Chapter 4
The Role of Lymphatics in Atherogenesis, Myocardial Infarction, Congestive Heart Failure, and Cardiac Transplantation

Shin Lin and Stanley G. Rockson

Abstract The lymphatic vasculature is a central biological participant in fluid, protein and cellular transport, and in immune responsiveness. Over the last 10 years, the biomedical investigation into the function of the lymphatic microvasculature has been vigorous, prompting reconsideration of the role of lymphatics in the genesis and progression of cardiovascular pathology. The lymphatic microvasculature of the heart and vascular wall likely participates in atherogenesis, myocardial infarction, congestive heart failure, and cardiac transplantation. Intensive exploration of lymphatic mechanisms of cardiovascular disease is likely to lead to enhanced insights and novel therapeutic approaches.

Keywords Atherosclerosis • Myocardial infarction • Congestive heart failure • Cardiac transplantation • Edema • Lymph • Lymphatics • Microvasculature

Introduction

The lymphatic vasculature plays an essential role in fluid homeostasis and in the trafficking of immunocytes [1] and is therefore critical to the edematous and immune-mediated sequelae of inflammation. In other words, the lymphatics actively participate in key structural and biological components of the inflammatory response and, thereby, represent a unique juncture for potential intervention. Active investigation into lymphatic mechanisms of disease, nevertheless, has suffered a relative lack of emphasis, due largely to an absence of suitable investigative tools and model systems [2]. Recently, powerful new lymphatic-specific markers, pharmacologic and genetic modulators, and novel investigative platforms have reinvigorated the

S. Lin • S.G. Rockson (✉)
Stanford University School of Medicine, Falk Cardiovascular Research Center,
300 Pasteur Drive, Stanford, CA 94305, USA
e-mail: rockson@stanford.edu

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Table 4.1 Genes involved in lymphatic development and function

Gene or gene product	Function
Angiopoietin-1	Growth factor [71]
Angiopoietin-2	Growth factor [72]
Chemokine (C-C motif) ligand 20 (CCL20)	Chemokine [73, 74]
Chemokind (C-C motif) ligand 21 (CCL21)	Chemokine [75]
Desmoplakin	Anchoring protein of intermediate filaments to the plasma membrane of adhering junctions [76]
Ephrin B2	Ligand of EphB receptors
FOXC2 (forkhead box C2)	Transcription factor [77, 78]
HGF (hepatocyte growth factor)	Growth factor [79]
Integrin α9	Adhesion molecule, possible VEGFR-3 co-receptor [80, 81]
LYVE-1	Hyaluronan receptor [58]
Macrophage mannose receptor 1	L-selectin receptor [82]
Neuropilin-2 (Nrp2)	Semaphorin and growth factor receptor [83]
Net (Elk3)	Transcription factor [84]
Plakoglobin	Connect cadherins to cytoskeleton in cell-cell junction [73, 81]
Prox1	Transcription factor [62, 85]
Podoplanin (T1α)	Transmembrane glycoprotein [86, 87]
Sex determining region Y-related high mobility group box (SOX18)	Transcription factor [88]
Syk and Src homology 2-domain containing leukocyte protein 76 (SLP-76)	Syk and SLP [89, 90]
Vascular endothelial growth factor-C (VEGF-C)	Growth factor [90, 91]
Vascular endothelial growth factor receptor-3 (VEGFR-3)	Growth factor receptor [92–94]

Source: Reprinted with permission from Nakamura K, Rockson SG [43]

study of lymphatic biology with the discovery of genes involved in lymphatic development and function (Table 4.1) [1, 3]. With this investigative renaissance, it is appropriate to reconsider lymphatic vasculature function within the context of the cardiovascular system and its complex role in the genesis, propagation, and therapeutics of cardiovascular pathology (Fig. 4.1).

