HHS Public Access

Author manuscript

Clin Sci (Lond). Author manuscript; available in PMC 2024 April 18.

Published in final edited form as:

Clin Sci (Lond). 2023 September 27; 137(18): 1451–1476. doi:10.1042/CS20220314.

Edema and Lymphatic Clearance: Molecular Mechanisms and Ongoing Challenges

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Abstract

Resolution of edema remains a significant clinical challenge. Conditions such as traumatic shock, sepsis, or diabetes often involve microvascular hyperpermeability, which leads to tissue and organ dysfunction. Lymphatic insufficiency due to genetic causes, surgical removal of lymph nodes, or infections, leads to varying degrees of tissue swelling that impair mobility and immune defenses. Treatment options are limited to management of edema as there are no specific therapeutics that have demonstrated significant success for ameliorating microvascular leakage or impaired lymphatic function. This review examines current knowledge about the physiological, cellular, and molecular mechanisms that control microvascular permeability and lymphatic clearance, the respective processes for interstitial fluid formation and removal. Clinical conditions featuring edema, along with potential future directions are discussed.

Keywords

Microvascular permeability; endothelial permeability; microvascular leak; lymph formation; lymphedema

Introduction

Edema, or swelling of tissues, can be a serious clinical problem because the excess interstitial fluid present in the tissue increases the distance for oxygen to diffuse from capillaries to cells and increases the potential for formation of oxygen radicals that can cause tissue damage [1,2]. Edema occurs when there is an imbalance in the generation of new interstitial fluid by microvascular filtration and the removal of excess interstitial fluid by lymphatic clearance. Edema can arise resulting from a variety of clinical conditions that affect circulation or lymphatic vessels, including injuries, poor nutrition, pregnancy, drug side effects, and diseases. Depending upon the clinical condition, severity can range from

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Author Contribution

J.W.B. conceived and drafted the original manuscript.

Competing Interests

The author declares that there are no competing interests associated with the manuscript.

a small and localized, such as the modest cutaneous swelling following a mosquito bite, to systemic and life threatening as in the case with severe burn injury [3].

Interstitial fluid has the important role of serving as a water and nutrient source for cells. Optimal interstitial fluid volume in the tissues ensures efficient delivery of O_2 removal of CO_2 to/from cells. The flow of interstitial fluid through tissues is important for removal of any metabolic waste products that do not easily diffuse into the blood, plus for delivery of potential antigens to the lymphatic system for immune surveillance. Understanding of the basic mechanisms that control interstitial fluid formation and removal is key for accurate diagnosis and proper treatment to resolve edema. In this review, these mechanisms will be discussed, with attention given to emerging developments in understanding of the cellular and molecular mechanisms that regulate microvascular permeability and lymph formation and flow. In addition, current challenges and future research questions will be identified and discussed.

Control of Interstitial Fluid Volume and Flow

Under physiological conditions, interstitial fluid flows through tissues from the capillaries and postcapillary venules toward draining lymphatic vessels (Fig. 1), maintaining a volume that is relatively small for optimal O_2 delivery, and a fluid pressure that is negative to prevent tissue expansion [3,4]. Interstitial fluid is formed by continuous filtration of blood plasma by capillaries and postcapillary venules [5]. Removal of interstitial fluid occurs when it is absorbed into initial lymphatic vessels, often commonly called lymphatic capillaries, and formed into lymph. This process provides a continuous bathing of cells in tissues with nutrients and allows for removal of waste products that do not easily diffuse back to the blood, plus recycling of plasma proteins and chylomicrons [6,7]. The rate of change of the interstitial volume (dV_i/dt) in a tissue at a given moment can be described by a simple relative rate equation:

$$dV_i/dt = dV_{PF}/dt - dV_{LF}/dt$$

where dV_{PF}/dt is the rate of plasma filtration and dV_{LF}/dt is the rate of lymph formation. Under normal conditions, there is an estimated margin of safety such that the capacity for lymph formation is estimated to be up to ten times higher than plasma filtration [8]. Edema forms when plasma filtration exceeds the rate of lymph formation. This can happen either when the filtration of plasma through capillaries and postcapillary venules becomes excessively high, when the ability of lymphatics to remove excessive interstitial fluid is impaired, or a combination of both.

Filtration across the capillary and postcapillary venular endothelium involves a combination of diffusive and convective forces. Simple diffusion of a solute (J_S; solute flux) is often described by Fick's First Law of Diffusion:

$$J_S = P_S A(C_{PL} - C_T)$$

Where P_S is the permeability coefficient for the solute, A is the surface area available for diffusion, C_{PL} is the concentration of the solute in the blood plasma, and C_T is the concentration of the solute in the tissue interstitial fluid. The P_S for albumin and other plasma proteins can be affected by a variety of inflammatory mediators, vascular endothelial growth factor (VEGF), metabolic byproducts, and drugs [3,9].

Convective transport of a volume of fluid (J_V) per unit of surface area (A) is typically described by the Starling Equation:

$$J_V/A = L_P[(P_C - P_I) - \sigma(\Pi_P - \Pi_I)]$$

Where L_P is the hydraulic conductivity of water (essentially "permeability" of the endothelium to water), P_C is the hydrostatic pressure inside the capillary, P_I is the hydrostatic pressure outside the capillary in the interstitial fluid, σ is the reflection coefficient for plasma proteins ranging from 0 (free movement across the endothelium) to 1 (impermeable), Π_P is the osmotic (or oncotic) pressure of plasma, Π_I and is the osmotic of interstitial fluid (Fig. 2). Considering this equation, J_V/A can become elevated by an increase in L_P, which can occur in response to inflammatory mediators, VEGF, ATP, and drugs [3,9]. Also, an elevation in P_C with all other variables remaining the same increases J_V/A. This scenario can occur when there is elevated blood flow to a capillary bed or when venous hydrostatic pressure is elevated, as $P_C = P_A - P_V$, where P_A is the arteriolar hydrostatic pressure and P_V is the venous hydrostatic pressure. The reflection coefficient σ is related to the diffusive permeability of plasma proteins across the endothelium. Stimuli that increase plasma protein permeability also reduce σ, which with all other variables unchanged increases J_V/A. A decrease in Π_P with all other variables remaining the same also facilitates an increase in J_V/A. This scenario occurs when there is a decrease in plasma protein concentration. Lastly, an increase in Π_I would also facilitate an increase in J_V/A. An increase in Π_I occurs when there is insufficient lymphatic clearance of protein from interstitial fluids. In theory, a decrease in P_I could also increase J_V/A, however the most likely source for a decrease in P_I would be elevated suction pressure generated by lymphatic vessels [10–12]. Based upon the relative rate equation above for dV_i/dt, an elevation in lymph formation caused by such a suction pressure would prevent rather than lead to edema. The dynamics of the system are also worth noting. If capillary J_V/A is elevated (regardless of cause) for an extended time, the increased extravasation of plasma over time will lead to increases in P_I and Π_I. In the absence of changes in other variables, an elevation in P_I, Π_I, or their combination would reduce J_V/A. Thus, elevated P_I and Π_I provide a certain margin of safety against additional edema formation [13,14].

Lymphatic clearance of interstitial fluid, synonymous with the term lymph formation, is an additional factor that ensures a margin of safety against formation of edema [8,15]. Lymphatic clearance also removes extravasated plasma proteins that generate osmotic force to pull fluid from capillaries into the interstitial space. Lymph formation occurs at the lymphatic capillaries, which consist of endothelial cells and do not have supporting mural cells or a basement membrane, making them very permeable to proteins and chylomicrons [16–18]. The mechanism of lymph formation is thought to involve transient pressure

gradients across the lymphatic wall that favor entry of interstitial fluid into the lymphatic capillaries, and the prevention of the newly formed lymph to efflux back to the interstitial space by microscopic one-way valves formed by the endothelial cells of lymphatic capillaries. These one-way valves are formed between discontinuous “button” junctions which differ from the continuous “zipper” junctions found in downstream lymphatic vessels or in blood vessels [19,20] (Fig. 3). Interstitial fluid pressure is lower than the average pressures measured within lymphatic vessels [21–24]. The lymphatic capillary networks coalesce into larger collecting lymphatic vessels that exhibit phasic contractions that: 1) generate transient suction pressures that travel through the lymphatic capillary networks that pull interstitial fluid into lymphatic capillaries [10,12], and 2) pump lymph toward the lymph nodes and eventually return lymph to the circulation [25,26]. Pumping force is generated by phasic contractions of a lymphatic muscle layer. In addition, periodic bicuspid valves (similar to venous valves) enable efficient pumping against gravity in a standing human [7]. Failure of either the pumping mechanisms or normal valve function impairs the transport of lymph through lymphatic networks, and in turn decreases the rate of lymph formation. When the rate of lymph formation becomes lower than the rate of plasma filtration, then interstitial volume increases and edema forms.

Clinical Conditions Featuring Edema

Conditions with elevated capillary hydrostatic pressure (P_C): Several clinical conditions feature edema caused by elevated P_C secondary to elevated venous hydrostatic pressure (P_V). Deep vein thrombosis (DVT) is a condition in which a venous blood clot forms in leg muscle. Patients who have hypercoagulability or venous stasis, such as those who have undergone recent surgery and are on bed rest are at particular risk. The clotting obstructs venous blood flow, elevating P_V, which in turn increases P_C. The result is elevated transcapillary filtration manifesting in edema with decreased Π_I [27,28].

Chronic venous insufficiency is a disease characterized by weakened venous valves, often initiated by DVT. Venous valves normally serve as gatekeepers to prevent retrograde venous blood flow against gravity. Venous valve dysfunction results in elevated P_V, which in turn increases P_C, resulting in elevated plasma filtration and edema [29].

