# Pathophysiology of Lymphatic Circulation in Different Disease Conditions

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## Contents



**Learning Objectives**

- To become familiar with the physiology and pathophysiology of lymphatic circulation
- To comprehend the causes and pathophysiology of lymphedema
- To learn about the newly discovered roles of lymphatic circulation in systemic diseases

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## 2.1 History

The first description of lymphatic vessels began in ancient Greece with Hippocrates (460–370 BC) and Aristotle (384–322 BC) who described vessels of the human body that may have been lymphatic vessels. More stringent reference to the lymphatic vessels came from Alexandria, where Erasistratus (ca. 304–250 BC) described milky arteries in the mesentery. Later, in the seventeenth century AD, Gaspare Aselli was the first physician/anatomist to document the functional lipid uptake and transport of lipid-rich meals in the white mesentery veins of dogs. The studies that followed Aselli's initial observations established that these vessels constituted a distinct vascular network that was separated but connected to the blood vascular system. The first gross anatomy of lymphatic vessels was definitely established at the beginning of the nineteenth century [1]. It was only at the end of the 1990s that investigators started to identify the receptor of vascular endothelial growth factor (VEGFR)-3 [2], the prosperous homeobox 1 (PROX1), the transcription factor [3], the membranes integral podoplanin glycoprotein (PDPN) [4], and hyaluronic receptor 1 of the lymphatic vessel (LYVE1) as specific lymphatic markers [5]. Subsequently, investigations



G. Mariani et al. (eds.), *Atlas of Lymphoscintigraphy and Sentinel Node Mapping*,
https://doi.org/10.1007/978-3-030-45296-4_2

in this field flourished through molecular genetic studies on embryo development, revealing more than 50 genes involved in the specific maturation of lymphatic vessels [6]. The lymphatic system was thus identified as an almost ubiquitous regulator of numerous physiological and pathological processes. Lymphatic vessels have been identified in organs which previously were thought not to exist, such as the eye, where they are involved in the regulation of intraocular pressure [7], or in the central nervous system, where they drain cerebral macromolecules and immune cells [8]. Moreover, pioneering studies have revealed lymph node lymphatic endothelial cells as antigen-presenting cells involved in the induction of peripheral immune tolerance [9]. These seminal findings have opened unexpected avenues for advancing knowledge on the lymphatic system in cardiovascular medicine [10].

**Key Learning Points**

- Recognition of the existence of lymphatic circulation has evolved slowly over the course of history, mainly because of the difficulties in visualizing the transparent vessels.
- We know now that lymphatic vessels are almost ubiquitous within different organs and show a remarkable heterogeneity, with different genes involved in development, reflecting their functional specialization.
- Unexpected advances in knowledge on the lymphatic system have recently emerged in cardiovascular medicine.

## 2.2 Physiology of the Lymphatic System

The lymphatic circulation should be considered as part of the peripheral cardiovascular system, as it interlinks closely with blood circulation both at its origins (the interstitial space) and at its final drainage point (the thoracic duct). To a large extent, the anatomy of lymphatic channels parallels that of the veins, and the two systems show many similarities in structure and function.

The lymphatic circulation includes the lymph, lymphatic vessels, lymph nodes (stations along the drainage route where fluid and cell exchange between blood and lymph occurs), and other lymphoid tissues, particularly the spleen and bone marrow. Through its own specialized cell, the lymphocyte, a close relationship exists between the peripheral lymphatic system, blood circulation, spleen, and liver (Fig. 2.1). Therefore, while lymph drainage has a predominant "plumbing" role, the lymphatic circulation does possess also important immunological roles.

The lymphatic vascular network consists of smaller blind-ended capillaries and larger collecting lymphatic vessels. The lymphatic capillaries are composed of a single layer of overlapping endothelial cells "oak leaf shaped" and lack a continuous basement membrane and pericytes. The distinctive oak leaf-shaped endothelial cells of initial lymphatics are loosely apposed with overlapping borders and linked each other by discontinuous, button-like junctions. Regions between buttons are open, so as to allow the entry of fluid and cells without repetitive formation and dissolution of intercellular junctions. These specific structures may function as primary lymphatic valves that prevent the tissue fluid taken up by lymphatic capillaries to be released back into the interstitial space (Fig. 2.2). Therefore, the lymphatic capillaries are highly permeable to interstitial fluid and macromolecules, such that, when the surrounding interstitial pressure changes, the lymphatics either expand and fill with lymph or contract and push lymph [12].

The capillaries drain into pre-collecting lymphatic vessels, which will merge into larger secondary collecting lymphatic vessels covered by smooth muscle cells, which provide contractile activity to assist lymph flow and possess a continuous basement membrane. Tissue fluid collected in the larger collecting lymphatics drains into the thoracic duct and is then returned to the blood circulation through lymphatic-venous connections at the junction of the jugular and subclavian veins.

At the distal capillaries, the systemic blood circulation loses about 2–4 L of fluid and about 100 g of protein into the interstitium per day. Normal physiology of lymphatics deals with draining from the tissue spaces these materials that cannot return to the bloodstream directly. Colloids, several types of cells (extravasated red cells, macrophages, lymphocytes, tumor cells, etc.), bacteria, and other microorganisms are channeled through the lymphatics, presumably as a protective mechanism to prevent noxious agents from directly entering the bloodstream. This is the reason why cellulitis and erysipelas can be a recurrent problem. Similarly, inorganic matters such as carbon and silica are removed by the lymphatics, as demonstrated by the black-stained pulmonary lymph nodes in coal miners.

**Key Learning Points**

- The lymphatic circulation should be considered as part of the peripheral cardiovascular system, as it interlinks closely with blood circulation.
- It consists of smaller blind-ended capillaries and larger collecting lymphatic vessels. The lymphatic capillaries are composed of a single layer of endothelial cells and lack a continuous basement membrane and pericytes, making them highly permeable to interstitial fluid and macromolecules.

