system is a unidirectional transport system. Fluid, cells, and macromolecules present in the interstitial space first enter blind-ended lymphatic capillaries (Figure 2). Compared with blood capillaries, lymphatic capillaries are irregular and have a relatively wide lumen (Figure 2). They are lined by oak leaf–shaped lymphatic endothelial cells (LECs) that contain overlapping endothelial flaps that function as primary valves (also called microvalves), making the lymphatic capillaries highly permeable. The capillary LECs have specialized discontinuous button-like junctions (Figure 3) located only at the sides of the flaps that contain proteins typical for both tight and adherens junctions. Unlike the blood capillaries, the lymphatic capillaries are not ensheathed by pericytes or smooth muscle cells (SMCs) and have little or no basement membrane. Instead, capillary LECs are attached to the extracellular matrix via elastic anchoring filaments (Figure 2) that prevent vessel collapse under conditions of high interstitial pressure. Increased interstitial pressure stretches the anchoring filaments, pulling open the endothelial flaps and allowing fluid and macromolecules to enter the vessel lumen.

From the lymphatic capillaries, the lymph is further transported via precollectors to collecting lymphatic vessels (Figure 2) and is returned to the blood circulation through the lymphaticovenous junctions between the thoracic or lymphatic duct and the subclavian veins. In the larger lymphatic vessels, the lymph is propelled forward by intrinsic SMC contractility, vasomotion, and the contraction of surrounding skeletal muscles. Relaxation of the lymphatic smooth muscle is dependent on nitric oxide, which is required for the lymphatic pump to reach maximal amplitudes and efficient flow. Retrograde flow of the lymph is prevented by bileaflet secondary valves in the precollectors and collecting vessels (Figure 2). In contrast to the lymphatic capillaries, the collecting lymphatic vessels are covered by basement membrane and pericytes/SMCs (Figure 2) and have continuous zipper-like endothelial junctions (Figure 3). Interestingly, the junctions in sprouting lymphatic capillaries stimulated by vascular endothelial growth factor (VEGF)-C revert from the button-like to the zipper-like organization, suggesting that the button-like morphology is a hallmark of resting lymphatic capillaries (Figure 3).

Molecularly, lymphatic capillaries and collecting vessels differ from each other in their expression of lymphatic endothelial markers such as lymphatic vessel hyaluronan receptor-1 (LYVE-1), the Prospero-related homeobox transcription factor 1 (Prox1), the forkhead box transcription factor Foxc2, the chemokine CCL21, and VEGF receptor (VEGFR)-3 (VEGFR-3), which are all highly expressed in all lymphatic vessels during development but downregulated in mature collecting vessels (Figures 2 and 3). In adults, the expression of Prox1, Foxc2, and VEGFR-3 remains high in collecting vessel valves, with lower levels in the interspersed lymphangions (Figures 2 and 3). LYVE-1, on the other hand, is largely absent from the collecting vessels. After maturation, lymphatic capillaries continue to express high levels of LYVE-1, Prox1, and VEGFR-3 and the cell surface mucoprotein podoplanin, whereas Foxc2 is expressed mainly in collecting vessels.

In addition to draining and transporting fluid, the lymphatic vascular system also plays an important role in immune responses by transporting extravasated leukocytes, antigens, and activated antigen-presenting cells. Collecting vessels are interrupted by lymph nodes, which act as hubs for lymphocytes and antigen-presenting cells and as filters for antigens. Antigen-presenting cells enter the lymph node via multiple
where specialized lymphatic capillaries called lacteals in the intestinal villi take up the lipid particles (chylomicrons) released by the enterocytes.

The lymphatic network permeates most organs in the body. Only avascular tissues such as the cornea, cartilage, and epidermis and some vascularized tissues like the central nervous system are devoid of lymphatic vessels. In addition to mammals, birds, fish and amphibians have a secondary lymphatic or lymphatic-like vascular system.

**Development of the Lymphatic Vasculature**

Lymphatic vessels develop only after the establishment of the blood circulation, at around embryonic week 6 to 7 in humans and at embryonic day 10 in mice. The best accepted view of lymphatic vascular formation, proposed more than a century ago by Florence Sabin, is that the lymphatic vessels arise by sprouting from the jugular veins. A study published in 1995 showed that high expression of VEGFR-3 specifically marks the developing lymphatic vessels. However, germline deletion of the Vgfr3 gene in mice showed that it is also required for blood vessel development before the emergence of the lymphatic vessels. The lymphatic vessels start to develop when a distinct subpopulation of endothelial cells (ECs) in the anterior cardinal vein start to express Prox1 and become committed to a lymphatic endothelial lineage. The spatially and temporally specific expression of Prox1 in the cardinal vein and the subsequent differentiation of LECs are induced by the transcription factor sex-determining region Y box 18 (Sox18). In addition to Sox18, the orphan nuclear receptor chicken ovalbumin upstream promoter transcription factor II (COUP-TFII), previously shown to specify venous EC fate, is required for initiation and early maintenance of Prox1 expression. Prox1 protein levels are also likely regulated by the microRNAs miR-181 and miR-31.

Subsequent to the induction of Prox1 expression in the cardinal vein, the LECs start to bud off from the cardinal vein, migrating in the direction of a gradient formed by VEGF-C, the first identified VEGF-3 ligand, expressed by mesenchymal cells dorsolateral to the cardinal veins. Mice with a targeted inactivation of the Prox1 or Vegfc gene fail to develop even the first primitive lymph sacs. In the Prox1- or Vegfc-deficient mice, the migration of the LECs from the cardinal vein is arrested, severe global edema develops, and the embryos subsequently die. Although Prox1 is also required for normal retinal, lens, brain, pancreas, and liver development, VEGF-C is the only growth factor specific to lymphatic vessel development. Interestingly, a recent study shows that in addition to VEGF-C, the collagen and calcium binding EGF domain 1 (CCBE1) protein is required for the budding and sprouting of lymphangioblasts from the venous endothelium and for thoracic duct formation in zebrafish.