Cardiogenic pulmonary edema arises from congenital heart disease, heart failure, or a mitral valve defect causing blood regurgitation. All these conditions elevate P_V in the pulmonary veins, leading to pulmonary edema [30,31]. The result is difficulty breathing and poor oxygenation of the blood, which can be life-threatening.

Lower limb edema is also associated with pregnancy. Expansion of the uterus can compress the veins to the lower body, elevating P_V. In turn, the increase in P_C results in edema in the legs. Interventions to reduce pregnancy-related lower limb edema include elevating the feet or using strategies to apply compression to the lower limbs, such as compression stockings or pneumatic intermittent compression [32,33].

Conditions with low capillary oncotic pressure (Π_P): Low Π_P can result from either from decreased production or accelerated loss of plasma proteins. Severe protein malnutrition impairs plasma protein production by the liver, causing low Π_P and ascites –

fluid accumulation in the abdominal cavity [34]. Kidney diseases such as renal failure or nephritis feature proteinuria due to escape of plasma albumin across the glomerular barrier. The loss of albumin leads to low Π_P which cases pitting edema (indentation of affected areas when pressed for a few seconds) in the lower extremities and puffy edema (swelling right under the skin) around the eyes [35,36].

Combination of low Π_P and high P_C: Liver diseases such as cirrhosis, hepatitis, or liver cancer can cause a combination of low Π_P and high P_C. The impaired function of hepatocytes to synthesize and secrete albumin lowers Π_P, while damage to the liver caused by these diseases causes portal hypertension, which increases splanchnic P_C. The combined low Π_P and high P_C cause ascites.

Another condition that can lead to low Π_P and high P_C is hemodilution due to intravenous infusion of crystalloid fluids, such as normal saline or lactated Ringers solution, particularly fast infusions [37–39]. Such infusions have been performed in trauma patients with severe hypotension to raise blood pressure to a sufficient level to maintain cardiac output. However, rapid crystalloid infusions can cause fluid overload can lead to edema systemically, including pulmonary edema that can be life threatening. Recent clinical trials point to better survival in hemorrhagic shock patients administered prehospital plasma rather than standard-of-care crystalloid fluids [40,41]. Whole blood may also provide additional benefit in the prehospital setting, yet requires additional study [42].

Increases in L_P or enhanced endothelial permeability: Several conditions impact the barrier function of the capillary and postcapillary venular endothelium, increasing diffusive permeability properties so that water and/or solutes may cross the semipermeable wall more easily. Inflammatory mediators and activated leukocytes are typical signals that elevate microvascular permeability. Depending upon the condition, a gradual rise in permeability may occur accompanying the development of chronic illnesses like diabetes mellitus, or the onset of microvascular hyperpermeability may be rapid as in the case with a cytokine storm that accompanies traumatic injuries. Also, in many illnesses or injuries the increase in microvascular permeability may be associated with additional circulatory problems that compound the problem [3,43,44].

Trauma caused by injuries or major surgery elicit an inflammatory response that disrupts the integrity of the capillary and postcapillary venular wall at sites remote from the location of injury. Likewise, sepsis – an extreme, systemic inflammatory response to infection that also features coagulopathy, vasodilation, and hemorrhage – can also elicit widespread microvascular hyperpermeability. Various mediators are released in systemic inflammation, such as bradykinin, histamine, cytokines and chemokines, and factors released from leukocytes including leukotrienes, lysosomal components, and oxygen free radicals, which all serve as signals to increase endothelial permeability. Major injuries caused by burns, blunt force trauma, penetration wounds, crush wounds, or hemorrhagic shock caused by excessive bleeding can all lead to a systemic inflammatory response syndrome (SIRS) in which inflammatory mediators activate leukocytes and increase microvascular permeability, causing malperfusion, hypoxia, and tissue dysfunction [3,44]. These conditions can lead to life-threatening organ dysfunction. For example, uncontrolled inflammation and edema

in the lungs causes poor ventilation due to the combination of excess fluid secretion into airways and increased diffusion distances for oxygen between the alveolar wall and capillaries or even alveolar flooding, leading to acute respiratory distress syndrome [45,46]. Another example is the ischemia that occurs in the splanchnic organs following severe blood loss and the activation of the baroreceptor response, which significantly reduces blood flow to the gut organs. Ischemia-reperfusion injury can occur when splanchnic blood flow is restored and is characterized as a microcirculatory disorder featuring no-reflow due to the combination of impaired vasoreactivity, microvascular hyperpermeability, and leukocyte plugging in capillaries and venules. Reactive oxygen species-derived oxidative stress that builds up during the ischemic period is considered a major player. Moreover, ischemia of the splanchnic circulation impairs reconstitution of the gut wall epithelium, impairing gut wall integrity and facilitating bacterial translocation and intestinal inflammation. The accompanying microvascular leakage into the abdominal cavity can raise peritoneal fluid pressure well above normal, leading to abdominal compartment syndrome (ACS). These scenarios cause tissue dysfunction that can elevated to the level of organ failure, and when the resulting imbalances stress other organs, ultimately multiple organ failure (MOF) occurs [44,47].

Microvascular hyperpermeability can also develop gradually with the pathogenesis and progression of diseases such as diabetes mellitus. Increased polyol pathway flux, oxidative stress, formation of advanced glycation end products (AGEs), and activation of multiple protein kinase C (PKC) family members contribute to diabetic microvascular hyperpermeability [48]. As diabetes becomes more advanced, the microvascular hyperpermeability is accompanied by impaired arteriolar reactivity, leukocyte activation, and pathological angiogenesis. Notably, vascular endothelial growth factor (VEGF), typically present during active angiogenesis, is also a very potent permeability factor [49]. The microvascular hyperpermeability caused by diabetes contributes local tissue and nerve damage systemically, with notable retinopathy, cardiomyopathy, peripheral neuropathy, and swelling of the lower limbs with poor wound healing and ulceration that often leads to the need for amputation [48].

Lymphedema: Lymphedema is caused by a failure of the lymphatic vessels to clear excess interstitial fluid. Lymphedema is broadly classified into primary and secondary lymphedema. Primary lymphedema is caused by inherited genetic mutations. Secondary lymphedema occurs following specific infections or injury – the most common injury being surgical excision of malignant lymph nodes [50]. Such injuries sever and block the pathway for normal lymph flow. After initiation of secondary lymphedema, pathologic wall thickening of collecting lymphatic vessels may contribute to additional obstruction of lymph outflow pathways [51].

To date, all genetic mutations associated with primary lymphedema appear to alter lymphatic endothelial cell biology. The most common primary lymphedema known, Milroy's disease, is caused by different mutations in the *FLT4* gene that encodes vascular endothelial growth factor receptor-3 (VEGFR3), leading to functional impairment of the lymphatic capillary network to convert excess interstitial fluid into lymph [52–56]. An apparent weak point in the lymphatic vessels is the intraluminal valves, as several

mutations that affect valve structure cause lymphedema. For example, inheritance of a single allele point mutation in the *FOXC2* gene causes lymphedema distichiasis, characterized by defective lymphatic valves [57,58]. Another hereditary lymphedema, Meige disease, is caused by mutations of *GJC2* (Connexin-47), which is highly expressed in valves [59,60]. Mutation of *GJA1* (Connexin-43) is connected to lymphedema associated with oculodentodigital dysplasia, affecting the eyes, face, teeth, and digits [61]. Notably, deficiencies of connexin-43 or -47 in mice disrupts lymphatic valve development [62]. The transcription factor *GATA2*, which is highly expressed in valves and important for their regulation [63] is connected to lymphedema associated with Emberger syndrome, myelodysplastic syndrome/acute myeloid leukemia and MonoMAC syndrome [63,64]. Mutation of *AKT1* in proteus syndrome, plus hyperphosphorylated Akt have both been linked to lymphatic malformations [65,66]. Interestingly, Akt also appears to be important for lymphatic valve formation and maintenance [67]. Thus, the intraluminal lymphatic valves are a key structure necessary for prevention of lymphedema. A variety of other gene mutations and associated syndromes also feature lymphedema and are reviewed in detail elsewhere [68,69].

While lymphedema is caused by lymphatic insufficiency, it is important to note that milder lymphatic dysfunction caused by a variety of different pathologies, in combination with elevated microvascular permeability may partially contribute to edema formation. Various inflammatory mediators and genetic mechanisms target both the microcirculation and lymphatic vessels, sometimes making causes of edema less clear [2,70]. A summary of all the conditions listed above leading to edema is provided in Fig. 4.

Mechanisms of Enhanced Microvascular Permeability

Endothelial cells of capillaries and postcapillary venules serve as the main gatekeepers for microvascular leakage, actively controlling the barrier function of these exchange microvessels. The control of microvascular permeability is determined by: 1) binding of ligands that activate their cognate receptors on endothelial cells; 2) second messengers and signaling pathways within the endothelial cells; and 3) structural effector molecules within the cells that ultimately change barrier integrity and active transport functions of the endothelium [3].

Ligands that stimulate increased microvascular permeability.—Several agonists released by cells in the blood or surrounding tissues act upon receptors on endothelial cells to modulate permeability of the microvascular wall. Inflammatory mediators such as histamine, bradykinin, platelet activating factor (PAF), tumor necrosis factor-α (TNFα), and interleukin-1β (IL-1β), or the growth factor vascular endothelial growth factor-A (VEGF-A) elicit increases in microvascular permeability through action on their receptors on endothelial cells [71–76]. Interactions with activated neutrophils and exposure to reactive oxygen species, extracellular histones, or AGEs also activate increased permeability of the endothelial wall [77–82]. These different stimuli act upon their cognate receptors, which include G-protein coupled receptors, receptor tyrosine kinases, or different types of cytokine receptors. Subsequently, second messengers and downstream signaling pathways are activated, that cause conformational changes in the cytoskeleton, focal adhesions, and

junctional adhesions between endothelial cells, eliciting alterations in barrier function of the endothelium [3].