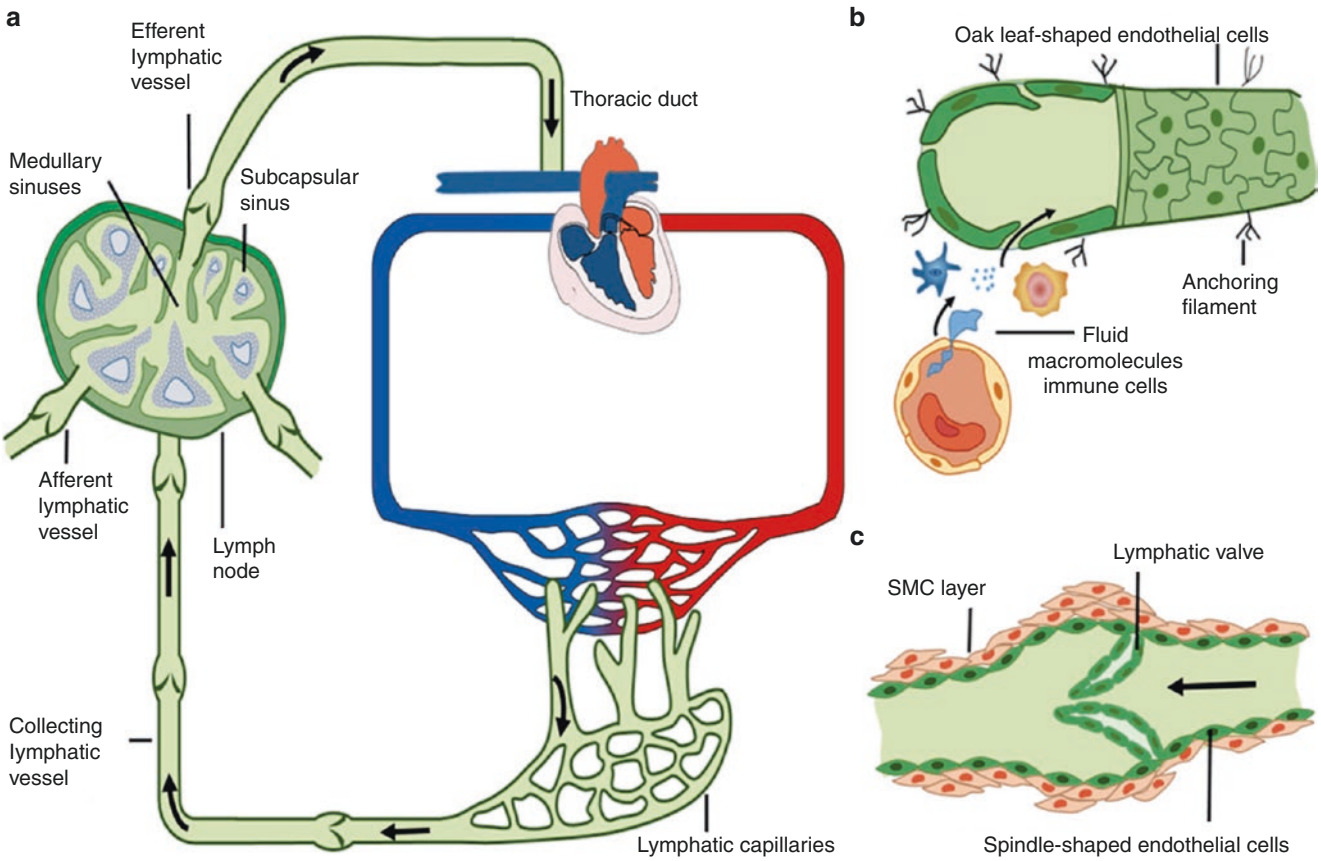


**Fig. 2.1** Schematic representation of the vascular circulation (arterial system, *red*; venous system, *blue*) and of lymphatic circulation (*green*), and their interrelationship. (**a**) Net of lymphatic capillaries drains tissue fluid and macromolecules from tissues through major lymphatic vessels and lymph nodes. The lymph is driven into the venous system through the left subclavian and the right subclavian veins, respectively. (**b**) Interstitial fluid, macromolecules, and immune cells are collected by lymphatic capillaries. (**c**) Lymphatic collectors contain intraluminal valve and SMC layers that permit the unidirectional lymph flow (*reproduced with permission from* [11])

- Tissue fluid collected in the larger collecting lymphatics drains into the thoracic duct and is then returned to the blood circulation through lymphatic–vasculature connections at the junction of the jugular and subclavian veins.
- Normal physiology of lymphatic circulation involves a daily drainage of about 2–4 L of fluid and about 100 g of protein from the tissue spaces directly to the bloodstream.

## 2.3 Lymphatic Circulation and Lipid Absorption

Lymphatic circulation is essential for the adsorption of lipids from the intestine. The major products of lipid digestion (fatty acids and 2-monoglycerides) enter the enterocyte either by simple diffusion or via a specific fatty acid transporter protein in the membrane. Once inside the enterocyte, fatty acids and monoglycerides are transported into the endoplasmic reticulum, where they are used to synthesize triglycerides. Beginning in the endoplasmic reticulum and continuing in the Golgi apparatus, triglycerides are packaged with cholesterol, lipoproteins, and other lipids into particles called chylomicrons. Transport of lipids into the circulation is different from what occurs with sugars and amino acids. In fact, instead of being adsorbed directly into capillary blood, chylomicrons are transported first into the lymphatic vessels that penetrate each intestinal villus.

Chylomicron-rich lymph then drains into the lymphatic system, which rapidly flows into blood. Blood-borne chylomicrons are rapidly disassembled and their constituent lipids utilized throughout the body. When large amounts of chylomicrons are being absorbed, lymph draining from the small intestine has a milky appearance, such that the mesenteric lymphatics are easy to see (first described as "venae alba et lacteae" or "white veins" by Aselli in the seventeenth century). Recent studies have shown that the lacteal is not simply acting as a passive duct; on the contrary, it is able to respond to autonomic nerve stimulation to the encircling smooth muscle cells and actively transport the absorbed lipid [13].

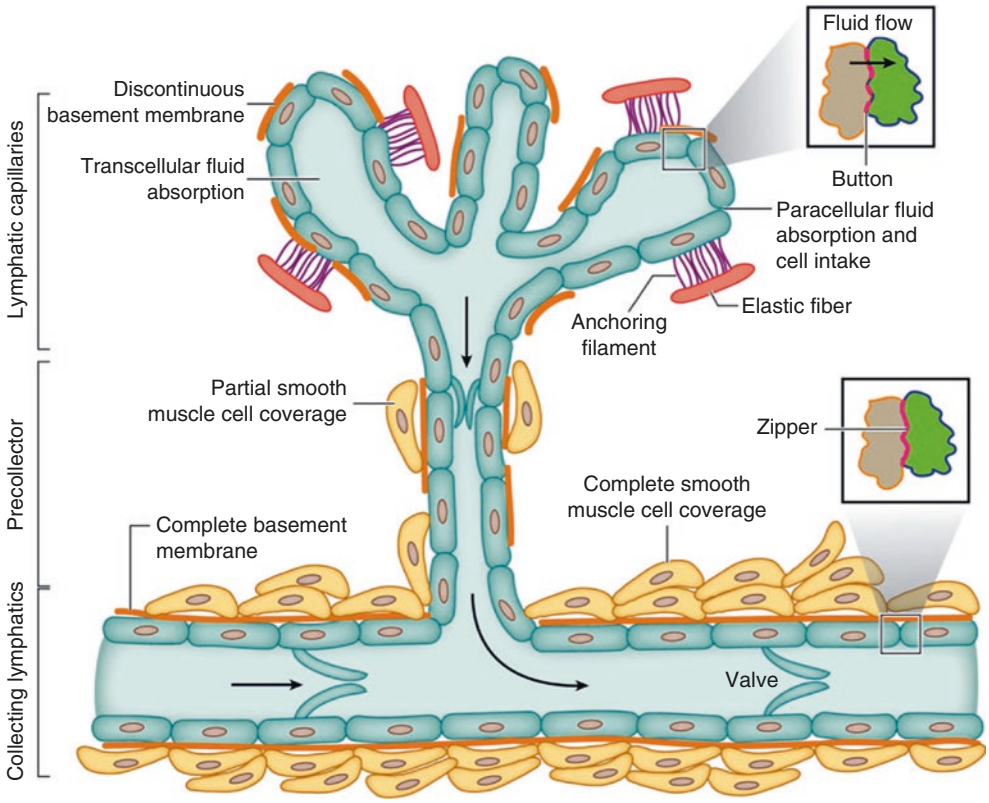


**Fig. 2.2** Schematic representation of the lymphatic system. The lymphatic capillaries are composed of a single layer of overlapping endothelial cells and lack a continuous basement membrane. Collecting lymphatic vessels are provided with smooth muscle cells, a basement membrane, and luminal valves that prevent lymph backflow. The unique structure of capillary lymphatic vessels accounts for the uptake of interstitial fluid, macromolecules, cells, and lipids that filtrate continuously from the blood capillary network (*reproduced with permission from* [11])

Compounds absorbed by the intestinal lymphatics drain via the cisterna chyli and the thoracic duct, thus entering systemic circulation at the junction of the left internal jugular vein with the left subclavian vein, thereby avoiding potential first-pass metabolism. Consequently, drug transport via the intestinal lymphatics may confer delivery advantages in terms of increased bioavailability and possibility of directing delivery to the lymphatic system.

**Key Learning Points**

- Lymphatic circulation is essential for the adsorption of lipids from the intestine.
- Instead of being adsorbed directly into capillary blood, the major products of lipid digestion, chylomicrons, are transported first into the lymphatic vessels that penetrate each intestinal villus.
- When large amounts of chylomicrons are being absorbed, lymph draining from the small intestine has a milky appearance.
- Compounds absorbed by the intestinal lymphatics drain via the cisterna chyli and the thoracic duct, into the systemic circulation at the junction of the left internal jugular vein with the left subclavian vein, thereby avoiding potential first-pass metabolism.
- Consequently, drug transport via the intestinal lymphatics may confer delivery advantages in terms of increased bioavailability and direct delivery.

## 2.4 Pathophysiology of Lymph Drainage Failure

Physiology of lymphatic circulation requires three intimately interconnected steps: (1) transport of prelymph across the interstitial space and into the initial lymphatics, (2) movement of lymph through the network of noncontractile initial lymphatics, and (3) active pumping of lymph through a series of contractile collecting trunks.

Under normal circumstances, water acts predominantly as a solvent or vehicle for the colloids, cells, and materials that can be drained only via the lymph route. Nevertheless, lymphatics also serve as an "overflow pipe" to drain excess interstitial fluid. This role of lymphatic circulation as "safety valve" against fluid overload involves the lymphatic system in every form of edema, although at variable degrees.

Edema is an excess of interstitial fluid, whose volume must increase by over 100% before edema becomes clinically detectable. Edema develops when the capillary filtration rate exceeds the lymphatic drainage rate for a sufficient period, a condition that results from an imbalance between capillary filtration and lymph drainage:

$$D_v / D_t = F_v - F_l$$

where $D_v/D_t$ is the rate of swelling, $F_v$ is the net capillary filtration rate, and $F_l$ is the lymph flow.