The Anatomy and Function of the Myocardial Lymphatics

Anatomy of Myocardial Lymphatics

The myocardium is permeated by a dense plexus of penetrating intracardiac channels that drain interstitial fluid from the subendocardium to the subepicardium, detectable initially as lymphatic capillaries and then coalescing into collecting

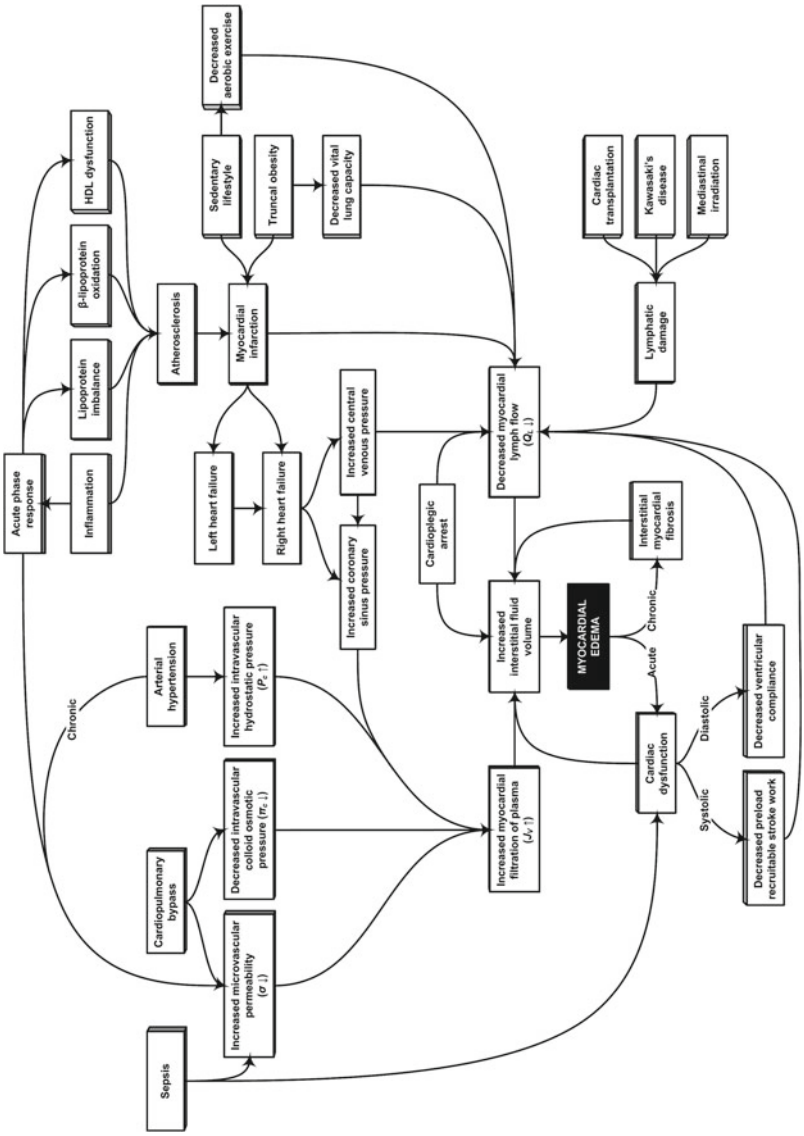

Fig. 4.1 The central role of myocardial edema in cardiac pathophysiology. A model of the theoretical causal relationships (*arrows*) in various cardiovascular pathologies as they might relate to lymphatic dysfunction and myocardial edema (Reprinted with permission from Nakamura and Rockson [43])

trunks [4]. The cardiac lymphatic trunk connects with the greater lymphatic vasculature at the cardiac lymph node before ascending to the thoracic duct and joining the central venous circulation close to the origin of the subclavian vein. In complete analogy to the peripheral lymphatic vasculature, unidirectional valves and rhythmic contraction of the adjacent tissues together ensure synchronous propulsion of myocardial lymph.

Lymphatic Function

The lymphatic system regulates interstitial fluid composition and volume both in health and in disease. The lymphatic system serves as a conduit for the by-products of normal cellular metabolism as well as those of specific pathological processes such as ischemia and necrosis [5]. As an example, inadequate perfusion of the myocardium leads to accumulation of anaerobic metabolites and disruption of myocardial fluid balance attributable to lymphatic disruption. Elucidation of these cardiac lymphatic mechanisms is expected to uncover novel strategies in the diagnosis and treatment of cardiac dysfunction.