Ligands that promote enhanced endothelial barrier function.—There are also a variety of agonists that act on specific endothelial receptors, leading to reduced permeability. Of these, the most well-studied is sphingosine-1-phosphate (S1P), which is thought to tighten the endothelial barrier primarily through action on S1P receptor-1 (S1PR1) [83–86]. Oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) also reduces microvascular permeability by binding to the receptor GRP78 and through transactivation of S1P receptor-1 to caveolin-enriched microdomains [87–89]. The prostaglandin receptor EP4 also has been reported to be involved in OxPAPC-mediated endothelial barrier enhancement [90]. It is worth noting that high concentrations of both S1P and OxPAPC cause endothelial barrier disruption, and this is attributed to activation of additional receptor subpopulations, notably other S1P receptors for S1P or VEGFR-2 for OxPAPC [86,91]. Recently, the sigma receptor-1 agonist PRE-084 was shown to reduce endothelial permeability *in vitro* [92]. *In vivo*, PRE-084 administration reduced brain microvascular hyperpermeability caused by amyloid-β injection [93].

Second messengers and downstream signals that control microvascular permeability.—Several of the agents listed above that increase microvascular permeability do so through activation of protein kinase C (PKC) isoforms. Initial experiments showed that pharmacological inhibitors of PKCs could attenuate increases in permeability *in vivo* caused by PAF, bradykinin, and VEGF [94–96]. These findings were supported by studies utilizing isolated, perfused coronary venules, showing PKC blockade inhibited VEGF-induced hyperpermeability to albumin [97] while phorbol esters, which activate PKCs, elicited increased permeability [98]. Similarly, in perfused rat lungs, PKC inhibitors attenuated neutrophil- or peroxide-induced extravasation [99,100]. Data from endothelial cell monolayer models showing that PKC blockade inhibited barrier dysfunction caused by application of hydrogen peroxide, VEGF, thrombin, TNFα, IL-1β, ischemia, or plasma from rats that underwent experimental burn injury also support a role of PKCs in stimulating increases in microvascular permeability [101–108]. Elevated vascular expression of the $PKC\beta_{II}$ isoform was reported in animal models of diabetes [71,109,110], while administration of PKC inhibitors decreased permeability of coronary venules from diabetic pigs [109] and attenuated microvascular complications such as diabetic neuropathy nephropathy, and retinopathy [111–113].

In contrast, cyclic AMP (cAMP) and its downstream effectors protein kinase A (PKA) and the exchange proteins directly activated by cAMP (EPAC) are generally endothelial barrier protective. Decreases in intracellular cAMP levels and PKA activity have been correlated with elevated microvascular permeability [114,115]. Inhibitors of PKA and EPAC increase endothelial permeability [116,117], while activation of PKA/EPAC leads to activation of the small GTPase Rap1 and termination of ischemia-induced microvascular hyperpermeability [118]. Administration of agents that increase cAMP levels decrease permeability and increase tight junction integrity between endothelial cells [119].

Activation of eNOS and eNOS-derived NO have also been shown to play an important role in both PAF and VEGF-induced microvascular hyperpermeability. While activation of eNOS leads to NO-dependent vasodilation of arterioles and increased blood flow and thus filtration, there are also specific mechanisms in the postcapillary venules that lead to increased permeability. Supporting this notion is evidence from isolated coronary venules, in which blockade of NO synthesis attenuates VEGF-, histamine-, or phorbol ester-induced hyperpermeability [98,120,121]. *In vivo*, application of PAF elicits increased endothelial NO production and microvascular leakage at postcapillary venules while also causing vasoconstriction of arterioles, yet pharmacological blockade of NO synthesis prevents the increased permeability [122–124]. Moreover, genetic knockout of eNOS attenuates PAF-induced microvascular hyperpermeability *in vivo* [125]. Location of eNOS within endothelial cells is thought to differentiate its impact on endothelial barrier function versus its vasodilatory function, as translocation of eNOS from the plasma membrane to the cytosolic fraction is needed for PAF-induced endothelial hyperpermeability [126–129]. S-nitrosation/nitrosylation of junctional proteins such as β-catenin and p120 catenin, plus regulators of the cytoskeleton such as vasodilator-stimulated phosphoprotein (VASP) appear to the downstream pathway for how NO increases endothelial permeability [130,131].

Multiple studies have shown mitochondrial wall disruption in postcapillary venules following hemorrhagic shock, causing release of mitochondrial cytochrome c into the cytoplasm in endothelial cells, activation of apoptotic signals, disruption of cell-cell junctions between endothelial cells, and reduced glycocalyx thickness [132–136]. In a similar fashion, TNF-α causes disruption of mitochondrial integrity and release of mitochondrial ROS in endothelial cells, leading to disruption of cell-cell junctions and increased permeability [137]. Prevention of mitochondrial wall disruption with the immunomodulator FK506 or administration of the antioxidant (-)-deprenyl attenuated the hemorrhagic shock-induced microvascular hyperpermeability [138,139].

The PI3K/Akt pathway has also been shown mediate VEGF- and TNF-α-induced increases in endothelial permeability. The regulatory subunit of PI3K associates with VEGF receptor-2 (VEGFR2) and PI3K activity increases when VEGF binds to VEGFR2 [140]. Pharmacologic inhibition of PI3K/Akt attenuates VEGF-induced increases in endothelial permeability both *in vivo* and in cultured endothelial monolayers [96,141]. Similarly, blockade of association of the p85 regulatory subunit of PI3K to the TNF-1 receptor with ropivacaine or lidocaine blocked TNF-α signaling and downstream endothelial barrier dysfunction [142]. PI3K/Akt signaling is thought to mediate increased permeability through activation of eNOS [96,141]

Activation of MAP kinases have been connected to increases in microvascular permeability. VEGF and the inflammatory disintegrin and metalloprotease ADAM15 Inhibition both elicit phosphorylation of ERK-1/2 on its activation site, while inhibition of ERK-1/2 inhibits increases in permeability [96,102,143]. Also, inhibition MEK-1/2, the upstream activator of ERK-1/2, attenuates histamine-induced increases in permeability of isolated porcine coronary venules [144]. The p38 MAP kinase has also been implicated in increases in endothelial permeability in response to histamine, VEGF, or PAF [76,141,145–147]. There

is evidence that p38 MAP kinase activation impairs the local lamellipodia at endothelial cell junctions that are thought to help maintain and repair cell-cell junctions [145].

The Src family of nonreceptor tyrosine kinases also has a key role in endothelial barrier function. Data from several studies suggest that Src mediates the increases in endothelial permeability caused by a variety of stimuli, including VEGF, C5a-activated neutrophils, TNF-α, AGEs, fibrinogen-γC terminal fragments, and high mobility group box-1 protein [148–153]. Src phosphorylates VE-cadherin on Y685 and β-catenin on Y654, leading to disruption of connections of the junctional cadherin complex with the actin cytoskeleton [151,154,155]. Recent evidence suggests that Src can be activated by eNOS-derived NO in the context of oxygen-induced retinopathy, leading to VE-cadherin phosphorylation, destabilization of endothelial junctions, and increased microvascular permeability [156]. There is also evidence that Src-mediated phosphorylation of moesin and focal adhesion kinase (FAK) are also involved in increased endothelial permeability [148].

Several lines of investigation have connected the Rho family GTPases to changes in microvascular permeability. Of these, activation of RhoA is generally linked to disruption of the endothelial barrier, while Rac1 has a protective role. Cdc42 has been studied to a lesser extent but may be involved in barrier restoration following hyperpermeability [157,158]. RhoA activates Rho-kinase (also called Rho-associated coiled-coil forming kinase or ROCK), which promotes actin polymerization, actin stress fiber formation, and activation of FAK [159–162]. Studies utilizing *Clostridium botulinum* exoenzyme C3 transferase to inhibit RhoA yielded enhanced barrier function of endothelial monolayers, and attenuated increases in permeability caused by histamine, thrombin, TNF-α, and TGF-β [157,163,164]. Inhibition of RhoA activity by expression of a dominant-negative RhoA mutant also reduces hyperpermeability of endothelial monolayers caused by histamine, thrombin, or AGEs [157,165]. Pharmacologic blockade of ROCK inhibits thrombin-induced increases in F-actin content and tyrosine phosphorylation of FAK along with hyperpermeability of endothelial monolayers, plus endothelial monolayer dysfunction caused by histamine, activated neutrophils or AGEs [76,165–169]. Likewise, pharmacologic inhibition of ROCK activity decreases baseline L_P in single-perfused rat mesenteric venules [170] and attenuates histamine-induced airway microvascular leakage *in vivo* [171], burn-induced mesenteric microvascular leakage *in vivo* [172,173], and hyperpermeability of isolated porcine coronary venules elicited by either activated neutrophils or VEGF [174,175]. Transfection of a constitutively active recombinant ROCK protein into endothelial monolayers or isolated coronary venules increases permeability [175]. There are other reports, however, in which blockade of RhoA/ROCK signaling failed to inhibit increases in permeability of endothelial monolayers caused by TNF-α or histamine or increases in microvessel L_P elicited by bradykinin or PAF [170,176,177]. In addition, there have been reports that RhoA/ROCK is also involved in sphingosine-1-phosphate (S1P)-induced endothelial barrier enhancement [178,179], suggesting that this pathway may serve a potential dual and complex role in endothelial barrier function.

