Chapter 9
VEGF Receptor Signaling in the Cardiac Lymphatics

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Abstract Since the discovery of angiogenic vascular endothelial growth factor (VEGF)-A in 1983 and lymphangiogenic VEGF-C in 1997, an increasing amount of knowledge has accumulated on the essential roles of VEGF ligands and receptors in physiological and pathological angiogenesis and lymphangiogenesis. We will review the properties of VEGF ligands and receptors concentrating on their lymphatic vessel effects first in noncardiac tissues and then in normal myocardium and cardiac disease. Tissue adaptation to several stimuli such as hypoxia, pathogen invasion, and inflammation often involves coordinated changes in both blood vessels and lymphatic vessels. As lymphatic vessels are involved in the initiation and resolution of inflammation and regulation of tissue edema, VEGF family members may have important roles in myocardial lymphatics and cardiac disease.

Keywords VEGF • Angiogenesis • Lymphangiogenesis • Cardiac lymphatics • Inflammation

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General Biology of the VEGF Family

VEGF-A (or VEGF) was first discovered as a tumor-secreted factor that induced vascular permeability and was therefore initially named as vascular permeability factor [1]. Subsequently, the VEGF family has expanded to a total of five mammalian ligands: placental growth factor (PlGF), VEGF-A, VEGF-B, VEGF-C, and VEGF-D [2–5].

The receptors for the VEGF family ligands include VEGFR-1, VEGFR-2, VEGFR-3, and neuropilins (NRP-1 and NRP-2). Upon binding of VEGF ligand dimers to their receptors, the corresponding receptors dimerize, phosphorylate tyrosine residues in the cytosol, and activate intracellular signaling pathways. The binding properties of VEGF ligands and the principal effects of VEGF receptors are summarized in Fig. 9.1. Heterodimerization of VEGF ligands and receptors, and splice variants and heparin-binding differences of ligands result in additional complexity and flexibility in VEGF ligand–receptor interactions [5]. Generally, the preferential expression of VEGFR-1 and VEGFR-2 in blood vascular endothelial cells (EC) and VEGFR-3 in lymphatic EC, and the binding properties of VEGF ligands to their corresponding receptors, underlies their angiogenic or lymphangiogenic predilection (Fig. 9.1). We first overview properties of the angiogenic VEGF ligands and receptors, and we then concentrate on the lymphangiogenic VEGF family members.

Angiogenic VEGF Members (VEGF-A, VEGF-B, PlGF, VEGFR-1, VEGFR-2, NRP)

VEGF-A binds to VEGFR-1, VEGFR-2, NRP-1, and NRP-2 and is the major regulator of the angiogenic switch. VEGF is produced by a variety of adult tissues, and vascular and inflammatory cells, and is induced by hypoxia, inflammation, and several growth factors [6–11]. VEGF-A elicits its effects mainly through VEGFR-2+ vascular endothelial cells (EC) and VEGFR-1+ monocytes and macrophages. VEGF induces EC migration, proliferation, and sprouting and results in angiogenesis. VEGF is essential for embryonic vascular development [12, 13]. The importance of VEGF in angiogenesis in adult is highlighted by the clinical success of anti-VEGF approaches in the treatment of cancer and eye disease [3]. However, the results of randomized clinical trials on VEGF-A-mediated therapeutic angiogenesis have been disappointing [14]. This may at least in part relate to the findings that the VEGF-A-induced new blood vessels are often leaky. VEGF-A is indeed intimately related to inflammation through increased vascular permeability and also through direct effects on VEGFR-1+ monocytes and macrophages [5].

PlGF was first detected in human placenta [15] and it binds to VEGFR-1 and NRP-1 [16]. PlGF is not essential for normal vascular development [3, 17], but it may enhance VEGF effects particularly in pathological conditions [18, 19], and it promotes cardiac hypertrophy [20]. PlGF has a clear inflammatory role as it activates both hematopoietic stem cells [1, 21] and monocytes and macrophages through VEGFR-1 [2, 22–24].