Within a 24-h period, more than half of the circulating blood protein content is extravasated into the interstitium, yet there is no direct path of reabsorption into the arteriovenous vasculature [6]. The return to the intravascular circulation of interstitial fluid, comprised of protein, water, and other components, is the primary function of the lymphatic vasculature. The concepts of filtration and flow are central to understanding the fluid dynamics that govern lymphatic function. According to the Starling equilibrium, hydrostatic pressures from within the microvascular capillary (P_c) and circumferentially in the peripheral interstitium (P_{int}) have antagonistic effects. Intravascular hydrostatic pressure is determined largely by upstream arteriolar pressure and downstream venular pressure. In addition, colloid osmotic pressure (π) exerts an opposite effect upon directional flow, causing reabsorption of interstitial fluid back into the intravascular circulation. The final operative variable upon fluid equilibrium is the permeability of the capillary membrane. Among these physiological variables, the most important factors in fluid balance are the capillary and interstitial hydrostatic pressures. A similar arrangement of forces governs the efflux of fluid from the interstitial compartment into the lymphatic lumen. Intramyocardial fluid homeostasis is, therefore, maintained through the equilibrium that is established between fluid filtration into the myocardial interstitium and fluid flow into the lymphatic vessels. The pool of interstitial fluid exists in steady-state balance, in which disturbance of either fluid filtration or lymph flow may result in myocardial edema [7, 8]. Compensatory mechanisms help to maintain physiological conditions; of these, the most protective is the capacity to increase the rate of lymph flow (Q_{VL}) during excessive plasma filtration [4, 7]. Such augmentation is driven by increased flow of interstitial fluid into the lymphatic vessel as a consequence of increased interstitial pressure and an inverse decrease in lymphatic resistance [4, 7]. To promote lymph flow, interstitial protein concentration is also diluted

in "protein washout" during higher rates of filtration [9], thereby reducing oncotic sequestration of fluid volume. Influences such as the phasic contraction of the myocardium and extracardiac thoracic movements further contribute to the dynamic regulation of fluid movement.

Anatomical and physiological study of the cardiac lymphatics in animals supports the importance of intact lymphatic function to the maintenance of tissue health [10]. Interruption of cardiac lymph circulation leads to tissue fluid stasis, inflammation, and fibrosis [10]. Acute lymphatic obstruction in the canine heart leads to epicardial lymphedema and lymphangiectasia [11]. With chronic impairment, early myocardial edema and subendocardial hemorrhage progress to endomyocardial fibrosis [10].

The Lymphatics in Cardiovascular Disease

Although recent study of the cardiac lymphatic vasculature has not been ample, the existing investigational literature suggests an important role for these vessels in the pathogenesis of atherosclerosis, myocardial infarction, congestive heart failure, and cardiac transplantation.

Atherosclerosis

Inflammation, infection, and fibrosis are the predictable consequences of lymphatic disruption in various settings of disease [12]. The presence of these events within the vascular wall may be particularly important; therefore, by inference, the loss of normal lymphatic function within the vascular wall may have a synergistic or augmenting effect upon the classically defined risk factors for atherosclerosis. In a study of human pathological specimens, it was observed that lymphatic vessels grow in areas that are rich in extracellular matrix, while regions rich in inflammatory cells are more prone to angiogenesis [13]. Furthermore, progressive atherosclerotic lesions that are rich in calcium and cholesterol crystal content demonstrate increased lymphangiogenesis in the vascular media.

Similar atherogenic mechanisms have been clinically documented following irradiation of mediastinal cardiac lymph nodes and during mediastinal lymphadenitis (which leads to severe coronary atherosclerosis in Kawasaki disease). The influence of the lymphatic system on atheroma formation may help to explain the discontinuous nature of atherosclerotic plaque formation along the axial length of the susceptible artery, as well as, potentially, the sparing of intramyocardial arteries in the face of systemically expressed risk factors.

The health of coronary arteries requires the nutritive support and metabolic equilibrium of a healthy, unimpeded circulation of the body fluids (both blood and lymph). This becomes particularly important in the context of the intramural entry

and survival of apolipoprotein B-containing particles and immune cells into the arterial wall, thereby leading to the generation of pro-inflammatory and pro-atherogenic mediators. Inasmuch as the cholesterol content of atherosclerotic plaque arrives within the arterial wall through plasma filtration and is removed in lymph [14], a role for the lymphatic system in lipoprotein-mediated atherogenesis can be hypothesized. In this view, the lymphatic supply of the vascular wall itself mediates atherosclerosis through its influence upon the degree to which the arterial intima is exposed to atherogenic lipoprotein. Inadequate lymphatic flow increases the transit time of circulating lipoproteins across the arterial wall, thereby prolonging susceptibility to oxidative damage and promoting entrapment within the arterial wall. Accordingly, the lipoprotein concentration within the lymph, and presumably within the tissue of the arterial wall, is inversely related to the rate of lymph flow [15].