Rac1 is generally thought to work in opposition to RhoA in endothelial cells and promotes enhanced endothelial barrier integrity. Rac1 produces a less dramatic actin polymerization than RhoA but also promotes formation of lamellipodia [180,181]. Rac1 activity decreases

concomitantly with thrombin-induced reductions in endothelial monolayer barrier function, and Rac1 activation occurs in conjunction with the rapid barrier enhancement elicited by S1P [181,182]. Alcohol also decreases levels of activated Rac1 in endothelial cell in conjunction with increased permeability [146] Toxin B, which inhibits Rac1, RhoA, and Cdc42, increases permeability of endothelial monolayers and L_P of single-perfused microvessels [170,183]. Inhibition of Rac1 was initially thought to be the reason for this outcome because blocking RhoA had been shown to tighten the endothelial barrier [184] while transfection of a dominant negative Cdc42 had no impact on endothelial monolayer baseline barrier function [157]. More direct inhibition of Rac1 with *clostridium sordellii* lethal toxin, which does not affect RhoA or Cdc42, also increased microvessel L_P, confirming this notion [185]. The pharmacologic inhibitor of Rac1, NSC23766, has also been shown to impair endothelial monolayer barrier function and increase permeability of isolated rat mesenteric venules [181]. In addition, overexpression of Rac1 in endothelial monolayers reduces permeability [181]. Interestingly, S1P also increases RhoA activity [181]. The S1P-induced RhoA activation occurs rapidly and at the cell periphery, and likely works in concert with Rac1 to promote formation of local lamellipodia at endothelial cell-cell junctions to help preserve the endothelial barrier [178].

It is also worth noting that termination of endothelial hyperpermeability appears to be built into responses to agonists such as PAF, VEGF, and thrombin, evidenced by 1) washout of the stimulus shortly after application still produces the same response as when it is left on endothelial monolayers, and 2) activation of terminating signals such as elevated cAMP and EPAC1 [186]. The activation of these barrier-enhancing signals is delayed compared to the initial signals that stimulate hyperpermeability [186]. These signals may be part of a pre-programmed response in which the endothelium reacts to opening of junctional clefts, in order to preserve vessel integrity.

Cellular structures that regulate microvascular permeability.—The signaling mechanisms mentioned above ultimately act on cellular structures including the glycocalyx, junctions, focal adhesions, and cytoskeleton to control the permeability of the endothelium (Fig. 5). The active control of cellular tension plus junctional and focal adhesion strength, in combination with the glycocalyx composition within the narrow paracellular clefts between endothelial cells, determine the permselectivity of the endothelium to solutes of different sizes and charges.

The endothelial glycocalyx is a fibrous surface layer that is carbohydrate-rich found on the apical surface and in wide regions of clefts between endothelial cells. The glycocalyx limits passage of plasma solutes in a charge and size-selective manner [187,188]. The layer itself also acts as a diffusive barrier for large solutes and limits adhesion of leukocytes and platelets to the endothelium [189,190]. Multiple studies have shown that endothelial glycocalyx degradation allows for increased leukocyte adhesion to the endothelium and elevated microvascular permeability [133,191–194]. Moreover, several injurious and inflammatory stimuli associated with increased microvascular leakage promote shedding of glycocalyx components, such as TNF-α-induced inflammation, hyperglycemia, ischemia-reperfusion, hemorrhagic shock/trauma, endotoxemia/sepsis [191,195–200]. On the other hand, S1P, which reduces microvascular permeability, stabilizes the endothelial glycocalyx

[133,201–203]. The regulation of the glycocalyx surface layer is likely due to a combination of synthetic pathways and degradation by enzymes such as heparinase, hyaluronidase, and matrix metalloproteinases [204,205]. In addition, changes in glycocalyx composition may occur in disease conditions such as sepsis, altering immune and inflammatory responses [206]. How changes in glycocalyx composition may affect permeability to plasma components remains to be determined.

Junctions between endothelial cells also have a prominent role in controlling microvascular permeability by regulating the sizes of clefts between adjacent cells. Key junctional protein complexes that restrict solute movement across the endothelium include members of the tight junction family and the adherens junctional complex. Tight junction proteins include occludins, claudins, and the zonula occludens (ZO) family members, while endothelial adherens junctions proteins include VE-cadherin, β-catenin, and p120 catenin. Most focus has been on VE-cadherin, which is visibly disrupted in microvessels displaying elevated permeability to plasma protein solutes [133,207]. In addition to potential stress placed on intercellular or junctional by contraction or retraction of endothelial cells, signals that affect the formation and maintenance of these sites of contact between endothelial cells can affect barrier integrity. For example, tyrosine phosphorylation of VE-cadherin at Tyr-658 and Tyr-731 impairs binding to other complex members β-catenin and p120-catenin [208]. A variety of studies provide evidence that disruption of the adherens junctional protein complex contributes to elevated microvascular permeability [148,209–211]. Binding of β-catenin to VE-cadherin links the adherens junction protein complex to the actin cytoskeleton and is required to establish strong, steady-state adhesions [212]. Binding of p120 prevents VE-cadherin degradation and promotes Rac1-mediated cell spreading [212–215]. Internalization of VE-cadherin has also been proposed to be an important regulatory mechanism that can affect junctional integrity [216]. Activation of moesin, a protein involved in linking the actin cytoskeleton to membrane proteins, has been implicated in VE-cadherin internalization [148]. More recently, moesin activation by its phosphorylation on Thr-558 has been implicated in suppressing VE-cadherin expression through a mechanism likely involving the transcription factor KLF4 [217].

Activation of focal adhesions, evidenced by elevated tyrosine phosphorylation of FAK and its substrate paxillin, was initially associated endothelial hyperpermeability [167,218]. Subsequent studies revealed that inhibition of FAK prevents hyperpermeability caused by activated neutrophils, VEGF, or fibrinogen-γC terminal fragments [152,219,220]. FAK can be phosphorylated by Src [221] and there is also evidence that of FAK activation downstream of the RhoA/ROCK pathway [167,222]. Paxillin has also been shown to be phosphorylated by the c-Abl tyrosine kinase in association with LPS-induced endothelial barrier dysfunction [223]. Interestingly, differential tyrosine phosphorylation of FAK, and distinct FAK localization patterns have been reported with thrombin-induced hyperpermeability and S1P-mediated endothelial barrier protection. Thrombin was reported to cause phosphorylation on Y397, Y576, and Y925, while S1P elicited phosphorylation on Y576, and this was attributed to differences in Src sensitivity [221]. The differential signaling leads to different outcomes for focal adhesions [224]. Thrombin promotes localization of FAK and paxillin on ends of stress fibers, while S1P promotes FAK and paxillin to be located near the cell periphery [221]. For S1P-mediated barrier enhancement,

the focal adhesion complexes have a key role in enabling lamellipodia-mediated closure of any openings in the clefts between endothelial cells. The small GTPase regulators GIT1 and GIT2 transiently relocate to peripheral focal adhesions in response to S1P [225]. FAK forms complexes with VE-cadherin and β-catenin, while paxillin forms complexes with α-catenin [226]. c-Abl-mediated phosphorylation of paxillin has also been implicated in S1P-receptor-1-mediated endothelial barrier enhancement [227].

The cytoskeleton is both a structural network and motor system for cell movement and determining cell shape. Of the three cytoskeletal components, the actin microfilaments, microtubules, and intermediate filaments, most work has centered on contractile bundles composed of actin and myosin, responsible for multiple types of cellular movements. In endothelial cells the thick bundles known as actin stress fibers have been proposed to produce tension that puts stress on intercellular junctions [175,228,229]. Many studies contain data suggesting that phosphorylation of the regulatory myosin light chains (MLC) on their activation site by MLC kinase (MLCK) causes formation of actin stress fibers, which produce contractile tension within endothelial cells that put stress on intercellular junctions and widening the junctional clefts, allowing more fluid and solutes to pass through [230–233]. Evidence for this viewpoint is supported by investigations in both cultured endothelial models and isolated venules, in which blockade of MLCK activity lowers permeability or attenuates increases in permeability caused by inflammatory stimuli [172,234]. Transference of active MLCK protein into endothelial cells or the walls of isolated venules also elevates permeability [230,235]. In vivo, knockout of the long form of MLCK in mice attenuates hyperpermeability caused by burn injury or combined lipopolysaccharide/ventilator-induced lung injury [236,237]. On the other hand, the MLC phosphatase (MLCP) helps preserve endothelial barrier function [238]. In addition, ROCK phosphorylates the MLC phosphatase and inactivates it, thus also facilitating actin stress fiber formation and formation of endothelial tension [175]. This being said, endothelial cells of postcapillary venules *in vivo* primarily have an actin ring near junctions with relatively few stress fibers [239], but there still seems to be involvement of a contractile mechanism considering the role of MLCK, evidenced by isolated venule studies [230,234]. Another more recent viewpoint is that intermittent, local lamellipodia located at junctions, under the control of the actin cytoskeleton, also control the structure of junctional clefts, and that disrupting the normal, intermittent local lamellipodia activity allows for opening of these clefts [145,181,240–243]. It is worth noting that after clefts open between endothelial cells, there appear to be targeted lamellipodia that close such openings [181], possibly as part of an endothelial-based programming to terminate hyperpermeability [186].