The initial budding of the LECs from the cardinal vein is Prox1 independent, but maintenance of the LEC identity requires constant Prox1 activity. Prox1 is a master gene controlling LEC fate and identity; overexpression of Prox1 in cultured blood ECs leads to induction of LEC-specific genes and downregulation of blood EC-specific genes. Recently, Prox1 has been shown to directly interact with COUP-TFII and in concert...
regulate LEC-specific gene expression. Interestingly, in line with these in vitro studies, COUP-TFI is also required for lymphangiogenic sprouting during later stages of development, possibly by directly regulating the expression of neuropilin-2, a VEGF-C coreceptor. In addition to developmental lymphangiogenesis, Prox1 mediates inflammation-induced lymphangiogenesis, in collaboration with the nuclear factor-κB transcription factor, through upregulation of VEGFR-3. Furthermore, interesting new results suggest a role for VEGF-C/VEGFR-3 signaling in the formation of the cardinal vein from a common precursor vessel in zebrafish; a somewhat similar principle may operate also in mammalian development.

An important step during lymphatic development is the separation of the forming lymphatic vessels from the blood vasculature. In adults, connections between the blood and lymphatic vasculature normally exist in only a few locations, such as the junction where the thoracic duct and the right lymphatic duct drain into the left and right subclavian veins, respectively. The tyrosine kinase Syk and the adaptor protein Slp-76 are essential for the lymphaticovenous separation of both PECAM-1 and VEGFR-3 in the lymphatic valves (arrow). Scale bars: 100 μm. C, Lymphatic capillaries are thin walled with a wide lumen and do not contain pericytes and a basement membrane. Anchoring filaments attach lymphatic capillaries to the extracellular matrix. Fluid, cells, and proteins that leak out from the blood capillaries enter the blind-ended lymphatic capillaries via overlapping edges of neighboring endothelial cells (microvalves). The lymph is then further drained into collecting lymphatic vessels. D, Lymphatic collecting vessels are surrounded by a basement membrane and pericytes/smooth muscle cells (SMC), and they contain luminal valves that prevent the backflow of the lymph.

After formation of the primary lymph sacs along the anteroposterior embryonic axis, they fuse to a single lymphatic plexus, which spreads by lymphatic sprouting—lymphangiogenesis—to form peripheral lymphatic vessels throughout the developing embryo. Subsequently, the lymphatic vessels undergo remodeling and maturation to form a hierarchical network of lymphatic capillaries and lymphatic collecting vessels (Figures 2 and 3). Several molecules have been implicated in the maturation of the lymphatic network. The forkhead transcription factor Foxc2 is highly expressed in the developing collecting lymphatic vessels and in lymphatic vessel valves in adults. The early development of
lymphatic vessels proceeds normally in the absence of Foxc2, but the subsequent remodeling fails; collecting lymphatic vessels in Foxc2−/− mice lack valves, and the lymphatic capillaries acquire an ectopic coverage by SMCs and basement membrane components.3 In addition, the collecting vessels fail to downregulate lymphatic marker molecules such as VEGFR-3, Prox1, and LYVE-1.3 These results indicate that Foxc2 controls the specification of the lymphatic capillary versus collecting lymphatic vessel phenotype. Our recent results also show that the nuclear factor of activated T cell (NFAT)c1 transcription factor cooperates with Foxc2 in regulating the lymphatic maturation3 (Figure 3). The NFATc1 activity in LECs is induced by VEGF-C via VEGFR-2 and/or VEGFR-2/3 heterodimers, leading to dephosphorylation and nuclear localization of NFATc1. The phosphatase activity of calcineurin can be blocked with cyclosporine A (CsA). Subsequently, FOXC2 and NFATc1 together regulate transcription of downstream genes. Lack of either FOXC2 or nuclear NFATc1 results in failure of collecting vessel maturation. *Mutations in VEGFR-3 and FOXC2 are a cause of primary lymphedema. C. Button-like and zipper-like endothelial cell-cell junctions. Left, On VEGF-C stimulation, the intraendothelial junctions of resting lymphatic capillaries change from button-like junctions to sprouting lymphatic capillaries with zipper-like junctions (triple arrow). Middle, The boundary of a resting lymphatic capillary with button-like junctions (arrowheads) and a collecting lymphatic vessel with zipper-like junctions. Arrow indicates flow direction. Staining for VE-cadherin (red). Sp indicates sprout; LC, lymphatic capillary; and cLV, collecting lymphatic vessel. Right, High Foxc2 expression in mouse venous valves (arrows). Staining for Foxc2 (red) and podoplanin (green). Unlike nearby lymphatic vessels (arrowheads), veins do not express podoplanin and are filled with red blood cells. Left and middle panel adapted from Tammela et al.4 Copyright © 2007, originally published in Nature Medicine.

controlling blood vessel remodeling.47 Mutant mice lacking the PDZ domain of ephrinB2 develop normal blood vasculature but display hyperplasia of the collecting lymphatic vessels, lack of luminal valves, and failure to remodel the primary lymphatic capillary plexus.48 Recent results suggest that ephrinB2 also controls lymphatic sprouting by regulating the internalization and downstream signaling of VEGFR-3.49 The angiopoietins Ang1 and Ang2 are involved in the maturation of the lymphatic vasculature, in addition to their role in blood vascular maturation. Mice lacking Angpt2 display defective remodeling and maturation of the lymphatic vasculature, including lack of lymphatic valves, defective sprouting, and abnormal recruitment of SMCs to lymphatic capillaries.50–52 Importantly, these defects resemble those found in mice with deficient Foxc2, NFATc1, or ephrinB2 signaling.3,9,48 Genetic rescue with Ang1 corrects the lym-
Lymphangiogenic Factors

The VEGF Family

Over the past 2 decades, the VEGFs and VEGFRs have been shown to be essential regulators of vasculogenesis, angiogenesis, and lymphangiogenesis.72 The mammalian VEGF gene family comprises 5 members: VEGF (also called VEGF-A), VEGF-B, VEGF-C, VEGF-D, and placenta growth factor. In addition, 2 nonmammalian growth factors, the VEGF homologs encoded by Orf viruses that are collectively called VEGF-E and VEGF-like proteins in snake venom called VEGF-F, are usually included in the family. The VEGFs mediate their signals via 3 receptor tyrosine kinases, VEGFR-1, VEGFR-2, and VEGFR-3, which consist of 7 extracellular immunoglobulin homology domains, a transmembrane part, and an intracellular tyrosine kinase domain (Figure 4).