The cellular expression of vascular endothelial growth factors VEGF-C and VEGF-D has been reported in human monocytes and macrophages [16]. These growth factors and their cognate receptor, VEGFR-3/Flt-4, are pro-lymphangiogenic regulators expressed during various stages of development and in post-embryonic life [17]. Given the role of VEGF-C in lymphangiogenesis during wound repair, its use has been invoked therapeutically for lymphedema [12, 18]. VEGF-D signaling, interestingly, induces apoptosis of human macrophages in vivo and mononuclear cells within advanced atherosclerotic plaques [16]. The mechanistic role of this apoptosis in atherogenesis is not been completely understood. Death of lipid-laden macrophages may reduce the progression to foam cell formation and the inflammatory index of atherosclerotic lesions, while the uninterrupted phagocytosis of apoptotic debris may perpetuate inflammation and disrupt plaque stability [19].

Inflammation is a key component in the initial development of atherosclerotic lesions, but it also perpetuates disease through the promotion of plaque instability and vulnerability [20]. Both angiogenic and lymphangiogenic events are found within the inflammatory foci of plaque [21]. VEGF-C cross-activates receptors responsible for both blood and lymphatic vessel development, whereas the biological activity of VEGF-D seems to be limited to lymphangiogenesis. Despite detection of both VEGF-C and VEGF-D expression in the intima of human coronary arteries, the observable neovascularization appears to be mediated primarily through VEGF-C and through angiogenesis [21]. Differential regulation of nascent vessel formation within the atherosclerotic intima may in fact disrupt arterial-to-lymphatic vessel balance, thus creating a disequilibrium in the forces that govern fluid homeostasis. The resulting intimal edema and lymph stagnation would promote atherogenesis, as previously mentioned.

During infection, the acute-phase response provokes and potentiates the local manifestations of inflammation. These processes affect lipoprotein activity and composition; in particular, several protective proteins of HDL are functionally inactivated or displaced, rendering the immediate intimal milieu vulnerable to further oxidation and inflammation [22]. Vascular permeability is increased by the vasoactive cytokines released by activated neutrophils [23]. This promotes plasma filtration into the interstitium, thereby enhancing delivery of pro-atherogenic lipids and

plasma proteins. Therefore, it is proposed that the coronary arteries become exquisitely sensitive to pro-atherogenic phenomena during the acute-phase response; paradoxically, this occurs when the lymphatics are least able to accommodate the pathological changes associated with lymph stasis [24].

In order to generate bulk lymphatic flow, the activity of the lymphatic system is predominantly modulated by the gross movements and positional changes of the thoracic cavity. Accordingly, the decreased thoracic movement and intrathoracic pressure observed in hypopnea reduces the flow of lymph, whereas aerobic exercise can increase lymph flow rates by nearly 300 % [15]. The epicardial lymphatics are especially dependent on extracardiac motion since lymph flow is impeded by the propulsive contractions of the heart, reducing their effective clearance capacity. Accordingly, the epicardial arteries are subjected to additional risk for lymph stasis and, thereby, to impaired maintenance of healthy vascular biology. Atherosclerosis is, indeed, limited nearly exclusively to the epicardial arteries [25], perhaps reflecting, at least in part, the lymphatic contribution. Common causes of sustained hypopnea, such as sedimentary lifestyle [26], decreased vital capacity, and truncal obesity [15], can thus confer independent risk for atherosclerosis explained by reduced lymphatic function. Age, hormonal status, and heredity are also implicated in the potential relationship between relative lymphatic vascular insufficiency and atherogenesis.

Myocardial Infarction

Chronic ischemia and myocardial infarction are the direct functional consequence of established and progressive atherosclerosis. When directly examined, there is a clear focal increase in the density of lymphatic vessels that is demonstrable in both acute and chronic ischemia [13]. However, this increase in lymphatic density is limited to specific pathological zones, such as necrotic edges, scars, and reactive pericarditis. Furthermore, ischemia is accompanied by neovascularization, since both blood and lymphatic vasculature demonstrate dilatation, branching, and sprouting.