The proteins VASP and Ena-VASP-like (EVL) are members of Ena/VASP family of proteins that mediate dynamic actin rearrangements in lamellipodia, cell-cell junctions, and focal adhesions [244,245]. Global VASP knockout mice feature enhanced bradykinin- and LPS-induced microvascular hyperpermeability [246,247]. Overexpression of EVL causes formation of larger focal adhesions in response to S1P in association with an amplified endothelial barrier enhancement. However, with thrombin challenge, EVL overexpression also reduces the number of focal adhesions [224]. EVL appears to interact with actin, cortactin, and profilin-2 to modulate actin polymerization and lamellipodia dynamics [248].

Mechanisms of Lymphatic Clearance

Several mechanisms along the lymphatic network are responsible for ensuring optimal clearance of excess interstitial fluid. As such, there are multiple weak points in the system that can potentially contribute to lymphatic insufficiency (Fig. 6). These mechanisms are described below.

Lymph Formation: The blind-ended initial lymphatics, also known as lymphatic capillaries, serve as the site of lymph formation, i.e., the point of entry for fluids into the lymphatic system [4,7]. Immune cells also enter the lymphatic system via the lymphatic capillaries [249–251]. For lymph to form efficiently, fluid needs to cross from the interstitial space into the lymphatic capillary lumen without backflow. This is accomplished with a unique discontinuous junction structure between lymphatic capillary endothelial cells that form microscopic one-way valves known as “button” junctions [19,20]. The buttons are rich in VE-cadherin, claudin-5, occludin, ZO-1, and JAM-A labeling where the cleft between adjacent cells is relatively tight, while the gaps between buttons tend to have PECAM-1 and LYVE-1 labeling [19]. These gaps form microscopic cell-membrane leaflet structures. When P_I exceeds the hydrostatic pressure inside the lymphatic capillary lumen (P_L), the leaflets between buttons are thought to open and permit fluid entry into the vessel, and when $P_L > P_I$ they close, acting as “primary valves” that prevent fluid escape back to the interstitial space [252–254]. The changes in P_I that drive lymph formation are thought to be oscillatory or momentary, driven by microvascular filtration of plasma and various tissue/organ movements [2,4]. In addition, suction pressures generated by flow in downstream collecting lymphatics (discussed in a later section) are thought to generate moments where $P_I > P_L$ in the lymphatic capillaries [10,12].

There is also evidence that lymphatic endothelial cells actively participate in lymph formation. Basal-to-apical chylomicron transport was observed in a bioengineered intestinal villus lymphatic capillary (lymph lacteal) model [255]. Elevated aquaporin-2 expression in response to increased transmural flow across lymphatic endothelial monolayers has been demonstrated, which may affect cell volume and shape [256]. In addition, growth factors, inflammatory mediators and shear stress affect endothelial cell shape and barrier function [257–259]. Various signals can also alter the abundance of button junctions on lymphatic capillaries. Angiopoietin 2 (ANG2) is needed for normal button junction development. An ANG2-blocking antibody impairs embryonic lymphangiogenesis and the formation of button junctions, resulting in impaired lymph formation [260]. In the airways, experimental *M. pulmonis* infection was reported to cause transformation of buttons to zippers in mouse tracheal lymphatic capillaries, which could be reversed when using dexamethasone to reduce inflammation [261]. In the gut, depletion of microbiota with antibiotics reduces the proportion of button junctions present in lymph lacteals due to reduced MyD88-dependent VEGF-C secretion from macrophages upon microbe recognition [262]. Genetic inactivation of the Notch ligand delta-like 4 (DLL4) within lymphatic endothelial cells reduces the proportion of buttons in lymph lacteals in mice [263]. Likewise, genetic deletion of neuropilin-1 and VEGFR-1 was reported to convert lymph lacteal buttons to continuous “zipper” junctions and cause defects in chylomicron uptake [264]. Collectively, the evidence suggests inflammatory or other pathologic stimuli that impact lymphatic

capillary endothelial cells, particularly those that negatively affect button junctions, may potentially impair lymph formation and by extension promote edema accumulation.

Lymphatic Pump Function: Lymph flow into and through the larger lymphatic vessel network is mainly driven by the pumping of collecting lymphatic vessels. Suction forces generated by this pumping is thought to pull newly formed lymph from lymphatic capillaries into the network [10,12]. The muscle layer of collecting lymphatics generates the phasic contractions produce the pumping forces, and is sensitive to changes in transmural pressure, shear stress due to lymph flow, and a variety of chemical and inflammatory mediators. The intraluminal "secondary" valves prevent backflow. Each segment between two secondary valves is called a "lymphangion" and is capable of contracting and relaxing either as an individual unit or in a coordinated fashion with adjacent lymphangions. This configuration, with a chain of lymphangions separated by intraluminal valves, distributes large hydrostatic pressure gradients that can be generated in a standing human into small steps. As such, failure of the intraluminal valves can severely impair the ability to propel lymph forward [7].

Collecting lymphatic vessels can adjust pump activity when the transmural pressure (difference of luminal and extraluminal pressure) changes. When transmural pressure is elevated, the phasic contraction frequency (CF) and the tone between phasic contractions both increase [265,266]. Increased force of phasic contractions also accompanies the increased CF when the lymph pressure in the downstream lymphangion (afterload) increases in the absence of an increase in lymph pressure of the upstream lymphangion (preload) [267,268]. Lowering the transmural pressure results in decreased CF and tone. It is worth noting that vessels have an upper limit defined by their maximal contractile strength [269]. The electrophysiology mechanisms are reviewed elsewhere [270] and essentially consist of action potentials in lymphatic muscle cells that elicit transient increases in intracellular free calcium and activation of actin-myosin-mediated contraction [271–278]. Failure of the pumping mechanisms that could potentially lead to edema may be caused by inflammation associated with age, metabolic disease, and other pathologies [265,279–291].

Collecting lymphatic vessels also possess endothelial-dependent responsiveness to wall shear stress. Elevated shear stress activates production of nitric oxide (NO) by endothelial NO synthase (eNOS). NO in turn causes cyclic GMP-dependent relaxation of lymphatic muscle, decreasing contractile force and CF [279,292–299]. When lymph flow is high, the pumping mechanism could potentially increase resistance to flow, so this relaxation mechanism is thought to allow collecting lymphatic vessels to act more like conduits. Aging appears to compromise flow-dependent relaxation of collecting lymphatics [283,300].

The secondary valves found in collecting lymphatic vessels also have a profound role on the ability of these vessels to efficiently pump lymph. Gene mutations known to cause primary lymphedema, such as those in *FLT4*, *GJC2*, *FOXC2*, *GJA1*, and others all affect the normal development and function of secondary valves [56–62,301]. While such genetic causes do cause problems with valves [302], there is also evidence of valve dysfunction elicited indirectly by poor lymphatic muscle tone [303]. In either case, impaired lymphatic pumping due to valve dysfunction will contribute to reduced clearance of excess interstitial fluid, leading to edema.

Collecting Lymphatic Permeability: The collecting lymphatic wall has been shown to be permeable to macromolecules in a similar fashion as microvessels [304–306], with magnitudes of permeability coefficients generally having an inverse relationship to solute size [307,308]. Junctional proteins such as VE-cadherin are important for maintaining lymphatic wall integrity, however transcellular transport via vesicles have also been shown to control normal passage of albumin across the collecting lymphatic wall [308]. In cultured lymphatic endothelial cell monolayer models, bacterial endotoxins, inflammatory mediators such as histamine, IFN-γ, IL-6, IL-1β, TNF-α, thrombin, and the growth factor VEGF-C [257–259]. The concern with elevated collecting lymphatic permeability is that it could potentially undermine lymphatic clearance function and facilitate edema formation.

There is evidence that hypercholesterolemia elicits elevated lymphatic permeability. Mice deficient in *ApoE* that are placed on an atherogenic diet become hypercholesterolemic. When an opaque dye is injected into the ear, the lymphatic vessels rapidly absorb, then leak this dye into the surrounding parenchyma, indicating severe leakage [309]. This was confirmed with isolated, perfused collecting lymphatic vessels [310]. In a later study, the same group demonstrated that the lymphatic leakage could be rescued by normalizing the plasma cholesterol levels, demonstrating that *ApoE* was dispensable for normal lymphatic function [311].

There is also evidence supporting a connection between elevated lymphatic vessel permeability with obesity and metabolic disorders. The first observation supporting this connection was that mice with global, single allele deletion of *Prox1*, a key transcription factor for lymphatic endothelial cell identity, have underdevelopment of the mesenteric lymphatic vessel network and leakage of lymph combined with adult-onset obesity [312,313]. A different approach, with ablation of lymphatic networks in FLT4-Cre$^{+/-}$DTR$^{+/-}$ mice led to dyslipidemia and insulin resistance [314]. After deletion of CD36 was induced in lymphatic endothelial cells, Prox1-CreER^{T2}-tdTomatoCD36$^{-/-}$ mice developed leaky mesenteric lymphatic vessels and insulin resistance [315]. There is also evidence that high-fat diets may affect collecting lymphatic permeability. Male C57BL6 mice fed a 16-week high-fat diet had nitrosative stress in the surrounding mesentery, with dilated and leaky lymphatics [314]. Male mice fed a 15- or 32-week high-fat diet were reported to have leaky mesenteric lymphatics, suspected to underlie development of insulin resistance and obesity [316]. Apelin knockout mice fed a high-fat diet developed obesity and had enlarged and leaky lymphatic and blood vessels, while Apelin transgenic mice had improved integrity of the lymphatic and vascular walls and were resistant to high-fat diet-induced obesity [317]. These findings suggest a role for leaky mesenteric lymphatic vessels in adipose deposition and the development of metabolic disorders.