(4) Similar to other receptor tyrosine kinases, the VEGFRs form homodimers and heterodimers and undergo transphosphorylation on ligand binding (Figure 4). The signals from VEGFRs typically promote cell proliferation, migration, and survival.74 Specific signals originating from VEGFR-2 also include destabilization of interendothelial junctions and activation of endothelial nitric oxide synthase.74 The main lymphangiogenic factor in both physiological and pathological settings is VEGF-C.52,75 Although VEGF-D has similar properties, its role as an endogenous regulator of lymphangiogenesis, with the exception of lymphangioloemomatosis (LAM; see below), is less clear.76 VEGF seems to stimulate mainly circumferential growth of lymphatic capillaries and, only indirectly, lymphangiogenesis via the recruitment of inflammatory cells.77 VEGF-B does not promote lymphangiogenesis or angiogenesis, but it has recently been shown to regulate endothelial fatty acid transport from blood to highly metabolically active tissues and to induce cardiac hypertrophy and growth of coronary arteries in transgenic rats.77–81

The VEGFs are secreted polypeptide dimers oriented in an antiparallel fashion with receptor binding sites at each end of the dimer (Figure 4). VEGF, placenta growth factor, and VEGF-B isoforms are formed through alternative splicing, whereas the main forms of VEGF-C and VEGF-D are produced by proteolytic processing. Both VEGF-C and VEGF-D are expressed as precursor proteins and are converted into active and mature forms by consecutive cleavages of the C- and N-terminal propeptides.82,83 The receptor binding affinity of VEGF-C and VEGF-D to VEGFR-3 is increased by the proteolytic cleavages, and only the fully processed forms can bind VEGFR-2.84 (Figure 4). In addition to the VEGF receptors, VEGF-C and VEGF-D also bind neuropilin-1 and -2 coreceptors, which modulate the signaling of VEGF receptors, providing specificity for their signal transduction and thus playing important roles in lymphatic vascular regulation.47,85,86 In addition, certain integrins and heparan sulfate proteoglycans can act as coreceptors for the VEGFs, providing additional ways to modulate the signaling.87,88

VEGF-C, the main lymphangiogenic factor of the family, induces proliferation, migration, and survival of ECs.89 During development, VEGF-C is expressed from embryonic day 8.5 on in mesenchymal cells at sites of lymph sac formation, adjacent to VEGFR-3–expressing ECs, and in adults mainly in vascular SMCs.22,90,91 In addition, VEGF-C expression remains high in adult lymph nodes.92 The pattern of VEGF-D expression includes the lungs, heart, skeletal muscle, and intestine.84 Both VEGF-C and VEGF-D are lymphangiogenic, but although Vegfc deletion leads to failure of lymphatic development, Vegfd deletion seems to be dispensable for the development of the lymphatic vasculature.22,93 In Vegfc heterozygous mice, lymph sacs form normally, but the mice display functional defects and hypoplasia of lymph vessels.72 Surprisingly, although VEGF-C and VEGF-D are the only known ligands for VEGFR-3, combined deletion of the 2 growth factors does not reproduce the early embryonic lethality observed in homozygous Vegfr3 knockout mice but instead essentially shows the same defects as single
Vegfc-null mutants. These results suggest that VEGFR-3 can exert ligand-independent signaling, eg, by being phosphorylated via integrin signals and cytoplasmic tyrosine kinases.

Overexpression of either VEGF-C or VEGF-D leads to sprouting lymphangiogenesis, and both factors have been successfully used to grow new lymph vessels in mouse models of lymphedema. VEGF-C or VEGF-D overexpression has also been shown to induce angiogenesis in various models. In skeletal muscle, the proteolytically processed, mature forms of VEGF-C and VEGF-D were shown to promote angiogenesis and lymphangiogenesis and to increase blood flow. Additional N-terminal proteolytic processing was shown to produce a minor form of VEGF-D capable of inducing only angiogenesis. It has further been suggested that VEGF-C is involved in regulating salt-dependent interstitial volume and blood pressure in response to sodium-induced expression of VEGF-C in mononuclear phagocytes, leading to modification of the cutaneous lymphatic capillary network and to VEGFR-2–mediated endothelial nitric oxide synthase activation.

Cross-Talk With Other Growth Factor Pathways

Although VEGF-C and VEGF-D, acting through VEGFR-3 and, to some extent, VEGFR-2, are the key growth factors that can directly stimulate the lymphatic endothelium, other growth factors have been implicated in lymphangiogenesis. Both VEGF and VEGF-E have been shown to promote the growth of lymphatic vessels, which express low levels of VEGFR-2. In addition, fibroblast growth factor-2, insulin-like growth factor-1 and -2, hepatocyte growth factor, lymphotoxin-α, and platelet-derived growth factor-B have been shown to induce lymphangiogenesis in experimental models. However, many of these effects may be secondary to the induction of VEGF-C and VEGF-D, or even VEGF, in a variety of cell types.

The angiopoietins, acting via the endothelial tyrosine kinase receptors Tie1 and Tie2, regulate blood vessel quiescence, vascular permeability, and EC survival. The Ang1 receptor Tie2 is expressed in cultured LECs and in lymphatic vessels in vivo. Analysis of gene-targeted mice has shown that Ang2 is essential for the proper patterning of