Several lines of evidence support the pathophysiologic role of altered myocardial lymph flow, studied largely in canine models of myocardial infarction (MI). Experimental obstruction of cardiac lymph drainage, without compensating cessation of interstitial fluid filtration, invariably produces myocardial edema within hours [27]. Immediately following an acute coronary artery occlusion, there is a decrease of fluid efflux into the interstitium, yet lymph flow increases dramatically within the first 30 min. This phenomenon likely reflects the impact of many factors, including partial recovery of myocardial function and collateralization of myocardial perfusion. Venoconstriction occurs in response to sympathetic activation, further augmenting intracapillary pressures and, as a consequence, plasma filtration. Additionally, ischemic injury to the capillary endothelium increases permeability to plasma, augmenting both plasma filtration rates and ultrafiltrate concentrations.

The interstitial content of protein and blood products progressively rises, while the pH of the myocardial lymph falls in proportion to increasing lactate concentrations. Within the first hours of ischemia, enzyme concentrations, including lactate dehydrogenase, serum glutamic oxaloacetic transaminase, and creatine kinase, are preferentially elevated in cardiac lymph when compared with venous serum. Concomitant increases in lymph flow elevate the fraction of extracellular fluid volume occupied by lymph, ensuring that these enzymatic changes are pronounced. In MI, release of creatine kinase from the heart correlates with the degree of myocardial necrosis but may be affected by variable transport and inactivation by lymph, thus complicating the use of these biomarkers for severity and prognosis.

In aggregate, lymph flow augmentation of >50 % is observed during experimentally induced MI. Such increases, however, cannot forestall the development of persistent edema in the interstitial and vascular spaces. When edema formation occurs within the interstitium of the freshly infarcted heart, structural and functional remodeling of the myocardium occurs, particularly in the ventricular endomyocardium where the metabolic demands are highest [5]. In canine models of myocardial interstitial edema, diminution of cardiac output of up to 40 % is observed for any given level of preload, demonstrating the profound functional consequence of extravascular fluid accumulation in the myocardium [8].

Within hours of lymphatic obstruction, acute structural alterations will include myofibril degeneration, subendocardial edema, and hemorrhage [11]. Fluid accumulation itself represents a restrictive loss of compliance and cardiac function [28]. Expansion of the interstitial fluid compartment increases the diffusion distance for oxygen and exacerbates the hypoxic state, increasing the rate and magnitude of infarct development [29]. The severity of the congestion induced by experimental ligation of the major cardiac lymphatic trunks in dogs is such that coronary capillaries are compressed [11], which exacerbates the generalized hypoxia of lymph stasis. In the chronic state, this directly provokes coronary arteriopathy, with subendothelial edema and degeneration of smooth muscle with fibrinoid necrosis [30]. In murine models of ischemic injury with subsequent obstruction of lymphatic flow, myocardial and cardiac vascular fibrosis is not uncommon, compromising cardiac output and compounding the ischemic damage caused by the antecedent anoxia [31]. These experimental findings were recently corroborated by histopathological study of human autopsy specimens [32]. Although the precise mechanism through which chronic myocardial edema promotes fibrosis remains poorly understood, it is conceivable that the pathophysiology mirrors the architectural changes observed in chronic, peripheral lymphatic vascular insufficiency [33] for which tissue inflammation is a hallmark [12].

Primary collagen accumulation is a plausible mechanism for the development of myocardial fibrosis [8]. This hypothesis is supported by recent evidence demonstrating the synthesis and deposition of collagen I and III within interstitial tissues following disruption of cardiac lymph flow in rabbits [25, 34]. Within 2 days of the onset of lymph stasis, lymphatic vessels become dilated and acute inflammatory cells infiltrate the perivascular tissues and release pro-inflammatory cytokines that ultimately cause fibrosis [30]. Arterial and lymphatic metabolism shifts towards

anaerobic glycolysis. These changes are most prominent in the most vulnerable vessels, including those with small luminal diameter or low reciprocity. Myocardial edema is, therefore, further exacerbated as the transport capacity of the lymphatics is overwhelmed. The accumulation of toxic by-products leads to lymphatic endothelial dysfunction and destruction and, ultimately, to complete decompensation of the lymphatic system.