However, there are other mouse models with underdeveloped lymphatic networks (K14-VEGFR3-Ig, VEGF-C$^{+/-}$, and Chy mice) that do not develop obesity on high-fat diets [318]. Chy mice have inactivating mutations of the VEGFR-3 tyrosine kinase domain and have chylous ascites develops at birth that resolves, with ongoing swelling of limbs [55]. However, no obesity develops when Chy mice are fed a high fat diet [318], possibly because these mice also have impaired absorption of triglycerides [319]. There is also the possibility that the obesity in *Prox1*$^{+/-}$ mice or mice that utilize a *Prox1*-driven Cre-recombinase for

knockout might be due to non-lymphatic factors due to the expression of *Prox*1 other cell types, such as hepatocytes or striated muscle, which may have altered metabolism [320,321]. It is also worth noting that leptin-receptor deficient mice (db/db strain on a C57Bl/KsJ background) did not develop increased permeability of collecting lymphatics until 20-30 weeks of age, after they had become obese and developed insulin resistance [304], suggesting that lymph leakage is not necessarily an early step in the development of obesity. Still, lymphatic permeability appears to be involved in metabolic disorders.

Ongoing Challenges

Edema remains a significant clinical problem for which there are limited therapeutic strategies. Several mechanisms leading to edema involve inflammation. However, there are both good and bad aspects of inflammation to consider. Inflammation is part of the healing process; however, inflammation is present in many pathologies, and uncontrolled, excessive inflammation leads to tissue dysfunction and damage. Another factor to consider is the vicious cycle that can occur with long-term microvascular hyperpermeability and propagation of inflammation. A key question is whether in advanced pathologies involving microvascular leakage, is the hyperpermeability reversible?

Another challenge is finding therapeutics that can selectively reduce microvascular hyperpermeability. Barrier enhancing agents have potential promise but need additional development. In addition, there are so many different compounds that can elicit microvascular hyperpermeability, and the solution to reducing edema among different groups of patients may require a personalized medicine approach. Development of a panel of laboratory tests for compounds that cause increased microvascular permeability might be a future approach. Genomic testing for mutations that may impair lymphatic and venous valves could possibly someday become part of a useful, personalized approach. Although a large body of knowledge has accumulated pertaining to the causes of microvascular leakage and lymphatic insufficiency, the ongoing challenges warrant additional research directed at understanding the molecular mechanisms that could potentially become future therapeutic targets to ameliorate edema.

Funding

This work was supported by the National Heart, Lung, and Blood Institute and the National Institute of General Medical Sciences of the National Institutes of Health under award numbers R56HL13542 and R35GM145379. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

Data Availability

Data sharing is not applicable to this review.

Abbreviations

A	surface area available for diffusion
ACS	abdominal compartment syndrome

ADAM15	a disintegrin and metalloproteinase domain-containing protein 15
AGE	advanced glycation end product
ANG2	angiopoietin 2
cAMP	cyclic adenosine monophosphate
CF	contraction frequency
CO_2	carbon dioxide
C_{PL}	solute concentration in plasma
C_T	solute concentration in tissue
DLL4	Notch ligand delta-like 4
dV_i/dt	change in interstitial volume over time
dV_{LF}/dt	rate of lymph formation
dV_{PF}/dt	rate of plasma filtration
DVT	deep vein thrombosis
eNOS	endothelial nitric oxide synthase
EPAC	exchange proteins directly activated by cAMP
ERK-1/2	extracellular signal regulated kinases 1 & 2
EVL	Ena-VASP-like
FAK	focal adhesion kinase
IFN-γ	interferon-γ
IL-1β	interleukin-1β
IL-6	interleukin-6
JAM-A	junctional adhesion molecule-A
J_s	solute flux
J_V	volume flux of a fluid
L_P	hydraulic conductivity
LYVE-1	lymphatic vessel endothelial hyaluronan receptor 1
MEK-1/2	mitogen activated protein kinase kinase 1 & 2
MLC	myosin light chains
MLCK	myosin light chain kinase

MLCP	myosin light chain phosphatase
MOF	multiple organ failure
NO	nitric oxide
O_2	oxygen
OxPAPC	oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine
PAF	platelet activating factor
PECAM-1	platelet endothelial cell adhesion molecule
P_C	capillary hydrostatic pressure
P_I	interstitial hydrostatic pressure
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKC	protein kinase C
P_S	solute permeability coefficient
Π_I	interstitial osmotic pressure
Π_P	plasma osmotic pressure
ROCK	Rho-associated coiled-coil forming kinase
ROS	reactive oxygen species
S1P	sphingosine-1-phosphate
SIRS	systemic inflammatory response syndrome
VASP	vasodilator-stimulated phosphoprotein
VEGF-A	vascular endothelial growth factor-A
TGF-β	tumor growth factor-β
TNFα	tumor necrosis factor α
VEGFR2	vascular endothelial growth factor receptor-2
VEGFR3	vascular endothelial growth factor receptor-3
ZO	zonula occludens

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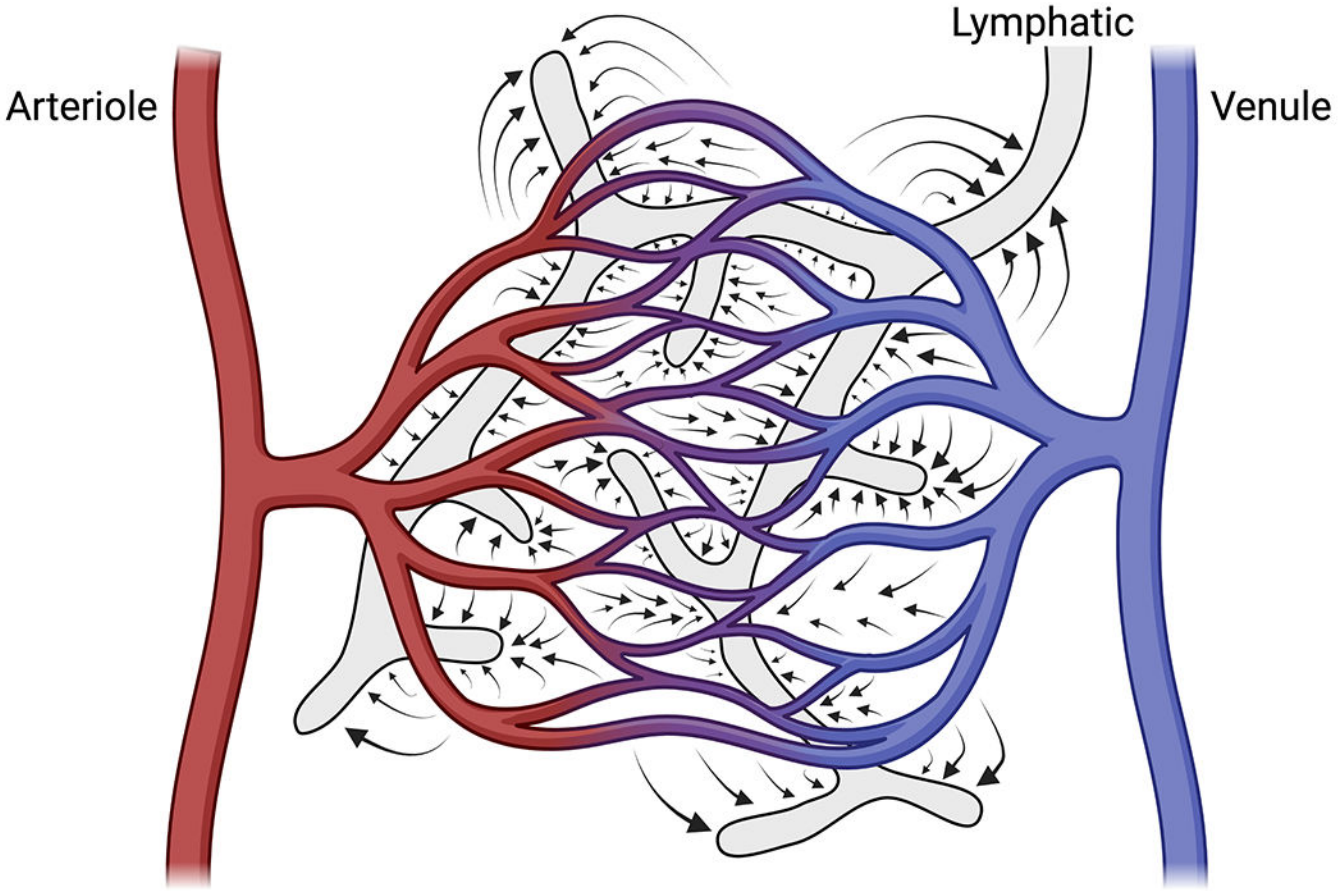

Fig. 1.
Microvascular leakage, interstitial flow, and lymph formation. Plasma filtration in capillary beds results in interstitial fluid formation. Lymphatic capillary networks, in close contact with blood capillaries, absorb interstitial fluid to form lymph.