Figure 4. Vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs). A, Schematic representation of the VEGF family growth factors (green shapes) and the VEGFR tyrosine kinases. The VEGFs are antiparallel dimers that induce dimerization and activation of their cognate receptors on binding. The VEGFRs are composed of 7 immunoglobulin-like domains (spheres) and a split tyrosine kinase domain (double ovals). The fifth immunoglobulin-like domain of VEGFR-3 is proteolytically cleaved. In adult tissues, VEGFR-1 and VEGFR-2 are expressed predominantly in blood vascular endothelial cells, whereas VEGFR-3 is expressed mainly in lymphatic vascular endothelium. VEGFR-2 is also found in LECs; therefore, VEGFR-2/VEGFR-3 heterodimers may form in these cells. VEGFR-2 and/or VEGFR-2/3 heterodimers on lymphatic endothelial cells are important for lymphatic maturation. 3 sVEGFR-1 indicates soluble VEGFR-1; ΔNΔC, fully processed forms of VEGF-C and VEGF-D lacking the N- and C-terminal propeptides; and PIGF, placenta growth factor. B, Crystal structure of VEGF-C in complex with VEGFR-2 domains 2 (D2) and 3 (D3). A cartoon and a molecular surface representation of the homodimeric VEGF-C (orange and green, respectively) and the 2 VEGFR-2 (D2-D3) chains (light blue; 1 chain is shown as a cartoon; the other chain is shown as a molecular surface representation) in a symmetrical 2:2 homodimeric:homodimeric complex (Protein Data Bank ID code 2X1X3). VEGF-C has an extended N-terminal helix that binds to the VEGFR-2 domains 2 (D2) and 3 (D3). Disulfide bonds and the N-linked glycans are shown as sticks and are yellow and gray. N and C termini, the VEGF ligand binding loops L1 through L3, and the N-terminal helix are labeled.
lymphatic vessels, whereas Ang1 is able to rescue the lymphatic phenotype in Ang2 gene–targeted mice, suggesting that both ligands act as receptor agonists in LECs. Ang1, Ang2, and Ang3/Ang4 have all been shown to promote lymphangiogenic sprouting, with Ang1 having the highest activity independent of Vegfr3 regulation. Another newly discovered lymphatic modulator is claudin-like protein of 24 kDa (Clp24), a hypoxia-regulated transmembrane protein of previously unknown function. In fish and frogs, the lymphatic development failed altogether on knockdown of Clp24, whereas the lymphatic vessels of Clp24−/− mice were dilated and showed abnormal recruitment of SMCs. CLP24 was found to interact with VEGFR-2 and VEGFR-3, and the Clp24−/− phenotype was further aggravated in mice also lacking 1 allele of either Vegfr2 or Vegfr3.

Furthermore, liprin β1 (also called PTPRF interacting protein, binding protein 1), a poorly characterized member of the family of leukocyte common antigen-related transmembrane protein, binding protein 1, regulating lymphatic vessel development, was also recently shown to be highly expressed in lymphatic vasculature and to play an important role in lymphatic vessel integrity. Knockdown of liprin β1 in Xenopus tadpoles resulted in edema, impaired lymphatic function, and defective assembly of lymphatic vessels.

Table. Newly Emerged Lymphatic Vascular Regulators

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Lymphatic Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK1, activin receptor-like kinase 1</td>
<td>Transforming growth factor-β type I receptor</td>
<td>Blocking ALK1 signaling results in remodeling defects of perinatal lymphatic vessels in mice</td>
<td>115</td>
</tr>
<tr>
<td>AM, adrenomedullin</td>
<td>Potent vasodilator regulating vascular permeability</td>
<td>Deletion of AM (or signaling components calcrl or RAMP2) leads to hypoplastic jugular lymph sacs and lymphedema; AM promotes lymphangiogenesis in vivo, possibly via the cAMP/MEK/ERK pathway; AM−/− mice die in utero</td>
<td>116, 117</td>
</tr>
<tr>
<td>Aspp1, apoptosis-stimulating protein of p53</td>
<td>Endothelial-specific activator of p53</td>
<td>Aspp1−/− mice display embryonic edema, disorganized lymphatic vessels, and abnormal drainage function; Aspp1−/− mice are viable</td>
<td>118</td>
</tr>
<tr>
<td>Calcrl, calcitonin receptor–like receptor</td>
<td>Adrenomedullin receptor</td>
<td>Deletion of RAMP2 (or signaling components AM or calcrl) leads to hypoplastic jugular lymph sacs and lymphedema; calcrl−/− mice die in utero</td>
<td>116</td>
</tr>
<tr>
<td>Clp24, Claudin-like protein of 24 kDa</td>
<td>Hypoxia-regulated transmembrane protein; previous function unknown</td>
<td>Clp24 knockout in fish and frog leads to complete failure of lymphatic development, whereas in Clp24−/− mice, the lymphatic vessels show abnormal patterning and smooth muscle cell recruitment; Clp24−/− mice are viable</td>
<td>119</td>
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<tr>
<td>Emilin-1</td>
<td>Extracellular matrix glycoprotein; component of elastic fibers and anchoring filaments</td>
<td>Emilin1−/− mice display hyperplastic, enlarged, and irregularly patterned lymphatic vessels with a reduced number of anchoring filaments; Emilin1−/− mice are viable</td>
<td>120</td>
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<tr>
<td>Liprin β1, PTPRF interacting protein, binding protein 1</td>
<td>Previous function unknown</td>
<td>Knockdown of liprin β1 in frogs results in edema, impaired lymphatic function and defective assembly of lymphatic vessels; highly expressed in lymphatic collecting vessels in mice, especially in lymphatic valves</td>
<td>121</td>
</tr>
<tr>
<td>miR-31</td>
<td>MicroRNA targeting PROX1</td>
<td>Injection of pre–miR-31 molecules in fish and frog leads to lymphatic vascular defects, including loss of lymphatic sprouting from the lymph hearts</td>
<td>21</td>
</tr>
<tr>
<td>RAMP2, receptor activity modifying protein 2</td>
<td>Deletion of RAMP2 (or the signaling components AM or calcrl) leads to hypoplastic lymph sacs and lymphedema; RAMP2−/− mice die in utero</td>
<td>116, 122</td>
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<tr>
<td>Rac1</td>
<td>Rho family GTPase</td>
<td>Endothelial-specific loss of Rac1−/− leads to deficient budding of lymphatic endothelial cells from veins; embryonic lethality</td>
<td>123</td>
</tr>
<tr>
<td>Spred-1/2</td>
<td>Negative regulators of growth factor–induced ERK activation</td>
<td>Combined knockout of Spred1 and Spred2 leads to severe lymphedema, subcutaneous hemorrhages, and blood-filled dilated lymphatic vessels; Spred-1 and -2 negatively regulate VEGF/CVEGFR-3 downstream signaling; Spred-1/2 double-knockout mice die in utero</td>
<td>45</td>
</tr>
<tr>
<td>Synectin, GIPC1</td>
<td>Scaffold protein</td>
<td>Knockdown of synectin in zebrafish and frog leads to defective lymphangiogenic sprouting; synectin genetically interacts with Vegfr3 and neuropilin-2a</td>
<td>124</td>
</tr>
<tr>
<td>Tbx1, T box 1</td>
<td>Transcription factor important in the development of large arteries</td>
<td>Endothelium-specific deletion of Tbx1 of leads to regression of lymphatic vessels and decreased Vegfr3 expression; mice are viable</td>
<td>125</td>
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VEGF indicates vascular endothelial growth factor; VEGFR, VEGF receptor.
In addition to the above-mentioned genes, several new players have been shown to act as regulators of the lymphatic vasculature. These factors are listed in the Table and include Aspp1, Emilin1, Spred-1 and -2, ALK1, the microRNA mir-31, signaling components of the adrenomedullin-RAMP2 pathway, the Rho GTPase Rac1, and the scaffold protein synectin.