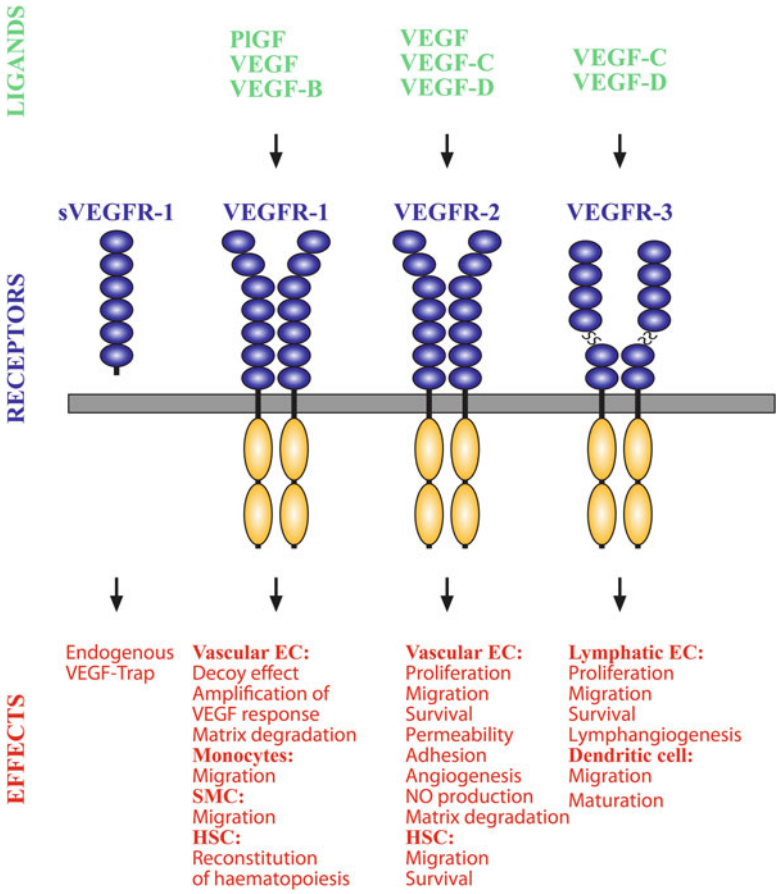

Fig. 9.1 VEGF ligands and receptors. VEGF ligands have different binding properties to VEGF receptors. VEGFR-1 modulates VEGF responses on endothelial cells and it is a chemotactic signal for monocytes and smooth muscle cells. VEGFR-1 also has a functional role in hematopoiesis, and the soluble sVEGFR-1 inhibits VEGF effects. VEGFR-2 elicits the main mitogenic and proinflammatory effects on vascular endothelial cells but has a functional role also in hematopoietic and vascular progenitor cells. VEGFR-3 signaling principally regulates the development and functionality of lymphatic endothelial cells and migration of antigen-presenting cells. *EC* endothelial cell; *HSC* hematopoietic stem cell; *NO* nitric oxide; *PlGF* placental growth factor; *SMC* smooth muscle cell; *VEGF* vascular endothelial growth factor; *VEGFR* VEGF receptor; *sVEGFR* soluble VEGFR (Modified from Nykänen A. Vascular growth factors and progenitor cells in cardiac allograft arteriosclerosis [dissertation]. Helsinki: University of Helsinki; 2007)

VEGF-B is expressed in skeletal muscle and heart, and also binds to VEGFR-1 and NRP-1 [3, 16, 25, 26]. Lack of VEGF-B does not impair vascular development, but it may yield to conducting defects and size reduction in the heart, and impaired recovery after myocardial ischemia [4, 27, 28]. VEGF-B is also involved in inflammatory angiogenesis [1, 29] and in cardiac arteriogenesis [5, 30] and hypertrophy [5, 31]. VEGF-B also has metabolic effects as it regulates endothelial lipid transfer and metabolism [6–11, 31, 32] as well as the development of diabetes [12, 13, 33].

VEGFR-1 (also known as fms-like tyrosine kinase, Flt-1) is a receptor for VEGF-A, VEGF-B, and PlGF [3, 16]. In addition to the membrane-anchored VEGFR-1, a soluble form of VEGFR-1 (sVEGFR-1) also exists [14, 34, 35]. VEGFR-1 is expressed in a variety of cells including EC, smooth muscle cells, monocytes and macrophages, and hematopoietic stem cells [3, 15–17, 36] and is upregulated by hypoxia-inducible factor-1 [18, 19, 37]. VEGFR-1 has high affinity for VEGF-A but low tyrosine kinase activity, and it has been viewed as a decoy receptor and a negative regulator of VEGFR-2 in ECs [20, 38]. Accordingly, VEGFR-1 deletion results in excessive endothelial progenitor cell proliferation, vascular disorganization, and embryonic lethality [39, 40]. Although the angiogenic effects of VEGFR-1 are subtle, VEGFR-1 may regulate arteriogenesis, pathological angiogenesis, myelomonocyte cell recruitment, and lipid metabolism [31–33, 41, 42].