Therefore, the pathogenesis of any edema involves either a high filtration rate or a low lymph flow, or a com-

bination of the two factors. Elevation of capillary pressure is usually secondary to chronic elevation of venous pressure caused by heart failure, fluid overload, or deep vein thrombosis. On the other hand, reduced plasma colloid osmotic pressure (e.g., hypoproteinemia) raises the net filtration rate and lymph flow; changes in capillary permeability (e.g., inflammation) increase the escape of protein into the interstitium, and water follows osmotically. Impairment of lymph drainage results in the predominant accumulation of protein and water in the interstitial space, since lymph is the sole route for returning escaped protein to the plasma.

Most edemas arise from increased capillary filtration overwhelming lymph drainage; therefore, any edema incriminates the lymphatic system through its failure to keep up with demand. Edema is initially soft and pitting, and then hard, non-pitting, accompanied by skin thickening. Impairment of local immune response leads to recurrent skin infections, further insult to the tissue, and worsening of lymphedema.

### 2.4.1 Role of Leukotrienes and Inflammation in Lymphedema

Leukotrienes are a group of short-lived lipidic mediators produced primarily by pro-inflammatory immune cells, like macrophages, neutrophils, eosinophils, mast cells, and dendritic cells. In response to diverse immune and inflammatory stimuli, these lipid mediators elicit potent inflammatory responses through binding to, and activation of, their cognate G protein-coupled receptors.

Recent investigations have demonstrated the mechanistic role of the leukotriene B4 (LTB4) in the molecular pathogenesis of lymphedema. LTB4 is a strong chemoattractant and activator of leukocytes and one of the most potent lipid chemotactic factors for neutrophils. The biological function of LTB4 is mediated primarily by BLT1 or BLT2 receptors. When acting through the BLT1 receptor, LTB4 regulates migration and activity of cells of both innate and adaptive immunity and plays essential roles not only in physiological defense against infection, but also in the pathogenesis of a number of chronic diseases, such as obesity, insulin resistance, type 2 diabetes, and atherosclerosis.

It has consistently been observed that $LTB_4$ promotes lymphedema development, thereby involving tissue inflammation as the mechanistic platform for the development of acquired lymphedema. When the mechanisms that explain the impact of $LTB_4$ in acquired lymphatic vascular insufficiency have been explored, lower concentrations of $LTB_4$ were surprisingly demonstrated to have a prolymphangiogenic effect, thus suggesting a possible role for this eicosanoid in lymphatic repair at more physiological levels [14]. Higher concentrations of $LTB_4$ inhibit both VEGFR3 mRNA expression and VEGFR3 protein phosphorylation. Additionally, higher $LTB_4$ concentrations inhibit Notch signaling, a pathway known to be important for both lymphatic development and maintenance [11].

Such new knowledge constitutes the pathophysiologic basis for pilot studies that have demonstrated the benefit of anti-inflammatory therapy with ketoprofen in patients with lymphedema, favoring the restoration of a failing lymphatic circulation [15].

### 2.4.2 Aging of Lymphatic Vessels

Lymphatic-related diseases, such as lymphedema, are prevalent in the elderly. The aging process induces changes in structure and function of the lymphatic system. In the 1960s, the specific "varicose bulges" in muscular lymphatic vessels were observed to increase with age [16]. Muscle cell atrophy, elastic element destruction, and aneurysm-like formations were also found in aged lymphatic vessels. Aging-associated alterations in lymphatic contractility decrease pump efficiency, a mechanism that results in excessive retention of tissue fluid within the interstitial spaces. Reduced responsiveness to inflammatory stimuli in aged lymphatic vessels decreases the normal capacity to react against foreign organisms. High permeability is caused by the loss of glycocalyx and the dysfunction of junctional proteins. In addition, increased caspase-3 activity, dissociation of the VE-cadherin/catenin complex, and low expression of actin cytoskeleton that occur in aged blood vessels are present also in aged lymphatic vessels [17] (Fig. 2.3).

Knowledge of the aging-related diseases of lymphatic vessels is critical to our understanding of lymphatic vessel-related diseases.

**Key Learning Points**

- Impairment of lymph drainage results in accumulation of protein and water in the interstitial space, which corresponds clinically to lymphedema.
- Lymphedema becomes clinically detectable when excess of interstitial fluid increases by over 100%.
- It has recently been recognized that leukotrienes, lipidic mediators with potent pro-inflammatory properties, are involved in the pathogenesis of lymphedema.
- Aging decreases the efficiency of lymphatic contractility pump, thus contributing to abnormal retention of tissue fluid within the interstitial spaces.

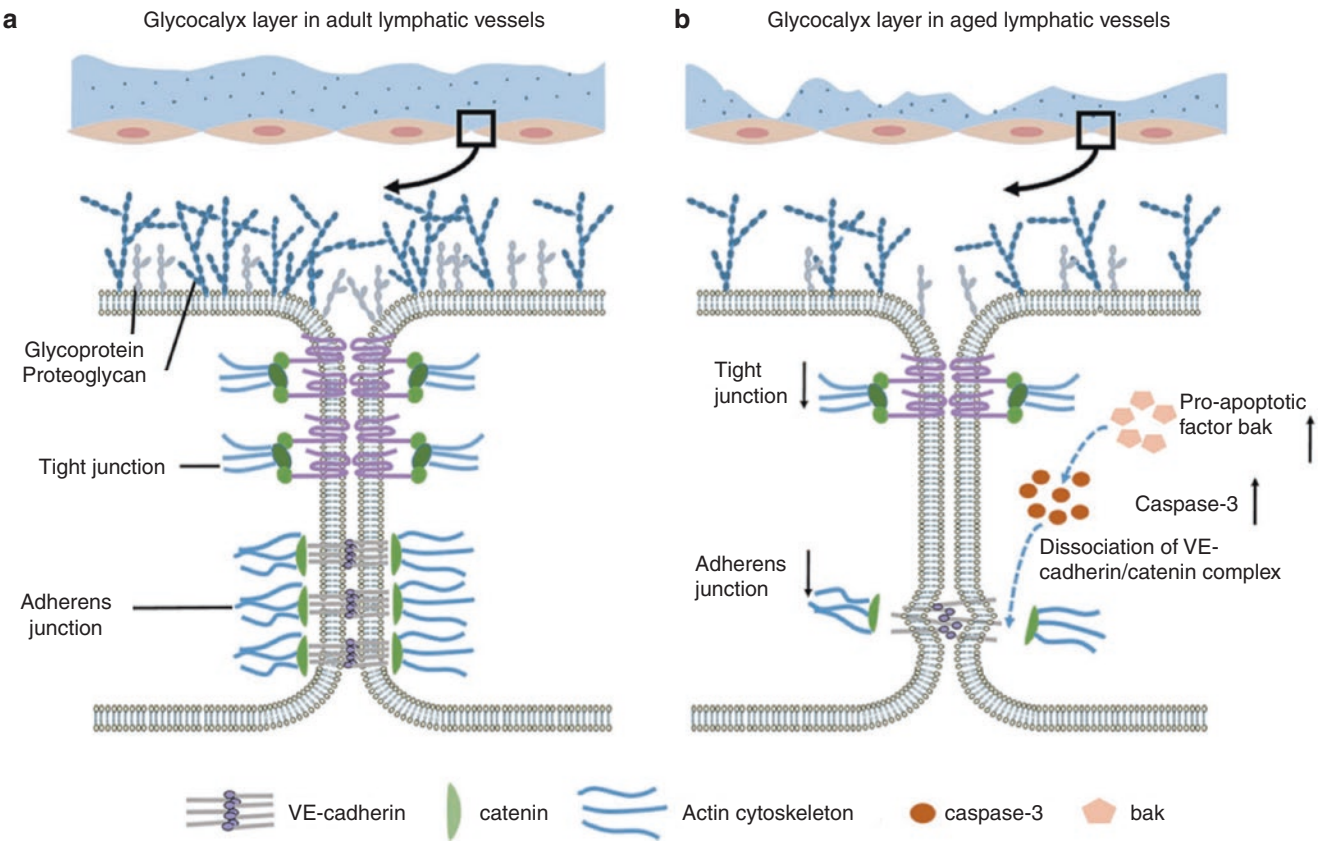


**Fig. 2.3** Glycocalyx layer and intercellular junctions of lymphatic vessels in aging process. (**a**) Continuous glycocalyx in adult lymphatic vessels. (**b**) Discontinuous glycocalyx in aged lymphatic vessel. Increased pro-apoptotic factor back activates caspase-3 to disrupt the downstream protein β-catenin, which leads to decreased adherent junctions and impaired barrier function (*reproduced with permission from* [17])

## 2.5 Lymphedema

Lymphedema is an edema arising from a failure of lymph drainage, which can be induced by several causes (see Table 2.1). First, there may be an intrinsic abnormality of the lymph-conducting pathways. Such conditions are referred to as **primary lymphedema**, which means that no other identifiable causes can be found. Primary lymphedema occurs because of the imperfect development of the lymphatic vascular system in utero. It can be familial (as with Milroy's disease and Meige's syndrome), or genetic, such as those associated with Turner and Noonan syndromes or the congenital vascular Klippel-Trenaunay syndrome, where malformed lymphatics coexist with an aberrant venous system. Sporadic cases of primary lymphedema are more frequent than the familial or genetic associated forms.