Reperfusion of ischemic myocardial tissue with hyperosmolar fluid ameliorates edema with a resultant reduction in infarct size [5, 28]. Similarly, treatment of myocardial infarction with hyaluronidase, a well-recognized historical lymphagogue, produced salutary results [35–37] in animal models and in early clinical trials. Hyaluronidase infusion during experimental ischemia/reperfusion injury significantly increases cardiac lymph flow, alleviating myocardial edema and accelerating functional recovery following reperfusion; this result is independent of any appreciable increase in coronary collateralization or blood flow [37]. Furthermore, several randomized controlled trials have demonstrated mortality benefit from hyaluronidase-based pharmacotherapy of myocardial infarction [38]. Nevertheless, the benefit was modest, at best, and required treatment within 6 h of chest pain onset, limiting widespread clinical applicability [38–41].

Reperfusion alone can restore lymphatic drainage capacity to physiologic levels [5], emphasizing the clinical imperative to restore coronary patency. In the interim, adjunctive therapy to revascularization may hasten edema resolution, particularly in situations of irreparable tissue necrosis and functional deficit. Acute MI represents a dynamic complex of multiple processes and a variety of potential therapeutic targets. Augmentation of lymphatic clearance by hyaluronidase represents one out of several evidence-based interventions that improve clinical outcome. Hyaluronidase depolymerizes specific acid mucopolysaccharides and reduces inflammatory exudates within the interstitium, thereby reducing resistance and improving both interstitial fluid and coronary blood flow [42]. During the evolution of MI, hyaluronidase facilitates recovery of homeostatic blood and lymph exchange [42] to attenuate hypoxia and ATP depletion, to limit reduced myocardial and cardiac lymph flow, and to minimize toxic metabolite accumulation. Hyaluronidase thus reduces the vulnerability of the myocardium to ischemic injury by increasing cardiac lymph flow [28]. Furthermore, the increased fluid flux through the interstitial space dilutes and clears the toxic metabolites that mediate reperfusion injury. This augmented fluid filtration during reperfusion is well tolerated and does not promote further edema formation, in view of corresponding increases in downstream lymph exchange [4]. As previously discussed, while the capacity of this compensatory mechanism is lost during ischemic insult, it can be restored following reperfusion.

Immunohistochemical analysis of the known markers of lymphatic vasculature suggests that there is increased lymphangiogenesis in ischemic hearts, both acutely and chronically [43]. The lymphatic neovasculature is most prominent in the epicardium. Of considerable interest as well is the evidence that suggests increased lymphangiogenesis in atherosclerotic lesions [13].

Heart Failure

Perturbation of myocardial fluid homeostasis will produce several well-documented consequences in both systolic and diastolic function [4, 8]. Preload-recruitable stroke work is directly correlated to the extent of myocardial edema in numerous experimental settings [4]. Decreases in inherent myocardial contractility translate into decreased cardiac output, establishing myocardial edema as an independent cause of functional cardiac impairment in systole. Lymph flow rates are reciprocally dependent upon the cardiac contractile capacity. In addition, administration of a positive ionotrope enhances myocardial lymphatic function in canine models [44].

Reduction of diastolic function is thought to be a consequence of decreased ventricular compliance. Interstitial fluid accumulation, for example, can reduce the potential for myocardial expansion and therefore decrease passive ventricular filling. The edematous myocardium is further stressed by increased metabolic demands. With each systole, the edematous heart must accommodate not only decreased lymph flow but also the added viscosity of excess interstitial fluid. The anatomical and histological architecture of the heart may also become deformed, further affecting myocardial efficiency [8]. The diffusion distance also increases with edema accumulation, as myocytes are displaced farther from the capillary delivery of oxygen. Hypoxic injury is typified by anaerobic evolution of toxic metabolites, decreased cardiac contractility, and increased microvascular permeability to proteins, thereby increasing interstitial colloid pressure and fluid accumulation [11]. Chronic edema induces fibrotic changes within the interstitium of the heart [8], as does edema secondary to insults such as hypoxic injury. There is interstitial collagen deposition [31] accompanied by decreased compliance and diastolic dysfunction, as previously discussed. Disruption of cardiac lymphatics in rabbits leads to significant decreases in left ventricular ejection fraction within the first 3 months following the lymphatic obstruction. This functional loss is accompanied by sustained elevations in levels of circulating plasma endothelin-1 and angiotensin II [45].