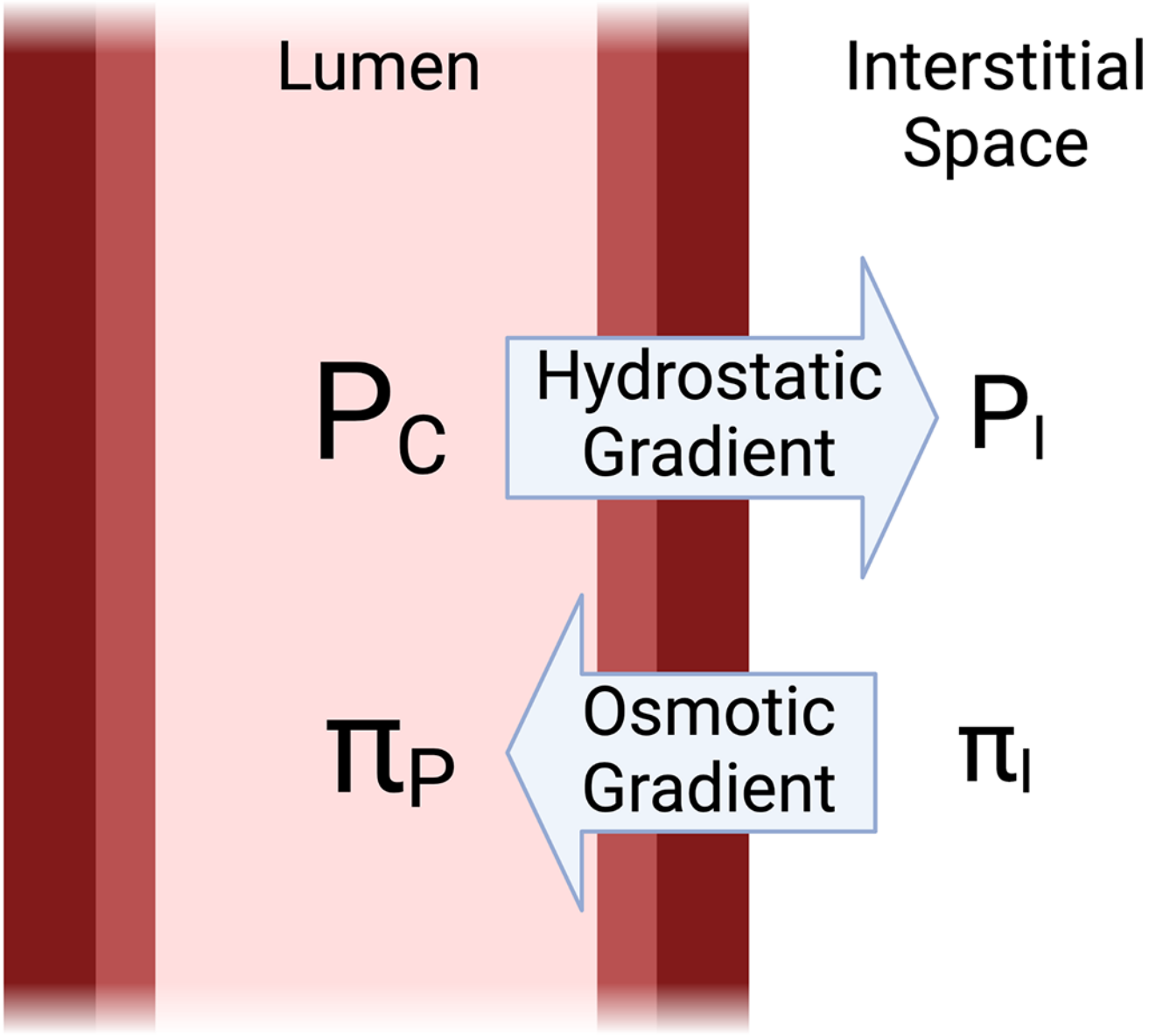

Fig. 2.

The Starling Forces. Hydrostatic pressure inside the capillary (P_C) generally exceeds the hydrostatic pressure in the interstitial space (P_I), causing a gradient for outward fluid flow. The osmotic pressure in the plasma (Π_P) generally exceeds the osmotic pressure in the interstitial space (Π_I), forming a gradient that promotes fluid flow into the capillary. Combined, there is generally a net flow of fluid out of the capillary.

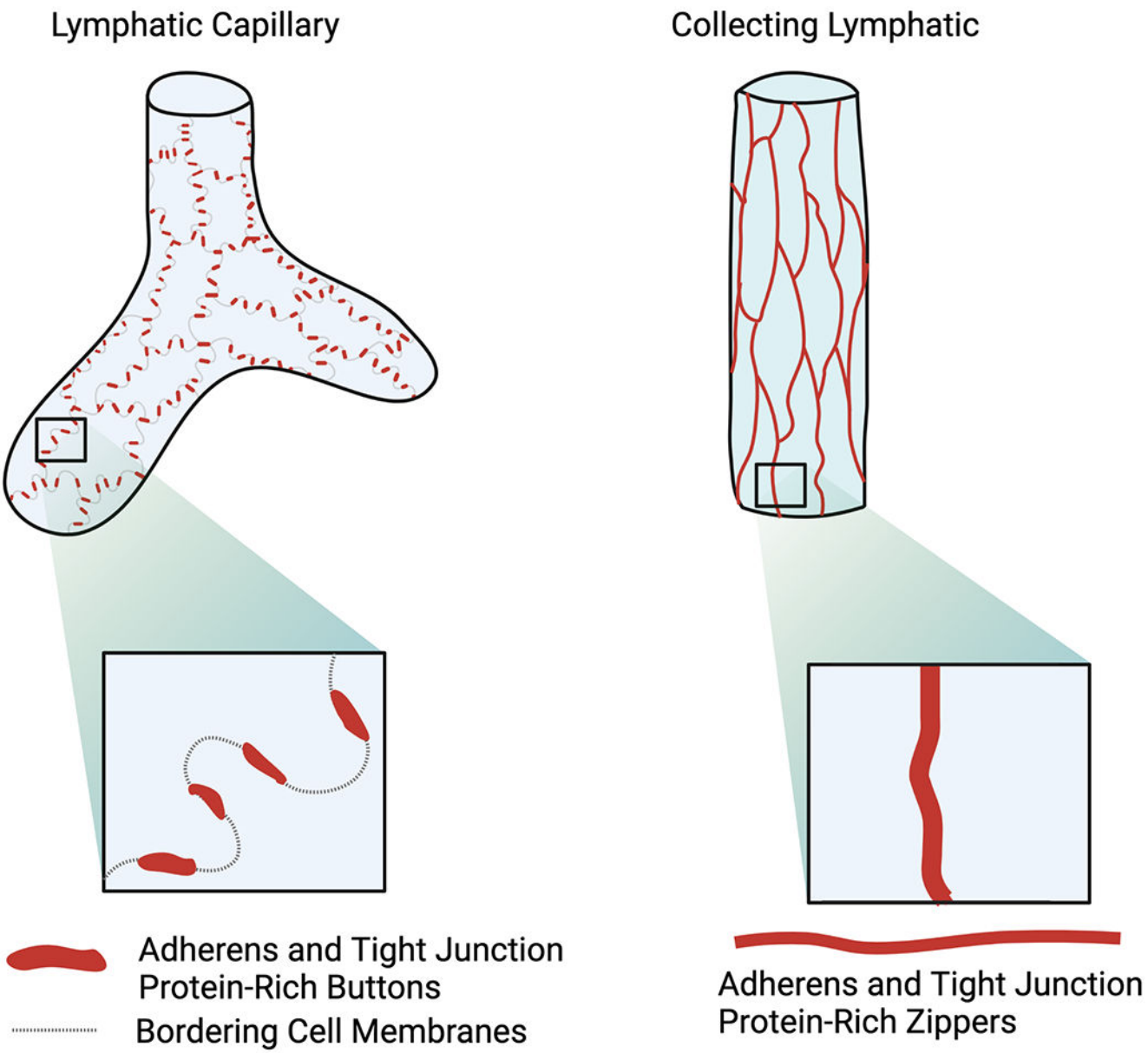

Fig. 3.

Lymphatic vessel junctions: buttons and zippers. The lymphatic capillaries feature intermittent button junctions, which are rich in adherens and tight junction proteins. Collecting lymphatic vessel endothelium has continuous junctions similar to those of blood vessels.