The identification of mutant genes in primary hereditary human lymphedemas has defined some crucial pathways during lymphovascular development and lymphangiogenesis. In particular, lymphangiogenesis requires adequate expression of the C isoform of the vascular endothelial growth factor (VEGF) which binds to its receptors (VEGFR-2 and VEGFR-3) under the control of the master regulator of lymphangiogenesis, the transcriptional factor Prox 1 [3]. VEGFR-3 is highly expressed on lymphatic endothelial cells. During the embryonic stage, VEGFR-3 acts as a receptor for both VEGF-C and VEGF-D, and its activation stimulates lymphatic vessel formation, thus representing the main molecular mechanism involved in the development and growth of the lymphatic system (Fig. 2.4).

**Table 2.1** Mechanisms and causes of lymph drainage failure

| Mechanism | Causes |
|---|---|
| Reduced lymph-conducting pathways | Aplasia-hypoplasia of the whole vessel |
| | Acquired obliteration of lymphatic lumen |
| Poorly functioning pathways | Failure of pump contractility |
| Obstructed pathways | Scar from lymphadenectomy, radiation therapy, or infection |
| Incompetent lymphatics with reflux | Megalymphatics |
| | Lymphatic hyperplasia |

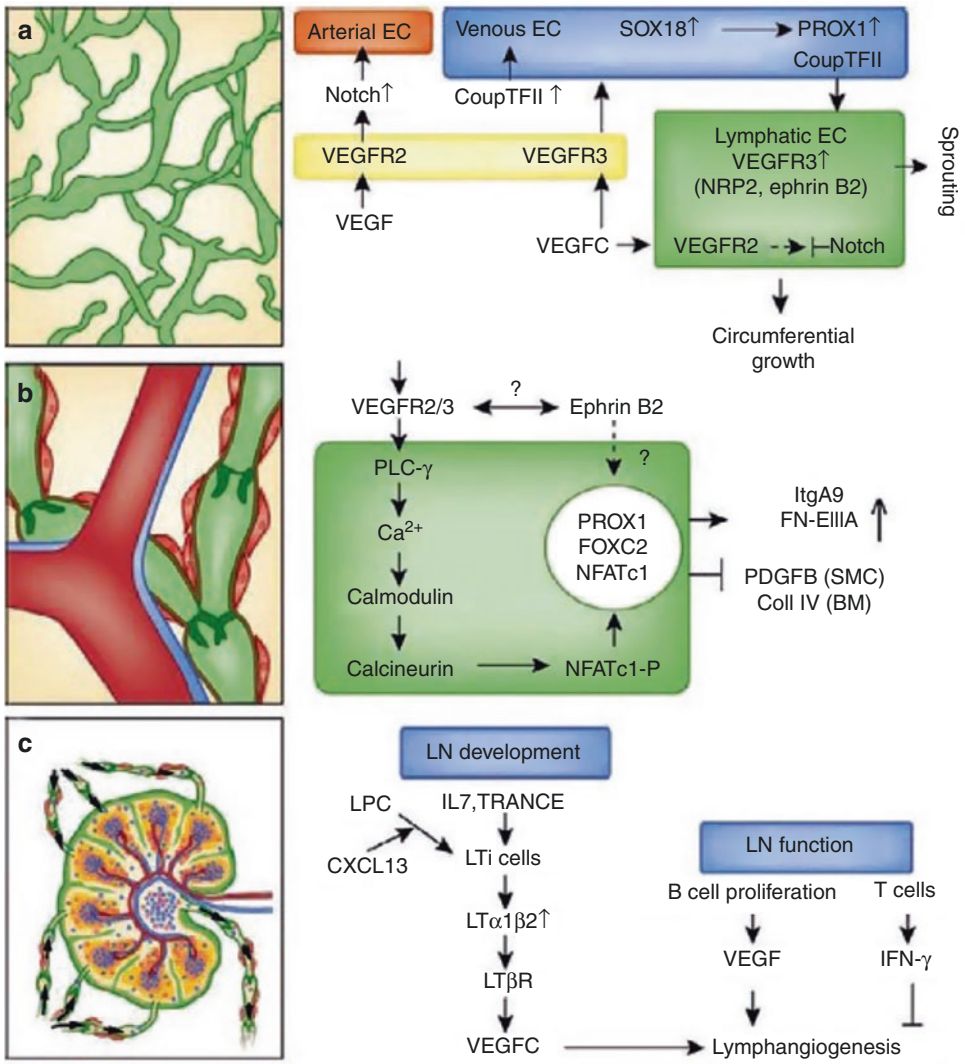


**Fig. 2.4** Main molecular mechanisms involved in the development and growth of the lymphatic system. (**a**) Lymphatic capillaries derive from venous endothelial cells. After arteriovenous differentiation controlled by Notch and COUP-*TFII* transcription factors, Sox18 activates Prox1, which interacts with COUP-TFII and induces lymphatic endothelial differentiation, involving enhanced expression of VEGFR-3. VEGF-C then induces the sprouting of the LECs to generate new vessels. VEGF-C and VEGF can also increase the size of lymphatic vessels by stimulating circumferential growth. (**b**) Formation of lymphatic valves requires a calcium-induced signal via phospholipase C-g and calmodulin to calcineurin that dephosphorylates the NFATc1 transcription factor, which enters the nucleus and induces valve-specific genes in a complex with FoxC2 (T. Petrova, University of Lausanne, personal communication cited in [18]). VEGFR-2, VEGFR-3, and ephrin B2 are upstream regulators of the pathways necessary for valve development. PDGF-B and collagen IV production is inhibited simultaneously. (**c**) In developing lymph nodes, lymphangiogenesis is first induced when IL-7 and TRANCE stimulate LTi cells that develop from lymphatic precursor cells under the influence of the chemokine CXCL13. The LTi cells produce LTα1β2, which activates VEGF-C expression via the LTb receptor in stromal organizer cells. In adult lymph nodes, B-cell proliferation stimulates VEGF-mediated lymphangiogenesis, whereas T-cell-derived cytokines restrict lymphangiogenesis by producing interferon-γ. *Notch* neurogenic locus notch homolog protein, *COUP-TFII* chicken of albumin upstream promoter transcription factor II, *Sox18* sex-determining region Y Box 18, *Prox1* prospero-related homeodomain transcript factor, *LEC* lymphatic endothelial cell, *VEGF* vascular endothelial growth factor, *NFATc1* nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1, *FoxC2* forkhead box protein C2, *ItgA9* integrin a9, *PDGF-B* platelet-derived growth factor, *IL-7* interleukin-7, *TRANCE* tumor necrosis factor (ligand) superfamily, member 11, *CXCL13* chemokine (cys × cys motif) ligand 13, *LTα1β2* heterotrimeric lymphotoxin (α1β2) (*reproduced with permission from* [18])

An example of genetic primary lymphedema, the first to be identified, is provided by the Milroy's disease, which is characterized by early-onset congenital lymphedema. In this syndrome, heterozygous missense mutations in the FLT4 (VEGFR3) gene inactivate the kinase activity of VEGFR-3. It is characterized by bilateral lower limb lymphedema, which is usually present at birth.

Another example, one of the most common primary lymphedemas, the lymphedema-distichiasis (LD) syndrome, is an autosomal dominant disorder with variable expression.

It is caused by mutations in the FOXC2 gene, which codes for FOXC2, a transcription factor involved in the development of the lymphatic and vascular system [19]. The LD syndrome is characterized by late childhood or pubertal onset lymphedema of the limbs, and by distichiasis (double row of eyelashes). While the latter is the most common expression of LD, venous insufficiency occurs in half of the patients. Other associations have been reported, including congenital heart disease, ptosis, cleft lip/palate, and spinal extradural cysts.