Development of pulmonary hypertension is an inevitable consequence of both acute and chronic left ventricular dysfunction and can be a prominent sequela of heart failure [8]. With increased resistance in the right ventricular outflow tract, the central venous pressure rises, reducing myocardial lymph transit into the central venous system. Increased lymphatic pressure is ultimately conveyed to the myocardial lymphatics [8]. Coronary sinus pressure is also affected [8], increasing coronary microvascular pressure, interstitial fluid filtration into the interstitium, and myocardial edema. Secondary right heart failure exacerbates the perturbations [8]. Conversely, increased pulmonary blood flow, as occurs in some forms of congenital heart defects, leads to functional and structural aberrations in lung lymphatics [46].

The impact of these various mechanisms is dependent upon the existing demands on the cardiac lymphatic vasculature [8]. Experimental elevation of coronary sinus pressure in chronic disease models produces measurable increases in myocardial water content [47] significantly earlier than a comparable intervention in healthy

animal subjects [8]. Thus, the burden of additional edematous forces is more apparent when auto-regulatory mechanisms are already taxed. Coronary vascular resistance is elevated in a direct linear relationship with myocardial edema [48] and can be conceptualized as a compensatory mechanism. Therefore, the contribution of the cardiac lymphatics to the propagation of chronic myocardial edema must not be overlooked. Loss of compensatory mechanisms is likely to play an important role in the evolution of congestive heart failure, independent of the primary pathogenesis. More recent work has shown that the heart responds by increasing myocardial lymphangiogenesis from the existing vascular tree, as opposed to de novo growth from circulating progenitors [49]. Moreover, the patterns of microvascular remodeling occurring during dilated cardiomyopathy differ from those of ischemic cardiomyopathy [50].

These phenomena have been studied in human tissues derived from patients with terminal heart failure due to ischemic (ICM) and dilated (DCM) cardiomyopathy [50]. When compared to control donor heart tissues, DCM hearts demonstrate a significantly higher density of LYVE-1 positive lymphatics ($p<0.05$), whereas no difference was seen for other markers. ICM hearts display a significantly higher density of D2-40 positive lymphatics ($p<0.01$) and a lower density of VEGFR-2 capillaries compared to control ($p<0.05$). Further research may help to elucidate the impact of extracellular matrix composition and VEGF-related angiogenesis on the myocardial microvasculature at various stages of heart failure.

Cardiac Transplantation

As a therapeutic intervention, cardiac transplantation poses multiple challenges to the maintenance of lymphatic function within the heart. Surgical disruption of the cardiac lymphatic vasculature during transplantation likely contributes to allograft failure through various mechanisms already discussed in this chapter, including vasculopathy and myocardial edema [51]. As is the case for the evolution of myocardial infarction, hypoxia reduces cardiac output and causes myocardial edema. Commensurate with the attempts of the autonomic nervous system to conserve perfusion capacity, there is a concurrent increase in the central venous resistance. Through similar mechanisms, experimentally induced increases in coronary sinus pressure also promote formation of myocardial edema. Cardiopulmonary bypass and cardioplegic arrest further promote myocardial edema by decreasing plasma colloid osmotic pressure and increasing plasma filtration while lymph flow diminishes [4]. The manipulations during organ procurement and transportation contribute only slightly to the overall degree of edema observed. Significant intracardiac interstitial fluid accumulation is seen only after reperfusion, reflecting the suppression of Starling equilibrium variables during cardioplegic arrest [52]. Echocardiographic studies suggest that the additional interstitial fluid distends the left ventricular wall, with spontaneous resolution over 3 months [53]. Persistence (or re-accumulation) of myocardial edema fluid precedes the cellular responses of

acute rejection [54]; considered in this light, edema detection could be considered as a prognostic surrogate for post-transplant patients. Impaired lymphatic flow across the myocardium further predisposes the pharmacologically immunosuppressed system to infection, whereby both host and graft vessels become damaged by the pathological responses. In particular, cardiac allograft vasculopathy may be a long-term consequence of lymphatic stasis [55]. Moreover, in the absence of transplantation, intramural coronary arteries are remarkably spared from atherosclerosis; it is only in the context of cardiac transplantation that significant intramural coronary atherosclerosis is encountered. Disruption of the transmural plexus of lymphatics surrounding the intramural coronary arteries may explain this phenomenon [4].