Intriguingly, the transcription factor FOXC2 binds to regulatory regions of the genes for several of these newly emerged lymphatic modulators or their family members such as Ramp1 and Ramp3, Spred-2, endothelin-1, transforming growth factor-β receptors, liprin β1, and integrin-α9, as well as to regulatory regions of more classic lymphangiogenic/angiogenic genes such as VEGF-C, PROX1, Angpt2, ephrinB2, and neuropilin-1, as determined in a genome-wide ChiP-chip analysis of primary LECs. Whether FOXC2 indeed regulates these genes in vivo remains to be determined, but the intriguing possibility exists that FOXC2 is a master regulator of a large number of genes involved in lymphatic development.

Lymphatic Dysfunction: The Therapeutic Horizon

Abnormal function of the lymphatic vasculature is involved in several human diseases. Impairment of lymphatic vessel function leads to lymphedema, whereas abnormal proliferation of LECs takes place in lymphangiomas and lymphangiosarcomas. In addition, the lymphatic vessels participate in inflammatory processes and are a major route for tumor metastasis. In this review, we focus on the molecular basis for therapeutic lymphangiogenesis. For more comprehensive reviews on tumor lymphangiogenesis and other lymphatic disorders, we refer the readers to previously published reviews.

Lymphedema

Impairment of the lymphatic vessels and insufficient lymphatic function cause interstitial accumulation of fluid, leading to chronic swelling of the limbs, or lymphedema. In addition to swelling, the protein-rich interstitial fluid induces an inflammatory reaction, leading to fibrosis, accumulation of adipose tissue, and impaired immune responses and wound healing. Although rarely lethal, lymphedema is a disfiguring and disabling condition, decreasing the quality of life. At the moment, there is no cure for lymphedema, but treatments to manage and reduce the swelling include physiotherapy, massage, and compression bandages. Lymphedema is divided into primary and secondary forms based on the underlying cause. Primary (hereditary) lymphedemas result from genetic damage, whereas secondary (acquired) lymphedema is a consequence of lymphatic failure resulting from trauma, surgery, radiotherapy, or parasite infection.

The genetic causes of primary lymphedema have provided valuable insight into the molecular mechanisms regulating the development and function of the lymphatic vascular system. Early-onset congenital lymphedema, also called Milroy disease (Online Mendelian Inheritance in Man (OMIM) No. 153100), has been linked to mutations in VEGFR3, for which a mouse model and treatment modality also have been described. Milroy disease has been reported to result in hypoplasia or aplasia of the superficial lymphatic capillary network. However, skin biopsies from swollen feet of patients have revealed the presence of abundant lymphatic vessels, suggesting that the mechanism of lymphatic failure in Milroy disease might be more complex than previously thought. Interestingly, VEGFR3 mutations in humans were recently shown to lead to incompetence of superficial veins, regardless of whether lymphedema was present or not, suggesting a role of this gene in venous as well as in lymphatic development.

Another form of primary lymphedema is lymphedema-distichiasis (LD; OMIM No. 153400), caused by mutations in the gene encoding FOXC2. Like Milroy disease, it is an autosomal-dominant inherited condition. Most mutations identified in FOXC2 are short insertions or deletions leading to a frame shift and resulting in prematurely truncated forms of FOXC2. Lymphedema-distichiasis is characterized by late-onset lymphedema of the lower extremities, usually manifested after puberty. In addition to lymphedema, the most highly penetrant feature in LD is congenital distichiasis, a double row of eyelashes, occurring in almost 100% of the patients. Less penetrant defects include varicose veins, ptosis, cleft palate, and congenital heart disease. Clinical investigation of LD has revealed lymph reflux in the lower limbs, suggesting primary valve failure in lymphatic vessels as an underlying cause of the lymphedema. Analysis of Foxc2-deficient mice further shows abnormal basement membrane coverage and recruitment of pericytes to the lymphatic capillaries, defects similarly observed in LD patients, implying additional causes for the lymphatic failure in LD. Interestingly, mutations in FOXC2 are also associated with venous valve failure, indicating that in addition to formation of lymphatic valves, FOXC2 is important for venous valve development and maintenance.

A very rare syndrome with associated lymphedema is hypotrichosis-lymphedema-telangiectasia (OMIM No. 607823), caused by mutations in the SOX18 gene, which acts upstream of PROX1 in lymphatic vessel development. This syndrome is characterized by the association of childhood-onset lymphedema in the legs, loss of hair, and telangiectasia, particularly in the palms.

Meige disease, or lymphedema praecox, is an idiopathic late-onset form of lymphedema, commonly detected around puberty. Meige disease is nonsyndromic, with lymphedema clinically indistinguishable from that found in the LD syndrome. Although sometimes mistakenly attributed to mutations in FOXC2, no underlying genetic cause has yet been found for these isolated cases of lymphedema. However, a recent publication reports mutations in the GJC2 gene (encoding connexin 47) in patients with dominantly inherited lymphedema (OMIM No. 613480). Connexin 47 is a gap junction protein, and until now, mutations in its gene were thought to cause only dysmyelination in the central and peripheral nervous system. In the lymphatic system, the GJC2 mutations are proposed to result in impaired gap junction channel activity, leading to impaired coordination of pulsatile lymphatic flow. Interestingly, connexin 47 is the...
first lymphedema gene that does not regulate lymphatic vessel development but instead is involved in regulating lymphatic function. Mutations in CCBE1, previously shown to control lymphatic endothelial cell sprouting in zebrafish, were found in patients with Hennekam lymphangiecatastaselymphedema syndrome (OMIM No. 235510), underscoring conservation of this pathway between species. In addition, lymphedema can occur as an accompanying feature in various other syndromes such as Turner syndrome, Noonan syndrome, and cholestasis-lymphedema syndrome.

The vast majority of lymphedema worldwide is caused by external factors, and hence is referred to as secondary lymphedema. The most common form of secondary lymphedema is lymphatic filariasis (also called elephantiasis), affecting >100 million people in tropical areas. Lymphatic filariasis is caused by infection of the lymphatic vessels by the mosquito-borne parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori*, leading to an inflammatory reaction that stimulates the production of VEGFs and consequentely leading to hyperplasia, obstruction, and scarring of the lymphatic vessels.