The currently known mutations of at least 19 different genes are involved in lymphatic development associated with primary lymphedema [20], the most frequent being GJC2, FOXC2, CCBE1, VEGFR-3, PTPN14, GATA2, and SOX18 (Table 2.2) [18].

Although genetic molecular investigation contributes to provide proper genetic counseling for parents of an affected child with congenital lymphedema, they cannot explain those forms presenting later in life (*lymphedema tarda*). In these cases, the latent period before swelling suggests that a failure of growth or regeneration following a damage or injury might be the real cause underlying edema, rather than an abnormal development of lymphatics since birth.

In **secondary lymphedemas**, damage to the lymph-conducting pathways may occur secondary to any number of causes originating primarily outside the lymphatic system. Secondary lymphedema is more common than primary lymphedema, as it can result from a wide variety of causes, such as the post-thrombotic syndrome, surgical or radiation therapy, trauma, and infections.

**Table 2.2** Gene abnormalities identified in different lymphedema syndromes

| Gene | Disease name | Clinical manifestation |
|---|---|---|
| FLT4 (VEGFR-3) | Hereditary lymphedema IA (AD) | Congenital lymphedema |
| GJC2 | Hereditary lymphedema IC (AD) | Lymphedema of the extremities, onset at <15 years of age |
| FOXC2 | Lymphedema-distichiasis syndrome | Lymphedema of mainly lower limbs, triple row of eyelashes, varicose veins |
| CCBE1 | Hennekam lymphangiectasia-lymphedema syndrome (AR) | Lymphedema of the extremities, intestinal lymphangiectasias, mental retardation |
| SOX18 | Hypotrichosis-lymphedema-telangiectasia syndrome (AD) | Lymphedema, alopecia, telangiectasia |
| PTPN14 | Lymphedema-choanal Atresia syndrome (AR) | Lymphedema of lower limbs in children, lack of nasal airways |
| GATA2 | Emberger syndrome (AD) | Lymphedema of lower extremities and genitalia, immune dysfunction, cutaneous warts, deafness |

*AD* autosomal dominant, *AR* autosomal recessive
Adapted from [18]

In chronic venous insufficiency, as in the post-thrombotic syndrome, most of the interstitial fluid is unable to return to the heart by way of the obstructed veins. Therefore, the volume of fluid transported by the lower extremity lymphatics increases to compensate for the venous occlusion. This safety valve function of the lymph vessels continues until the lymphatic valvular mechanism becomes insufficient; then, reflux occurs, swelling of the limb increases, and ulcers develop.

Lymphedema frequently coexists with lipedema, a condition affecting only women. Lipedema is a bilateral, symmetrical swelling of the lower extremities extending from the pelvic brim to the ankles. Histologically, its hallmark is a gross increase in the subcutaneous fat layer, only limited to the areas mentioned above. The patient may be normal in weight, or even thin in the upper half of the body, but grossly obese from the pelvic brim down. Lymph vessels in lipedema are coiled, the prelymphatic canals and initial lymph vessels are abnormal, lymph transport velocity is reduced, and lymphedema involves both lower extremities (including the feet).

The most common **secondary upper-arm lymphedema** is to be expected after axillary lymph node dissection for breast cancer surgery, and/or radiation therapy in the upper arm. The incidence of breast cancer-related lymphedema, however, ranges widely between 6 and 70%; it may be a common underreported morbidity, considering the relation between lymphatic drainage of the upper extremity (UE) and of the breast in the caudal part of the axilla. Sentinel lymph node (SLN) groups for the UE and breast share connections in 24% of cases, which could explain lymphedema after surgery if damaged [21]. Lymphedema is generally localized at the arm on the side of breast surgery, and patients with a high peripheral blood vascular filtration rate seem to be predisposed to this complication [22, 23]. GJC2 (CX47) mutations are associated with a predisposition toward the development of postmastectomy lymphedema [24]. A clear relationship between the number of lymph nodes removed and the risk of lymphedema has not been definitely established, and clinical trials are focusing on the reduction of axillary lymph node dissections, even in the presence of a positive SLN [25].

The most common cancers in which treatments cause **secondary lower limb lymphedema** are melanoma, sarcoma, and pelvic tumors (including cervix, uterus, and prostate); it is noteworthy that pelvic cancers and infiltrating sarcomas can present with lymphedema, probably as failure of lymphatics to regenerate and re-anastomose satisfactorily through scarred or irradiated tissue secondary to cancer treatment.

Trauma of the lymphatic channels (either from elective surgery or by accident) has to be extensive in order to induce lymphedema. Indeed, the experimental reproduction of lymphedema is extremely difficult, owing to the highly efficient regenerative powers of lymphatics.

**Parasitic lymphedema** is the most common cause of lymphedema worldwide. It is caused by the microfilariae of *Wuchereria bancrofti* and *Brugia malayi*, which can be transmitted to humans by different mosquito species. When these microfilariae reach the lymph vessels, they develop into adult worms; the resulting inflammation and fibrosis cause progressively increasing lymphatic obstruction. Elephantiasis is the end result of repeated infections, developing over many years.

Another tropical form of lymphedema is podoconiosis (endemic nonfilarial elephantiasis), a noninfectious geochemical disease of the lower limb lymphatic vessels evolving from chronic barefoot exposure to red-clay soil originating from volcanic rock. The hypothesis is that mineral particles in red-clay soils are absorbed through the skin of the foot and phagocytized by macrophages in the lymphatic system of the lower limbs, thus causing an inflammatory reaction in the lymphatic vessels resulting in fibrosis and vessel obstruction [26].

As common remark, all chronic limb lymphedemas cause functional impairment, disfiguration of the limb, and severe psychological damage.

***Intracavitary lymphedema*** is the accumulation of chyle in the peritoneal cavity. Chyle can be defined as "milky lymph" which flows from the lacteals of the gut through the cisterna chyli and then through the thoracic duct. Chylous ascites occurs after abdominal surgeries or neoplastic infiltration of the abdominal lymphatic structures, as well as following traumas, lymphatic dysplasia, intraperitoneal lymphatic fistula within the framework of congenital defects of the lymphatic system, rupture of the lymphatic cyst, as well as an infectious and inflammatory process in lymph nodes. Ascitic fluid with triglyceride levels greater than 110 mg/dL is diagnostic of chylous ascites; total protein content varies usually from 1.4 to 6.4 g/dL, with a mean of 3.7 g/dL.

Chylothorax is a form of lipid pleural effusion characterized by the presence of chyle in the pleural space, which can be the result of obstruction or disruption of the thoracic duct, or one of its major tributaries. A triglyceride concentration >110 mg/dL is virtually diagnostic, but the presence of chylomicrons confirms the diagnosis. However, chylothorax defined by these criteria represents a heterogeneous group of clinical entities. The presence of chylomicrons or triglyceride levels >110 mg/dL in a pleural effusion should be considered evidence of chyle leakage of indeterminate clinical significance. In the case of an acute or chronic chylothorax due to possible injury of the thoracic duct injury, this evaluation is crucial, as surgical ligation of the thoracic duct is often entertained. In contrast, a cholesterol-rich effusion is typically the result of long-standing pleurisy with elevated cholesterol levels in the pleural space; most cases of cholesterol pleural effusions are attributed to tuberculous or to rheumatoid pleurisy. Distinguishing between a chylothorax and a cholesterol effusion is critical. A chylothorax develops after injury or obstruction of the thoracic duct, leading to leakage of chyle into the pleural space, and is characterized by an increased triglyceride concentration and by the presence of chylomicrons. In contrast, a cholesterol effusion is a long-standing effusion associated with an elevated cholesterol concentration (usually greater than 250 mg/dL) and with a thick pleural rind; this condition represents a form of lung entrapment. The accumulation of chyle in the pericardial space, or chylopericardium, occurs most frequently after trauma, and cardiac and thoracic surgery, or in association with tumors, tuberculosis, or lymphangiomatosis. When its precise cause cannot be identified, it is called primary or idiopathic chylopericardium, a quite rare clinical condition. Etiology can be primary or the result of various clinical situations, particularly trauma (thoracic duct lesions), neoplasms (primary such as lymphangioma or through invasion of the lymphatic system by other neoplasms), and filaria infection. Primary forms are the result of malformation of the intestinal lymph circulation and its relationship with the systemic circulation, resulting in megalymphatics that develop fistulas following even minimal trauma, and that can be located at atypical sites in the body.

**Key Learning Points**

- The cardinal manifestation of lymphatic dysfunction is lymphedema.
- Primary lymphedemas have been identified in hereditary diseases associated with specific genetic defects.
- Secondary lymphedemas occur secondarily to any causes originating primarily outside the lymphatic system.
- Secondary lymphedemas are more common than primary lymphedemas; the post-thrombotic syndrome, surgical or radiation therapy, trauma, infections, and neoplasms are the most frequent etiologies.
- Intracavitary lymphedema may occur because of accumulation of lymph in the peritoneal cavity, as well as in the pleural or in the pericardial space.