The utility of hyaluronidase to limit myocardial edema has been demonstrated in an experimental model of acute rejection following heart transplantation [56]. The underlying mechanisms are not specific to transplantation, but likely apply to the more general phenomenon of myocardial edema. Analogous to observations in MI [28], administration of hyaluronidase during cardioplegic arrest promotes active drainage of cardiac lymph and reduces interstitial edema. With decreased myocardial water content, endpoint surrogates of aerobic metabolism and post-ischemic recovery of cardiac function improve [37].

Recent histological evidence corroborates the physiologic studies of the lymphatic vasculature in cardiac transplantation. This work is aided by the discovery and use of specific immunohistochemical markers of lymphatic endothelial cells [3]. Two of the best recognized markers, LYVE-1 and Prox1, are down-regulated following heart transplantation [57], while expression of VEGFR-3, the cognate receptor for the pro-lymphangiogenic factors VEGF-C and VEGF-D, remains unaltered. LYVE-1 is a transmembrane glycoprotein receptor for the extracellular matrix glycosaminoglycan, hyaluronan, among other molecules including osteopontin, collagens, and matrix metalloproteinases. Functionally, these molecules play a role in a variety of cellular processes, including lymphocyte migration and activation, hematopoiesis, and tumor metastasis [58, 59]. Although LYVE-1 is closely associated with lymphatic endothelium early in development and throughout maturity, the precise function of the receptor remains unknown (beyond its putative role in hyaluronan homeostasis) [60]. The primary receptor for hyaluronic acid, CD44, is known to facilitate cell migration by removing pericellular matrix surrounding fibroblast and epithelial cells, suppressing intercellular adhesion during wound healing, inflammation, and tumor progression [61]. Thus, LYVE-1 may play a functional role in both physiological and pathological lymphangiogenesis through its ability to transport hyaluronic acid across the lymphatic vessel wall. Nearly exclusive localization to the lymphatic endothelium throughout the vasculature, together with convenient assay techniques, renders LYVE-1 aptly as useful a molecular and histochemical marker of lymphatics, helping to distinguish them from blood vasculature. In addition, prospero-related homeobox 1 (Prox1), a nuclear transcription factor, is exclusively expressed on cells of committed lymphatic lineage during development [62]. This is in contrast to LYVE-1 and VEGFR3, which are also expressed on a limited population of non-lymphatic endothelial cells [63]. Although Prox1 is necessary and sufficient for lymphatic

commitment [63], the molecular milieu in which Prox1 operates is not known; both downstream initiating and regulatory factors and other upstream requisites or supplemental events are still under investigation [64, 65]. In the context of cardiac transplantation, the postsurgical decrease in the density of LYVE-1 and Prox1, with preserved levels of VEGFR-3, suggests that the phenotype of the lymphatics within the graft is altered from wild type [57]. Alternatively, it is conceivable that a reduction in the population of lymphatic endothelial cells induces a compensatory up-regulation of the VEGFR-3 expression and, thus, the lymphangiogenic signal. It is perhaps of greater significance that VEGFR-3-positive cell density inversely correlates with the observed incidence of graft incidence [57]; observations within an experimental animal model suggest that the resumption of adequate immune modulation leads to rapid restoration of inner lymphatic vessels [66]. Further investigation of the various converging biological processes (myocardial fluid regulation, lymphocyte trafficking, and inflammation) warrants further investigation. Such studies are likely to lead to enhanced mechanistic insights and therapeutic approaches.

Future Perspectives

Molecular and ultrastructural study of the lymphatic vasculature is still in its infancy. From the foregoing discussion, it should be evident that, in future, individuals with a variety of cardiovascular pathologies may benefit directly from the enhanced insights to be gained from research into the lymphatic mechanisms that contribute to the genesis and propagation of these and other systemic diseases [67]. Progress will entail enhanced imaging modalities for the dynamic function of lymphatic vasculature within the cardiovascular structures, perhaps aided by the application of molecular imaging using a nanotechnology approach. Exploration of the direct role of lymphatic mechanisms of lipoprotein homeostasis within the arterial wall is quite desirable and may lead to new therapeutic applications. The recent identification of lymphatic mechanisms that contribute to chronic transplant rejection in other organ systems [68–70] may have direct applicability to the treatment and prevention of cardiac allograft rejection.

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