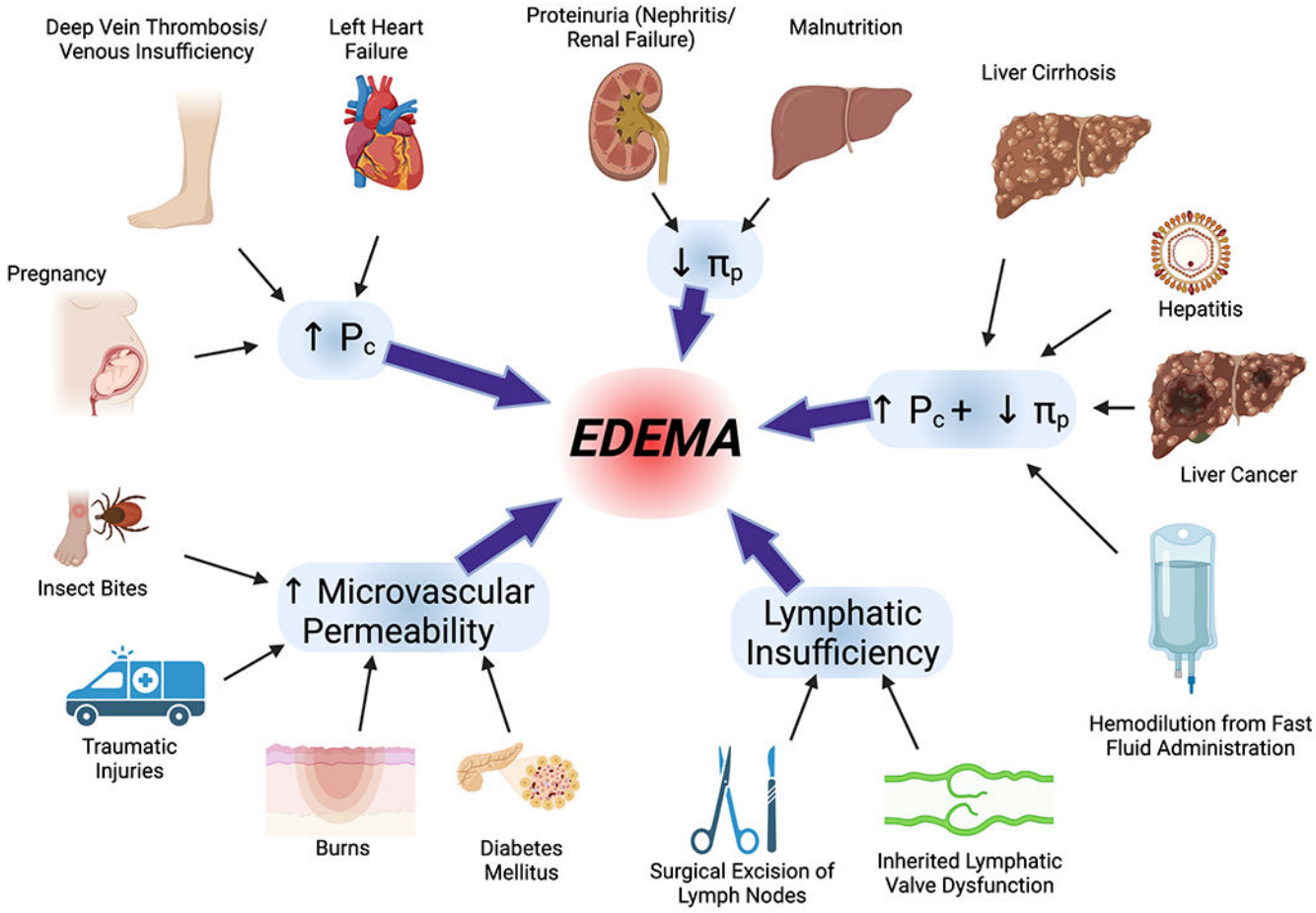

Fig. 4.

Summary of several different clinical conditions that can lead to edema. Imbalances in the Starling forces, microvascular hyperpermeability, of lymphatic insufficiency can all manifest as edema.

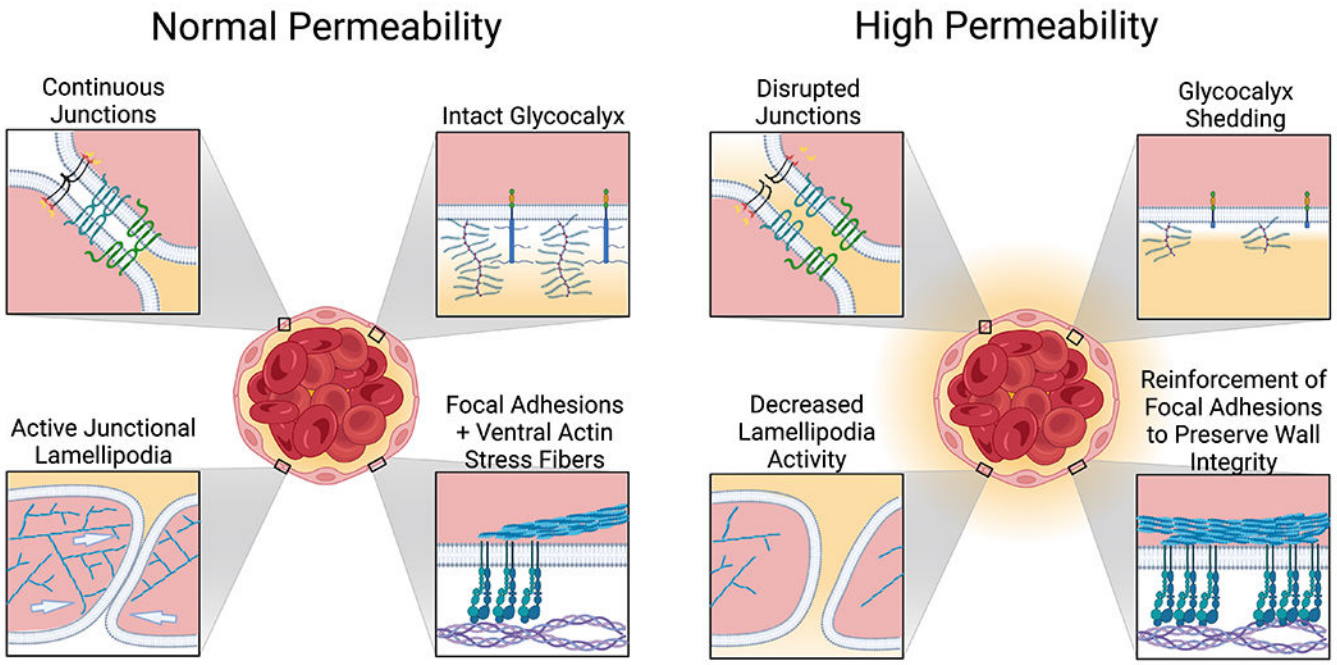

Fig. 5.

Structural mechanisms that control microvascular permeability. Inflammatory mediators cause partial disruption of endothelial cell-cell junctions, shedding of the luminal glycocalyx, and decreased junctional lamellipodia activity at cell borders, which all contribute to facilitating paracellular movement of fluid and solutes across the vascular wall. In reaction to the elevated permeability, increased actin stress fiber and focal adhesion reorganization is observed, which likely improves cell anchoring and stiffness, preserving wall integrity and allowing for reattachment of junctional proteins.

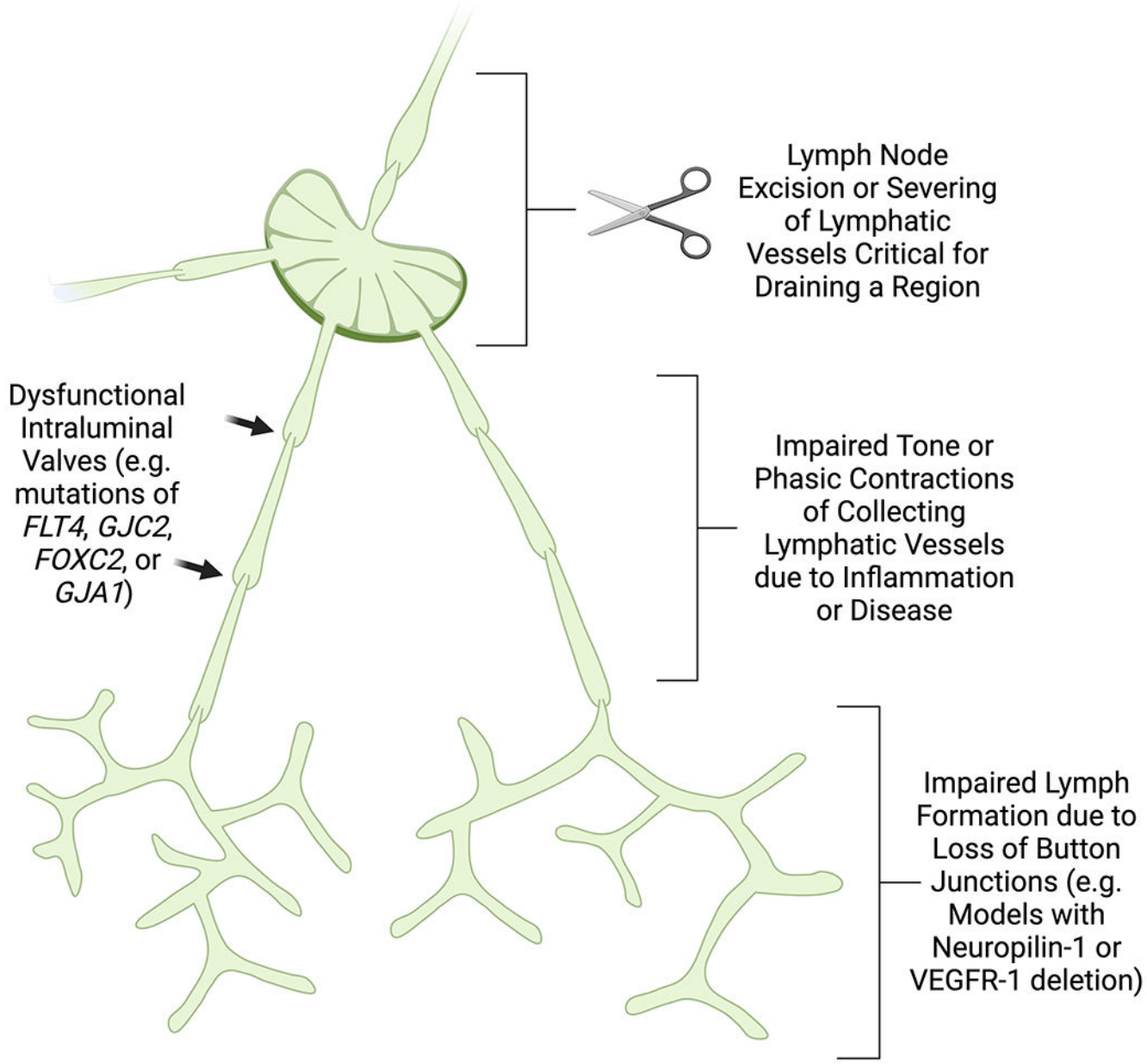

Fig. 6.

Conditions that can lead to lymphatic insufficiency. Severing of key lymphatics or excision of lymph nodes critical for draining a region can lead to severe tissue swelling. Gene mutations that cause dysfunction of the luminal valves of collecting lymphatics lead to impaired lymph flow and thus reduced lymph clearance. Dysfunction of collecting lymphatic pumping can also occur if lymphatic tone or phasic contractions are impaired. Deletion of certain genes has been found to cause “zippering” of button junctions in lymphatic capillaries, impairing lymph formation.