## 2.6 Lymphatic Malignancies

Solid tumors can originate in the lymphatic tissues. Lymphangiosarcoma, a malignant tumor of unknown molecular pathogenesis, causes primary or secondary lymphedema. Lymphangio-leiomyomatosis is a tumor characterized by the infiltration of abnormal smooth-like cells through the pulmonary interstitium, perivascular spaces, and lymphatics of young females, and it leads to lymphatic disruption and final respiratory failure. Kaposi's sarcoma is an angiogenic tumor of lymphatic endothelial cells (LECs). The lymphatic vessels also provide a route for tumor cells to metastasize, and the lymph node microenvironment may select tumor cells with increased metastatic potential.

**Key Learning Points**

- Tumors can originate in the lymphatic tissues.
- Most frequently, the lymphatic circulation serves as the primary route for the metastatic spread of tumor cells to regional lymph nodes.
- Propagation to lymph nodes is one of the main prognostic factors in patients with solid epithelial cancers: after reaching the SLNs, tumor cells will spread into distant lymph nodes and other organs.
- On the other hand, direct hematogenous metastatic spread can occur independently from metastatic spread through the lymphatic system.

## 2.7 Lymphangiothrombosis and Acute Lymphangitis

Obliterative processes consequent to lymphangiothrombosis or recurrent lymphangitis might occur in the same way as for veins, since lymph can clot in the same way as blood; unfortunately, there is no clinical investigation for ascertaining the presence of lymph thrombosis.

Lymphangitis, an inflammation of the lymphatic collectors, is clinically evident as a red streak up the limb corresponding to the inflamed vessels. Edema is often an accompanying feature. Infection is generally limited to the lymph nodes, and lymphadenitis may give rise to painful swelling in the groin or axilla (depending on the site of infection). Lymphangitis can be recurrent. When lymphatic insufficiency exists and the local system fails in its host defense duty, recurrent infection can occur, presenting clinically as recurrent erysipelas.

Erysipelas is a skin infection that is usually caused by β-hemolytic group A streptococci. After having had erysipelas in an extremity, a significant percentage of patients develops persistent swelling or suffers from recurrent erysipelas. Although persistent swelling after erysipelas is most likely caused by secondary lymphedema, a study [27] proved that patients presenting with a first episode of erysipelas often have signs at lymphoscintigraphy of preexisting lymphatic impairment in the other, clinically non-affected, leg. This means that subclinical lymphatic dysfunction of both legs may be an important predisposing factor. Therefore, treatment of erysipelas should focus not only on the infection, but also on the lymphological aspects; in this regard, long-term treatment for lymphedema is essential in order to prevent recurrence of erysipelas and aggravation of the preexisting lymphatic impairment.

Based on the clinical experience that lymphangitis or cellulitis is not always followed by the development of lymphedema, it can be speculated that lymphedema is the result of vulnerable lymphatics with preexisting lymphatic insufficiency, although proving which came first—the cellulitis or the lymphatic insufficiency—is still difficult.

**Key Learning Points**

- There are no clinical investigations for ascertaining the presence of lymph thrombosis.
- Lymphangitis, an inflammation of the lymphatic collectors, is clinically evident as a red streak up the limb; edema is often an accompanying feature, and lymphangitis can recur.
- Erysipelas is a skin infection usually caused by β-hemolytic group A streptococci.
- Subclinical lymphatic dysfunction may be an important predisposing factor for erysipelas.
- Therefore, treatment of erysipelas should focus not only on the infection per se, but also on the lymphatic components of the clinical problem.
- This includes long-term treatment of lymphedema in order to prevent recurrence of erysipelas and further worsening of the preexisting lymphatic impairment.

## 2.8 Pathophysiology of Lymphatic Circulation in Systemic Diseases

The new emerging concept of lymphangiogenesis strongly emphasizes the contribution of lymphatic circulation to the initiation, maintenance, natural history, or therapeutic approach to a broad array of systemic diseases, including obesity, atherosclerosis, and cardiovascular diseases.

### 2.8.1 Obesity

Abnormality in the pathophysiologic regulation of lymphatic circulation might be involved in the pathogenesis of obesity.

The link between lymphatic function and adipose biology has recently been recognized [28]. Lymph nodes and collecting lymphatic vessels are usually embedded in visceral or subcutaneous fat, thus suggesting a relationship between lymphatic vessels and adipose metabolism. Additionally, ectopic growth of adipose tissue is observed in edematous regions of patients suffering from chronic lymphedema. In rats, chronic inflammation of the peripheral lymph nodes increases the number of adipocytes surrounding the nodes. Moreover, increased deposition of subcutaneous fat in edematous regions has been described in lymphedema-carrying Chy-mice with heterozygous inactivating mutation in VEGFR3, thus supporting the hypothesis that the lymph is adipogenic. Indeed, mice with heterozygous Prox1-inactivating mutation were found to have leaky lymphatic vessels and to develop obesity and inflammation resembling late-onset obesity in humans [29]. Prox1 heterozygous mice constitute the first in vivo model of lymphatic mediated obesity, where the leading cause of the obese phenotype is the abnormal lymph leakage due to disruption in lymphatic vascular integrity, particularly of the mesenteric lymphatic vessels; leaking of lymph exerts a potent adipogenic stimulus, although the exact factor responsible for such stimulus is presently still unknown.

Obese adipose tissue expansion is an inflammatory process that results in dysregulated lipolysis, increased circulating lipids, ectopic lipid deposition, and systemic insulin resistance. Lymphatic vessels provide a route of fluid, macromolecule, and immune cell clearance, and lymphangiogenesis increases this capability. Indeed, inflammation-associated lymphangiogenesis is critical in resolving acute and chronic inflammation, but it is largely absent in obese adipose tissue. Enhancing adipose tissue lymphangiogenesis could, therefore, improve metabolism in obesity. Furthermore, an improvement in obesity metabolism enhances lymphangiogenesis of adipose tissue. This hypothesis has recently been proved in transgenic mice with inducible expression of vascular endothelial growth factor (VEGF)-D under a tissue-specific lymphangiogenesis tightly controlled during obesity high-fat diets of 16 weeks. VEGF-D adipose overexpression induced de novo lymphangiogenesis in murine adipose tissue. When increasing VEGF-D signaling and lymphangiogenesis specifically in adipose tissue, the immune accumulation associated with obesity is reduced and the metabolic response is favored [30].

### 2.8.2 Arterial Hypertension

In animal models of hypertension it has been observed that extrarenal control of sodium balance and blood pressure can reside in the glycosaminoglycans, macrophages, and lymphatic vessels of the skin. Sodium ions in the skin interstitium can be stored in an osmotically inactive form bound to glycosaminoglycans that can provide an actively regulated interstitial $Na^+$ exchange mechanism that participates in volume and blood pressure homeostasis [22]. $Na^+$ magnetic resonance imaging of human body sodium distribution demonstrates that sodium is primarily accumulated in the skin and muscles [31]. In a recent clinical investigation [32] of skin biopsies in 91 patients (both hypertensive and with normal blood pressure) who had elective surgery with abdominal skin incision, the content of $Na^+$ and water, accumulation of macrophages (CD68), and density of lymphatic vessels (D2-40) and blood vessels (CD31) were calculated in the specimens of abdominal skin together with plasma NT-proANP, vascular endothelial growth factor (VEGF)-C, and VEGF-D concentrations. In the hypertensive group, the skin expression of CD68 and the serum concentration of VEGF-C were different than in the group with normal blood pressure. The results of this investigation suggest that cutaneous accumulation of $Na^+$ is associated with the presence of hypertension and is also correlated with the presence of CD68 macrophages and with decreased prevailing levels of VEGF-C, thus implying that the lymphatic system contributes significantly to this cascade of events leading to hypertension. These human observations suggest that further exploration of the lymphatic contribution to the pathogenesis and maintenance of hypertension might lead to novel interventions directed at the prevention and treatment of this highly morbid condition.

### 2.8.3 Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the arterial wall that develops silently over decades, evolving to fatty streaks characterized mainly by macrophages loaded with cholesterol esters. Continuous recruitment of monocytes into plaques drives the progression of this chronic inflammatory condition, sustained at least in part by the deposition of cholesterol crystals and immunity against cholesterol-associated apolipoproteins.