VEGFR-2 (also known as kinase insert domain receptor, KDR/fetal liver kinase, Flk-1) binds VEGF-A, VEGF-C, and VEGF-D. VEGFR-2 is mainly expressed on vascular EC and is considered responsible for the majority of VEGF-A-induced angiogenic and permeability effects [3, 5]. VEGFR-2 expression is essential for embryonal hematopoiesis and vasculogenesis [43, 44]. In adults, VEGFR-2 expression is usually downregulated and presents only at sites of active angiogenesis such as wound healing, tumors, and after myocardial infarction [45, 46]. In myocardial infarction and sepsis, VEGFR-2 is a major regulator of vascular permeability and cardiac dysfunction [47, 48].

Neuropilin receptors NRP1 and NRP2 are involved in neuronal development and bind semaphorins [16]. Neuropilins also interact with VEGF signaling as NRP1 is a co-receptor for VEGFR-1 and VEGFR-2, and NRP2 for VEGFR-3 [16]. In the vascular system, NRP1 is expressed predominantly in arteries and potentiates the binding and activity of VEGF-A on VEGFR-2 [49]. Interaction of VEGFR-3 and its co-receptor NRP2 modulates the function of veins and lymphatic vessels [50, 51]. NRP1 also participates in lymphatic vessel and valve development through semaphorin interaction [52].

Although the VEGF ligands and receptors described above are considered mainly angiogenic, some overlap on lymphangiogenic effects have been described [53]. The reason for this overlap may be in part the fact that VEGF-A recruits macrophages that may in turn drive lymphangiogenesis [54].

Lymphangiogenic VEGF Members (VEGF-C and VEGF-D, VEGFR-3, NRP)

VEGF-C binds to VEGFR-2 and VEGFR-3 and is a major regulator in the development of lymphatic vasculature [2, 55–57]. VEGF-C is produced as a precursor protein that undergoes proteolytic modification [58]. It is produced in areas of active lymphangiogenesis in the embryo, and the expression is maintained in lung, heart, liver, and kidney in the adult [59]. VEGF-C is not activated by hypoxia, but is increased by serum and its components, platelet-derived growth factor, epidermal

growth factor, and transforming growth factor-β [60]. VEGF-C mRNA is also upregulated by proinflammatory cytokines TNF-α, IL-1α, and IL-β [61]. Inflammatory cells such as macrophages, dendritic cells, and CD4+ T lymphocytes are a rich source of VEGF-C [54, 62–65].

Loss of one VEGF-C allele causes prominent lymphatic hypoplasia, whereas loss of both VEGF-C alleles results in embryonic lethality [66]. Similarly, inhibition of VEGF-C/D/R3 signaling in the adult regresses existing lymphatic vessels and results in lymphedema [67]. In contrast, VEGF-C overexpression results in lymphatic hyperplasia [2, 57] and in therapeutic lymphangiogenesis in lymphedema [68]. VEGF-C is upregulated in many cancers and participates in lymphatic metastasis of tumors [69, 70]. Interestingly, salt-induced hypertension is counteracted by macrophage VEGF-C upregulation and tissue lymphangiogenesis [71]. Another noteworthy finding suggests that, in addition to clear lymphatic effects, VEGF-C may also have angiogenic effects through VEGFR-2 binding, by regulating a subtype of vascular EC that express VEGFR-3 [72–74], and also by attracting VEGF-A-producing macrophages [75].

VEGF-C is intimately involved in many inflammatory conditions that involve lymphangiogenesis such as bacterial infection [62], rheumatoid arthritis [76], skin inflammation [77], and organ transplantation [64, 78, 79]. In addition to inducing lymphangiogenesis, VEGF-C modifies lymphatic vessel properties by, for example, upregulating CCL21—a chemokine that attracts CCR7+ tumor cells and dendritic cells [64, 80]. VEGF-C has also direct effects on VEGFR-3+ dendritic cells and induces their migration [81] and maturation [78]. VEGF-C thus modifies immune reactions through direct effects on lymphatic EC and also on macrophages and dendritic cells [64, 78, 81, 82]. In addition to participating in antigen-presenting cell traffic and the initiation of immune responses, VEGF-C-induced lymphangiogenesis may also balance tissue inflammation by promoting lymphatic drainage and the resolution of tissue inflammation [83–85].

Human VEGF-D binds to VEGFR-2 and VEGFR-3, whereas mouse VEGF-D binds only to VEGFR-3 [86]. Like VEGF-C, VEGF-D also undergoes proteolytical modification, is involved in lymphangiogenesis, and has angiogenic properties. In contrast to VEGF-C, VEGF-D is not essential for the development of lymphatic vessels [87]. Adenovirally delivered VEGF-D induces a potent angiogenic and lymphangiogenic response in skeletal muscle and is associated with elevated vascular permeability [88], but the relative effects on angiogenesis and lymphangiogenesis may depend on the tissue used [89]. VEGF-D is involved in the metastatic spread of cancer [90].