A recent study shows that antigens produced by the nematodes can induce the proliferation of LECs and the formation of lymphatic tube-like structures. Doxycycline has been shown to be an effective treatment for some forms of lymphatic filariasis by killing worm symbiotic bacteria.

In industrialized countries, cancer therapy, particularly of breast cancer but also gynecologic cancer, is the leading cause of secondary lymphedema. Metastatic tumor cells frequently spread to the lymph nodes, necessitating radical surgery, including lymph node removal, which, together with radiotherapy, destroys the lymphatic vessel network, especially the collecting vessels, leading to impairment of afferent lymphatic flow. Between 20% and 30% of patients who have undergone radical axillary lymph node dissection during breast cancer surgery develop lymphedema of the upper limb. Interestingly, patients with a high peripheral blood vascular filtration rate, ie, higher demand for lymphatic drainage, were recently shown to be predisposed for breast cancer–related lymphedema of the arm. Other causes of lymph vessel damage can be bacterial infections of the skin (eg, erysipelas) or in the lymphatic vessels themselves (lymphangitis).

**Lymphatic Imaging**

The lack of techniques for imaging dynamic lymph flow in real time has made it difficult to functionally characterize the various forms of lymphedema and to map sentinel lymph nodes noninvasively to evaluate tumor metastasis in cancer patients. More important, this has also been a large factor contributing to the impedance of the development of new treatments for pathological conditions involving the lymphatic vessels. Until recently, only 2 common techniques, lymphangiography and lymphoscintigraphy, have been available for direct imaging of the lymphatic vessels (reviewed elsewhere). Lymphangiography, which involves administration of contrast agents through cannulated lymphatic vessels, has largely been abandoned because of risk of complications from the contrasting agents and the technical skill required for vessel cannulation. In lymphoscintigraphy, radiolabeled colloid particles are injected intradermally or intraparenchymally, taken up by the lymphatic plexus, and then visualized 2-dimensionally. Although lymphoscintigraphy is successfully used to visualize major trunks of the lymphatic plexus and to identify sentinel lymph nodes, the long integration time of the γ camera, the particle size, and the dermal backflow prevent its use in imaging dynamic lymph flow quantitatively. A new optical imaging technique using near-infrared fluorescence involves injection of fluorescent dyes (mainly indocyanine green) into the patient. This method has a clearly higher sensitivity compared with radioactive lymphoscintigraphy and has recently emerged as a useful technique for quantitatively imaging lymph flow, especially when highly stable liposomal formulations are used. Nevertheless, limitations still exist, such as inadequate signal penetration of deep tissues. Additional concerns were raised by the possibility that indocyanine green dye injection stimulates lymphatic smooth muscle contractions. Nevertheless, near-infrared imaging will likely soon allow better functional characterization of the various forms of lymphedema and more precise mapping of sentinel lymph nodes. Immuno-positron emission tomography with radioactively labeled antibodies against LEC surface antigens such as LYVE-1 enables early visualization of tumor- and lymph node–associated lymphangiogenesis. Moreover, a new imaging approach based on optical frequency-domain imaging is also becoming a highly useful optical imaging technique. Optical frequency-domain imaging, a second-generation optical coherence tomography technology, uses entirely intrinsic mechanisms of contrast and hence overcomes the limitations of tissue penetration and the extravasation and other confounding effects of fluorescent tracers. Optical frequency-domain imaging has been successfully demonstrated in high-resolution, wide-field, and deep imaging of tumor microvasculature, as well as in functional lymphangiography. Another emerging, highly promising technique is ultrasound array–based real-time photoacoustic microscopy, which allows noninvasive high-speed 3-dimensional imaging of human pulsatile dynamics. In addition to arterial pulsatile motion and hemoglobin concentration dynamics, this photoacoustic imaging technique has been used to map sentinel lymph nodes with high accuracy and shows promise for imaging lymphatic dynamics in a highly qualitative and quantitative manner.

**Lymphangiogenic Therapy**

Studies of mouse models have greatly advanced our understanding of therapeutic lymphangiogenesis as a means to treat lymphedema. VEGF-C gene transfer via adenoviruses (Ad), adeno-associated viruses, or naked plasmids, as well as the use of recombinant VEGF-C protein, stimulated the formation of new lymphatic capillaries and reduced edema in several preclinical animal models of lymphedema. These results point to a promising means of restoring lymphatic vessels in lymphedema patients. However, these studies have been limited mainly to analysis of lymphatic capillaries, whereas a comprehensive molecular analysis of the collecting lymphatic vessels, which commonly are damaged in secondary lymphedema, has been lacking.
To study secondary lymphedema in a clinically relevant setting, we have established a mouse model involving the dissection of all axillary lymph nodes and associated collecting vessels, which frequently are damaged in humans after breast cancer treatment (Figure 5). The lymph node removal was followed by administration of adenoviral vectors into the surrounding tissues. Mice dissected of the axillary lymph nodes and treated with control adenoviruses developed lymphedema of the paws and demonstrated impaired return of lymph from the paw to the bloodstream. In contrast, mice treated with AdVEGF-C or AdVEGF-D displayed decreased edema and restoration of lymphatic drainage, which continued to improve over time (Figure 5). Histological analysis of axillae treated with AdVEGF-C or AdVEGF-D demonstrated robust growth of the lymphatic capillaries, which gradually underwent an intrinsic remodeling, differentiation, and maturation program into functional collecting lymphatic vessels. These vessels acquired all hallmarks of collecting vessels, including formation of continuous, zipper-like EC-cell junctions, intraluminal valves, and SMC coverage, although the vessels remained smaller in diameter than in normal nonoperated axillae even at 6 months after surgery.

The process of lymphatic vessel maturation is likely to be analogous to arteriogenesis, which can be induced by prolonged stimulation with VEGF or placenta growth factor and by shear stress caused by increased flow in the vessels. Interestingly, VEGF-C is a chemoattractant for monocytes/macrophages, which are important for arteriogenesis; these cells may also play a role in lymphatic vessel maturation. Prolonged VEGF-C stimulation may also directly promote LEC differentiation. In fact, the lymphatic vessels formed in response to VEGF-C stimulation resemble the lymphatic vascular plexus that forms early during development, and it is likely that the intrinsic developmental mechanisms governing lymphatic vessel maturation are reactivated in these vessels. The molecular players regulating this process are likely to involve at least the transcription factors Foxc2 and NFATc1 because they regulate the remodeling and maturation of collecting vessels during development, including the formation of lymphatic valves. Moreover, it is possible that flow of the lymph in the nascent vessels contributes to the remodeling.