Although the link between cholesterol and inflammation that drives disease progression is not completely understood, it is established that removal of cholesterol from the arterial wall constitutes a step toward regression of atherosclerosis.

The presence of lymphatics in the arterial wall was described more than 100 years ago [33]. The presence of lymphatic vessels in the adventitia of arteries adjacent to small blood vessels, the *vasa vasorum*, that are expanded in atherosclerotic plaques, suggests that lymphatic vessels have a role in the development of atherosclerotic plaques [34]. Recently the existence of lymphatic vessels has been demonstrated in the adventitia as well as in intraplaque regions of human carotid endarterectomy specimens [35].

It is speculated that the lymphatic circulation could provide a protective pathway for lipid and inflammatory cell efflux from the arterial wall, thus counteracting the development of atherosclerotic plaques. The multistep process of cholesterol mobilization from extravascular tissues to biliary and non-biliary excretion is termed reverse cholesterol transport (RCT). Cholesterol removal from macrophage stores involves hydrolysis, mobilization, and efflux of cholesterol esters to lipoprotein acceptors such as apoAI, which results in the formation of HDL. HDL leaves the interstitial tissue and is transported through the bloodstream into the liver for disposal as biliary cholesterol and bile salts, or to the intestinal wall for trans-intestinal cholesterol efflux.

Although the initial and final steps of RCT have been well characterized, it was only recently shown that HDL primarily uses lymphatic vessels in the efflux from the interstitium to the bloodstream. Indeed, experimental evidences showed that induction of lymphangiogenesis by the administration of VEGF-C into the footpad improved lymphatic function, decreased footpad cholesterol content, and improved RCT in *ApoE−/−* mice [36]. In contrast, surgical disruption of collecting lymphatic vessels in the popliteal area reduced RCT from the footpad by as much as 80%. In another study, surgical ablation of lymphatic vessels in the tail also blocked RCT. In Chy-mice, which selectively lack dermal lymphatic vessels, RCT from the rear footpad was impaired by ≤77% [36].

The relevance of lymphatic RCT in atherosclerotic plaque is a surprising finding, and it would be important to determine why HDL prefers lymphatic vessels instead of the postcapillary venous system to exit the interstitial space.

Overall, animal models indicate that RCT is critically dependent on lymphatic vessels, and that the venous system is not enough to sustain RCT. Furthermore, inducing lymphangiogenesis could constitute a strategy to enhance RCT. This could be especially important in the case of hypercholesterolemia and obesity, conditions that were shown to directly impair lymphatic vessel function.

Despite extensive investigations, it remains to be assessed whether inducing intimal or adventitial lymphangiogenesis could enhance RCT and reverse atherosclerosis in patients with cardiovascular disease [37].

### 2.8.4 Myocardial Infarction

The presence of lymphatic vessels in the arterial wall and their role in atherosclerosis have stimulated investigations on whether lymphangiogenesis might improve myocardial function, particularly in the infarcted or dilated heart, where wall tension is grossly elevated [35].

Following myocardial infarction, cardiac lymphatic vessels undergo a profound lymphangiogenic response; in experimental settings in mice, ectopic VEGFC stimulation augments the lymphangiogenic response resulting in a transient improvement in post-myocardial infarction (MI) cardiac function. Therefore, inducing lymphangiogenesis could provide a pathway for inflammatory cell efflux to tip the balance in favor of wound healing within the injured adult heart. Despite the endogenous cardiac lymphangiogenic response post-MI, the remodeling and dysfunction of collecting ducts contribute to the development of chronic myocardial edema and inflammation—aggravating cardiac fibrosis and dysfunction. Therefore, therapeutic lymphangiogenesis may be a promising new approach for the treatment of cardiovascular diseases [38]. The roles of lymphatic vessels in MI and atherosclerosis are summarized in Fig. 2.5.

**Key Learning Points**

- Current knowledge leads to speculation that lymphatic circulation is involved in the pathogenesis of cardiovascular diseases, including obesity, hypertension, atherosclerosis, and myocardial infarction.
- Expansion of the adipose tissue in obesity is an inflammatory process that results in dysregulated lipolysis, increased circulating lipids, ectopic lipid deposition, and systemic insulin resistance.
- Lymphangiogenesis is critical in resolving acute and chronic inflammation, but it is largely absent in the adipose tissue of obese subjects; enhanced adipose tissue lymphangiogenesis could, therefore, improve metabolism in obesity.
- The presence of lymphatic vessels in the adventitia of arteries adjacent to *vasa vasorum* (that are expanded in atherosclerotic plaques) and the presence of lymphatic vessels inside human carotid endarterectomy specimens suggest that lymphatic vessels have some role in the development of atherosclerotic plaques.
- Cholesterol mobilization from extravascular tissues to biliary and non-biliary excretion, termed reverse cholesterol transport (RCT), is critically dependent on lymphatic vessels, and the venous system is not sufficient to sustain RCT. The relevance of lymphatic RCT is a surprising finding.
- Cardiac lymphatic circulation might improve myocardial function, particularly in the infarcted or dilated heart.
- Despite extensive investigations, it remains to be assessed whether inducing intimal or adventitial lymphangiogenesis could enhance RCT and thus reverse atherosclerosis in patients with cardiovascular disease.
- Therapeutic lymphangiogenesis might constitute a promising new approach for treating cardiovascular diseases.

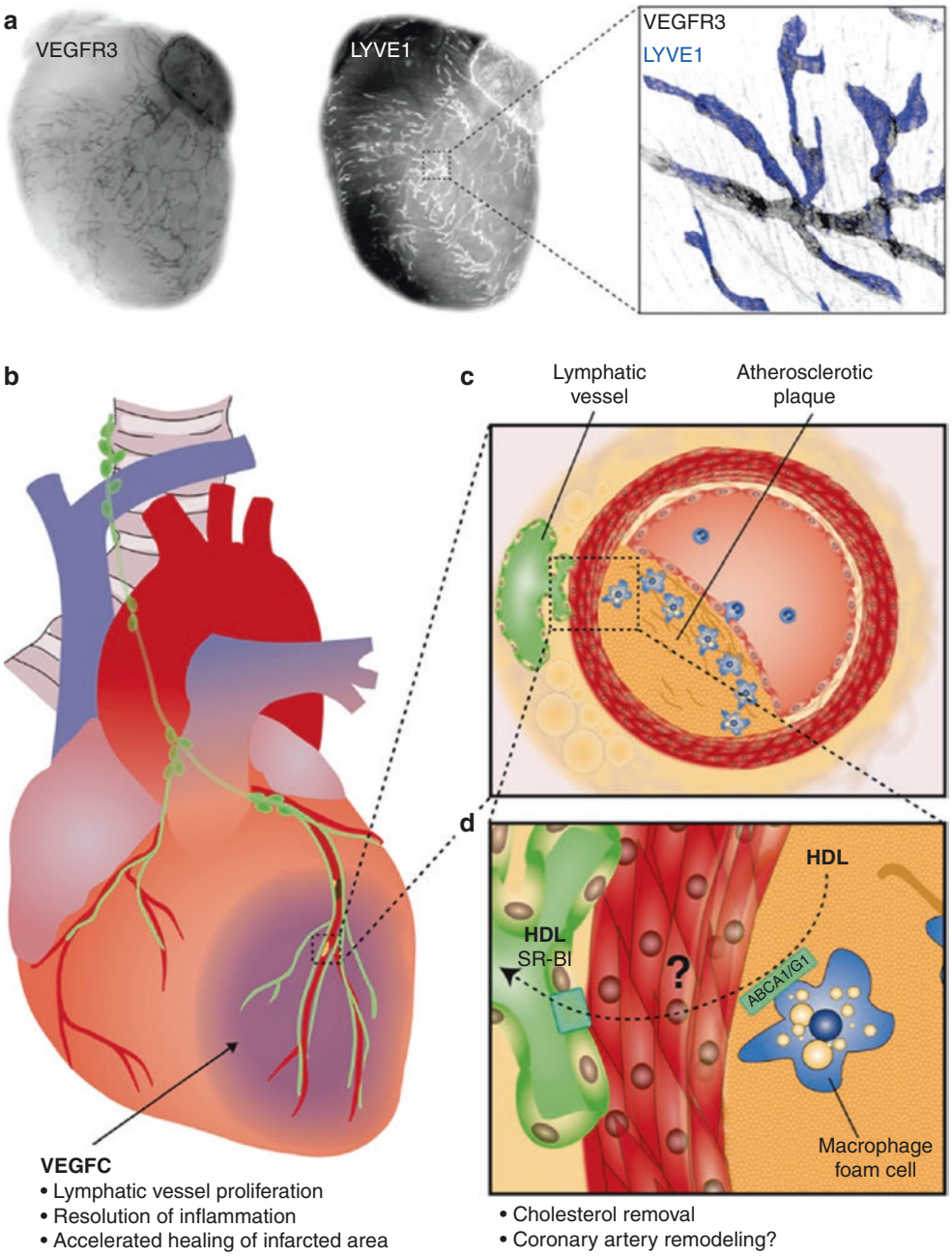


**Fig. 2.5** Role of lymphatic vessels in cholesterol metabolism, atherosclerosis, and myocardial infarction. (**a**) Epicardial lymphatic vessels stained for VEGFR3 and LYVE1. (**b**) Schematic overview of the heart with myocardial infarction caused by the occlusion of the atherosclerotic coronary artery. (**c**) Cross section of an atherosclerotic coronary artery and an adventitial lymphatic vessel. (**d**) Hypothetical model for the role of lymphatic vessels in high-density lipoprotein (HDL)-mediated cholesterol removal from atherosclerotic plaques (*adapted from* [10])

## 2.9 Concluding Remarks

The cardinal manifestation of lymphatic malfunction is lymphedema. Overall data from the last decade, however, indicate that lymphatic circulation is critically involved also in the pathogenesis of cardiovascular disease, including hypercholesterolemia, atherosclerosis, and obesity.

The concept that inflammation promotes lymphedema has opened new pharmacological strategies and therapeutic targets. Enhanced insights into the interplay between inflammation and pathological tissue remodeling are required to pave new therapeutic avenues in lymphedema and in other forms of lymphatic disorders.

The venous system is not sufficient by itself to sustain the multistep process of cholesterol mobilization from extravascular tissues to biliary and non-biliary excretion, termed reverse cholesterol transport. In this regard, the lymphatics play an important role, and promoting lymphangiogenesis could constitute a novel strategy to enhance reverse cholesterol transport.

Despite extensive investigations suggesting that therapeutic lymphangiogenesis may be a promising new approach for the treatment of cardiovascular diseases, it still remains to be assessed whether inducing intimal or adventitial lymphangiogenesis could enhance RCT and thus reverse atherosclerosis in patients with cardiovascular disease.

**Acknowledgements** This chapter is a revision of the original chapter written by R. Di Stefano, P. A. Erba, and G. D'Errico in the previous edition of the book.

## References


1. Lord RS. The white veins: conceptual difficulties in the history of the lymphatics. Med Hist. 1968;12:174–84.
2. Kukk E, Lymboussaki A, Taira S, et al. VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development. Development. 1996;122:3829–37.
3. Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. Cell. 1999;98:769–78.
4. Breiteneder-Geleff S, Soleiman A, Kowalski H, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am J Pathol. 1999;154:385–94.
5. Banerji S, Ni J, Wang SX, et al. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J Cell Biol. 1999;144:789–801.
6. Yang Y, Oliver G. Development of the mammalian lymphatic vasculature. J Clin Invest. 2014;124:888–97.
7. Thomson BR, Heinen S, Jeansson M, et al. A lymphatic defect causes ocular hypertension and glaucoma in mice. J Clin Invest. 2014;124:4320–4.
8. Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatic vessels. Nature. 2015;523:337–41.
9. Cohen JN, Guidi CJ, Tewalt EF, et al. Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. J Exp Med. 2010;207:681–8.
10. Aspelund A, Robciuc MR, Karaman S, et al. Lymphatic system in cardiovascular medicine. Circ Res. 2016;118:515–30.
11. Jiang X, Nicolls MR, Tian W, et al. Lymphatic dysfunction, Leukotrienes, and lymphedema. Annu Rev Physiol. 2018;80:49–70.
12. Brown P. Lymphatic system: unlocking the drains. Nature. 2005;436:456–8.
13. Choe K, Jang JY, Park I, et al. Intravital imaging of intestinal lacteals unveils lipid drainage through contractility. J Clin Invest. 2015;125:4042–52.
14. Tian W, Rockson SG, Jiang X, et al. Leukotriene B4 antagonism ameliorates experimental lymphedema. Sci Transl Med. 2017;9:eaal3920.
15. Rockson SG, Tian W, Jiang X, et al. Pilot studies demonstrate the potential benefits of antiinflammatory therapy in human lymphedema. JCI Insight. 2018;3.
16. Zerbino DD. [Senile changes in the outflow lymphatic vessels]. Arkh Anat Gistol Embriol. 1960;39:37–42.
17. Shang T, Liang J, Kapron CM, et al. Pathophysiology of aged lymphatic vessels. Aging (Albany NY). 2019;11:6602–13.
18. Alitalo K. The lymphatic vasculature in disease. Nat Med. 2011;17:1371–80.
19. Sutkowska E, Gil J, Stembalska A, et al. Novel mutation in the FOXC2 gene in three generations of a family with lymphoedema-distichiasis syndrome. Gene. 2012;498:96–9.
20. Brouillard P, Boon L, Vikkula M. Genetics of lymphatic anomalies. J Clin Invest. 2014;124:898–904.
21. Pavlista D, Eliska O. Relationship between the lymphatic drainage of the breast and the upper extremity: a postmortem study. Ann Surg Oncol. 2012;19:3410–5.
22. McLaughlin SA. Lymphedema: separating fact from fiction. Oncology (Williston Park). 2012;26:242–9.
23. Stanton AW, Modi S, Mellor RH, et al. Recent advances in breast cancer-related lymphedema of the arm: lymphatic pump failure and predisposing factors. Lymphat Res Biol. 2009;7:29–45.
24. Finegold DN, Baty CJ, Knickelbein KZ, et al. Connexin 47 mutations increase risk for secondary lymphedema following breast cancer treatment. Clin Cancer Res. 2012;18:2382–90.
25. Wojcinski S, Nuengsri S, Hillemanns P, et al. Axillary dissection in primary breast cancer: variations of the surgical technique and influence on morbidity. Cancer Manag Res. 2012;4:121–7.
26. Tekola Ayele F, Adeyemo A, Finan C, et al. HLA class II locus and susceptibility to podoconiosis. N Engl J Med. 2012;366:1200–8.
27. Damstra RJ, van Steensel MA, Boomsma JH, et al. Erysipelas as a sign of subclinical primary lymphoedema: a prospective quantitative scintigraphic study of 40 patients with unilateral erysipelas of the leg. Br J Dermatol. 2008;158:1210–5.
28. Harvey NL. The link between lymphatic function and adipose biology. Ann N Y Acad Sci. 2008;1131:82–8.
29. Harvey NL, Srinivasan RS, Dillard ME, et al. Lymphatic vascular defects promoted by Prox1 haploinsufficiency cause adult-onset obesity. Nat Genet. 2005;37:1072–81.
30. Chakraborty A, Barajas S, Lammoglia GM, et al. Vascular endothelial growth factor-D (VEGF-D) overexpression and lymphatic expansion in murine adipose tissue improves metabolism in obesity. Am J Pathol. 2019;189:924–39.
31. Kopp C, Linz P, Dahlmann A, et al. $^{23}$Na magnetic resonance imaging-determined tissue sodium in healthy subjects and hypertensive patients. Hypertension. 2013;61:635–40.
32. Chachaj A, Pula B, Chabowski M, et al. Role of the lymphatic system in the pathogenesis of hypertension in humans. Lymphat Res Biol. 2018;16:140–6.
33. Hoggan G, Hoggan FE. The lymphatics of the walls of the larger blood-vessels and lymphatics. J Anat Physiol. 1882;17:1–23.
34. Nakano T, Nakashima Y, Yonemitsu Y, et al. Angiogenesis and lymphangiogenesis and expression of lymphangiogenic factors in the atherosclerotic intima of human coronary arteries. Hum Pathol. 2005;36:330–40.
35. Kutkut I, Meens MJ, McKee TA, et al. Lymphatic vessels: an emerging actor in atherosclerotic plaque development. Eur J Clin Invest. 2015;45:100–8.
36. Martel C, Li W, Fulp B, et al. Lymphatic vasculature mediates macrophage reverse cholesterol transport in mice. J Clin Invest. 2013;123:1571–9.
37. Csanyi G, Singla B. Arterial lymphatics in atherosclerosis: old questions, new insights, and remaining challenges. J Clin Med. 2019;8(4). pii: E495. https://doi.org/10.3390/jcm8040495.
38. Henri O, Pouehe C, Houssari M, et al. Selective stimulation of cardiac lymphangiogenesis reduces myocardial edema and fibrosis leading to improved cardiac function following myocardial infarction. Circulation. 2016;133:1484–97; discussion 1497.