In an attempt to restore the anatomy of the axilla after surgery in our secondary lymphedema mouse model, we combined AdVEGF-C therapy with lymph node transplantation. Such an approach has previously been undertaken without growth factor therapy, but in these experiments, the autologously transplanted lymph nodes incorporated into existing lymphatic vasculature at a low frequency. The lymph nodes transduced with AdVEGF-C survived, formed both afferent and efferent connections with the preexisting lymphatic vessel network, and could even trap metastatic tumor cells, whereas the majority of control-treated nodes regressed, suggesting that VEGF-C therapy improves the success rate of lymph node transplantation. These findings demonstrate for the first time that growth factor therapy can be used to generate functional and mature collecting lymphatic vessels. VEGF-C combined with lymph node transplantation allows complete restoration of the lymphatic system in damaged tissues and provides a model for future treatment of lymphedema in patients.

Our findings, however, are based on a mouse model, which has several limitations when considering direct extrapolation to the human patient setting. First, the hydrostatic conditions are dramatically different in mice because humans are considerably larger. This also means that the absolute area damaged by axillary lymph node dissection in humans is larger and the regenerating lymphatic vessels must span a longer distance to form anastomoses with both the distal and proximal ends of the lymphatic vascular tree. However, this gap could be bridged by the transplantation of chains of lymph nodes from another location in the patient, whereas VEGF-C could be used to form the microvascular anastomoses. This is supported by recent findings showing that, at
least in embryos, myeloid cells expressing large amounts of VEGF-C and VEGF-D are capable of inducing lymphatic hyperplasia and ultimately blood-lymphatic shunts. On the other hand, maturation of the lymphatic vessel plexus induced by VEGF-C therapy could be more complete in humans compared with mice because of a longer lifespan. Initial experiments in large animal models have demonstrated that these therapeutic approaches could be feasible in humans. It is important to note, however, that VEGF-C has been shown to promote lymphatic metastasis of tumor cells, to increase blood vascular permeability (although not as strongly as VEGF), and to stimulate extravasation of lymph from the lymphatic vessels during the initial phase of growth factor–assisted lymphatic repair. In light of these findings, patient safety is an important issue that must be considered when identifying patients for future clinical trials.

**Tumor Lymphangiogenesis and Antilymphangiogenic Therapy**

Lymphatic vessels act as a conduit not only for immune cells but also for tumor cells. Tumor metastasis to regional lymph nodes is a major step in cancer progression and fatality and is in most cancers an important prognostic indicator of the disease. Many types of tumors express the lymphangiogenic growth factors VEGF-C and VEGF-D, and several studies have shown that expression of these growth factors actively induces tumor-associated lymphangiogenesis, leading to lymphatic invasion, lymph node and distant metastasis, and subsequently poor patient survival.

To block tumor lymphangiogenesis and metastasis, antilymphangiogenic strategies using neutralizing antibodies against VEGFR-3, VEGF-C, and VEGF-D, as well as soluble VEGFR-3 fusion proteins (VEGF-C/D trap) and VEGF-C siRNA molecules, have been used. The efficacy of neutralizing antibodies against VEGFR-3 was shown to be increased through the use of a combination of 2 distinct classes of antibodies directed toward functionally different regions of the receptor that are involved in ligand binding and receptor dimerization. Some inhibition of tumor-induced lymphangiogenesis is also provided by antibodies against the neuropilin-2 coreceptor, which, however, is also expressed in a variety of neuronal tissues. Moreover, an interesting recent report shows that an alternatively spliced soluble form of VEGFR-2 may provide an additional inhibitor of lymphangiogenesis. Interestingly, even blood EC–associated VEGFR-2 may prevent local lymphangiogenesis by clearing VEGF-C from the extracellular milieu. However, blocking the VEGF-C/VEGFR pathway does not completely prevent tumor lymphangiogenesis or metastasis. This indicates that additional pathways should be targeted for more efficient tumor-associated antilymphangiogenic therapies. Because LECs themselves produce tumor cell–attracting chemokines such as CCL21 or CXCL12 (SDF-1), which will further facilitate the dissemination of CCR7- or CXCR4-expressing cancer cells, blocking these pathway may represent an additional treatment strategy.

Because lymphangiogenesis normally does not occur in adults, it has been expected that antilymphangiogenic strategies do not interfere with normal lymphatic vessel function. Indeed, inhibition of the VEGFR-3 pathway in adult mice does not affect normal lymphatic vessels and hence suggests that antilymphangiogenic therapy can be safely applied in adults.

**Kaposi Sarcoma**

Kaposi sarcoma (KS) is an angiogenic tumor consisting of proliferating KS-associated herpesvirus–infected cells that form irregular microvascular channels. KS-associated herpesvirus infection is endemic in sub-Saharan Africa, where KS is currently the most common childhood malignancy resulting from widespread infection with KS-associated herpesvirus and human immunodeficiency virus. Tumor cells in KS lesions are spindle cells in the latent phase of the viral life cycle, when only a subset of viral gene products are expressed. Kaposi sarcoma-associated herpesvirus has been demonstrated to induce cellular reprogramming of blood ECs to a more LEC-specific phenotype, suggesting that the virus is capable of modulating EC differentiation. Moreover, KS-associated herpesvirus can activate VEGFR-3 and induce endothelial proliferation and migration. Thus, inhibition of VEGFR-3 may represent a promising treatment option in KS.

**Lymphangioleiomyomatosis**

LAM is a rare cystic pulmonary disease that affects primarily women of child-bearing age (for reviews on LAM, see elsewhere). LAM is characterized by the infiltration of abnormal smooth muscle–like cells (LAM cells) through the pulmonary interstitium, perivascular spaces, and the lymphatics, leading to obstruction of small airways and subsequent respiratory failure or to disruption of the lymphatics, resulting in chyous pleural effusion. LAM is also characterized by the presence of pulmonary cysts and angiomylipomas, tumors made up of LAM cells, adipose tissue, and underdeveloped blood vessels. LAM occurs in about one third of women suffering from tuberous sclerosis complex, which is caused by mutations in the tumor suppressors tuberous sclerosis complex-1 or -2 involved in mammalian target of rapamycin signaling or is associated with renal angiomylipomas. Although a fraction of LAM patients are responsive to the mammalian target of rapamycin inhibitor rapamycin, patients with progressive disease may require pulmonary transplantation. LAM cells are often closely associated with the lymphatic endothelium, may be present in lymph nodes, and express abundant VEGF-D, readily detectable in patient serum. Thus, the progression of LAM may be dependent on the ability of LAM cells to invade the lymphatic system, which suggests that inhibitors of lymphangiogenesis should be evaluated in treatment of LAM.

**Obesity and Lipedema**

The lymphatic vessels carry out a crucial function in the absorption of dietary lipids. Interestingly, the lymphatic vessels may have a similar role in tissues such as the skin and mesentery. Mice born with hypoplastic lymphatic vessels survive but accumulate subcutaneous and peritoneal fat over time. The Chy mice, a mouse model for Milroy disease that has a heterozygous VEGFR-3–inactivating mutation resulting in hypoplastic cutaneous lymphatic vessels, similarly show...
accumulation of subcutaneous adipose tissue. Interestingly, although mice heterozygous for Prox1 deletion have normal-looking lymphatic vessels, they develop adult-onset obesity. Similarly, patients suffering from hereditary or acquired lymphatic insufficiency gradually accumulate subcutaneous fat. These findings suggest that lymphatic vessels are required for trafficking of lipids in peripheral tissues, which may have implications in the treatment of obesity.

Lipedema is an inherited disorder of adipose tissue, occurring almost exclusively in women. The underlying cause, however, is still unknown. Lipedema occurs as a bilateral, gradual accumulation of fatty deposition in the lower extremities and is often mistaken for lymphedema. In later stages, lipedema can, however, cause secondary lymphedema as a result of increased pressure of the expanding adipose tissue on the lymphatic vasculature. Lipedemic fat cannot be lost by diet or exercise, and treatment is designed primarily to treat the accompanying secondary lymphedema.

Conclusions

Considerable progress has been made in understanding the mechanisms of lymphatic vascular regulation, but this progress is still lagging behind our knowledge of the blood vascular system. Additional studies are needed to solve important questions concerning both basic mechanisms and, more important, therapeutic aspects of lymphatic regulation. One remaining question is whether the pathological changes observed in chronic lymphedema such as late-stage fibrosis, fat accumulation, and chronic inflammation can be reversed by prolymphangiogenic therapy. This question is also intimately associated with the optimal duration of therapy. On one hand, VEGF-C therapy will initially promote lymphatic vessel dysfunction, but the growth of a sufficiently dense network of lymphatic capillaries requires several days at least. Thus, VEGF-C expression needs to be shut off at the right time to minimize edema and to allow vessel remodeling. This small window of time may favor the application of VEGF-C as a recombinant protein rather than via viral gene transfer vectors, because this type of application would allow more careful monitoring of dose and treatment duration. Future therapies will be subject to scrutiny in terms of safety. It is clear that patients with a high risk of dormant cancer should be excluded from therapy, but application of specific prolymphangiogenic factors should otherwise be well tolerated. Monitoring of treatment efficacy would ideally involve the use of the newly described imaging techniques such as near-infrared, optical frequency-domain imaging, or ultrasound array–based real-time photoacoustic microscopy (see above), which allow quantitative and qualitative dynamic imaging of the lymphatic vasculature. Another interesting approach would be to use prolymphangiogenic therapy in conjunction with proangiogenic therapy, because the latter is frequently accompanied by vascular leakage and subsequent edema that could be cleared by the generation of new lymphatic channels.

In conclusion, important questions remain to be answered, especially in terms of treatment efficacy and patient safety. Nonetheless, recent findings have given us important new insights, which in time will likely allow us to manage aspects of lymphangiogenesis for the therapeutic benefit of patients.

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In the article by Norrmén et al, “Biological Basis of Therapeutic Lymphangiogenesis,” which published in the March 29, 2011 issue of the journal (Circulation. 2011;123:1335–1351), the authors included an outdated view on fluid balance in the microcirculation. In the second paragraph of their article, the sentence:

“Most of the extravasated interstitial fluid is absorbed back by colloid osmotic pressure into the blood capillaries on the venous side of the capillary bed, while the remaining fluid and macromolecules are taken up by the lymphatic vessels (Figure 1).”

Has been revised to state the following:

“Most of the extravasated interstitial fluid and macromolecules are absorbed back by the lymphatic vessels, whereas some reabsorption may also occur in the venules, depending on the tissue (Figure 1).”
Figure 1 has also been replaced to reflect this correction. The correct figure appears as follows:

**Figure 1.** Contributions of the blood and lymphatic vascular systems to tissue fluid homeostasis. *A* through *D*, Mechanisms leading to tissue edema. Normal fluid homeostasis in tissues is schematically illustrated in *A*: Colloid proteins and associated water are constantly filtrated from the arterial side of the capillary bed into the interstitial space (red arrows). The majority of the filtrate is collected by the lymphatic capillaries (green arrows); some of the fluid may be reabsorbed into the capillaries on the venous side of the capillary bed (blue arrows). *B*, Under the conditions of increased blood vascular permeability, such as in inflammation, the amount of filtrate is dramatically increased. Although the lymphatic vessels have a remarkable capacity to increase their drainage, sometimes the system is overwhelmed and net edema remains. *C*, Obstruction of the veins, for example, due to venous thrombosis or venous insufficiency will impair reabsorption (Reabs) and increase blood pressure within the capillary bed, leading to increased filtration (Filtr). Again, the lymphatic vessels are capable of increasing drainage, yet net edema is generated. *D*, Inherited or acquired damage to the lymphatic vessels, such as surgery or radiation therapy, blocks lymphatic drainage. This will lead to gradual accumulation of edematous fluid in tissues. The units in the bar graphs are arbitrary. Note that only the underlying reasons for edema formation are given in each figure, and secondary effects due to, for example, increased interstitial fluid pressure in edematous conditions are not accounted for.
Reference 1, which is cited in the Figure legend, has also been updated. It now refers to:


These changes have been made to the current online version of the manuscript. The authors regret the errors.

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