VEGFR-3 binds VEGF-C and VEGF-D, and it is a key regulator for lymphatic growth [2, 66]. VEGFR-3 signaling regulates cardiovascular development in embryos [91], but later in development and in adulthood, it more selectively regulates the growth and maintenance of lymphatic vessels [67]. VEGFR-3 may also have angiogenic effects in adults, since VEGFR-3 is expressed in stalk cells [72, 74], and VEGFR-3+ macrophages produce VEGF-A as well [75]. VEGFR-3 is defective in primary lymphedema [92, 93] and is induced in vascular malformations [94]. VEGFR-3 is also upregulated during lymphangiogenesis in cancer, wound

healing, and inflammation [95]. Inflammation upregulates lymphatic EC VEGFR-3 expression trough the activation of inflammatory transcription factor NF-κB. This renders the VEGFR-3+ lymphatic ECs responsive to VEGF-C and VEGF-D [96]. In addition to lymphatic EC, VEGFR-3 is also expressed in macrophages and dendritic cells and regulates their migration [63, 81, 97].

Neuropilins are co-receptors for VEGFR-2 and VEGFR-3. NRP2 binds VEGF-C and VEGF-D, and is a co-receptor for VEGFR-3. In addition to its effects on veins, NRP2 also regulates lymphatic vessel sprouting [50, 51] and is upregulated in vascular malformations [94]. The other neuropilin NRP1 is also involved in lymphatic vessel maturation and valve formation, but this involves semaphorin and not VEGF-A [52].

Lymphatic-Specific VEGF Expression and Signaling in the Heart

VEGF Expression and Signaling in Cardiac Lymphatics During Embryogenesis and in Healthy Adult

The developing vasculature of the heart requires a variety of signals, including endothelial growth factors. The details of lymphatic development are very well described, mainly in noncardiac tissues of mouse, but the general features seem to be universal.

The first LECs differentiate from venous endothelial cells at midgestation, induced by VEGF-C [66, 98]. The LECs are distinguished by expression of specific molecules such as prospero-related homeodomain transcription factor (Prox1), vascular endothelial growth factor receptor-3 (VEGFR-3), the membrane glycoprotein podoplanin, and lymphatic vessel hyaluronan receptor-1 [66, 98, 99]. In mouse, starting from embryonic day (E) 9.5, a complex sequential activation of the transcription factor SOX18, Prox1, and the venous nuclear receptor COUP-TFII initiates the LEC differentiation program in the anterior cardinal vein [66, 98].

The paracrine secretion of VEGF-C is crucial for the further dorsolateral sprouting, migration, and survival of the first LECs and the formation of lymph sacs [66]. The VEGF-C co-receptor neuropilin-2 (NRP-2) and the Eph tyrosine kinase ligand ephrin B2 are required for efficient sprouting of lymphatic capillaries [51, 100]. The Notch1-Dll4 signaling pathway is essential for postnatal lymphatic development [101]. Interestingly, in adult tissues, lymphatic sprouting induced by VEGF-C is not restricted by Notch, whereas VEGF does not promote efficient lymphatic sprouting unless Notch signaling is inhibited [102].

According to the study of endothelial growth factor distribution in the human fetal heart [103], their localizations at different gestational ages are similar. Lymphatic vessels are only detected in the epicardial layer, and they are negative for VEGFR-1 but strongly positive for both VEGFR-2 and VEGFR-3. Very weak VEGFR-3 signals are also observed in some myocardial capillaries but not in the

endocardium in 13- to 30-week fetuses. However, the VEGFR-3 expression seems to be downregulated in the blood vessels during the first trimester. Thus, although VEGFR-3 is needed for early cardiovascular development [104], it later serves a more specialized biological function mainly in lymphatic endothelia [103].

Only a few studies have described the pattern of VEGF receptor expression in the lymphatics of healthy adult heart [64, 105, 106]. In a study by Geissler et al. [105], human myocardial biopsies have been used for immunohistochemical stainings of VEGFR-3-positive lymphatics: the density of VEGFR-3-positive vessels was calculated to be around 50 per mm^2, and their average diameter was about 3 microns. As described for the healthy adult rat heart, the density of VEGFR-3-positive vessels is generally lower in the myocardium than in the epicardial area. In addition, vessel VEGFR-3 immunoreactivity colocalizes with LYVE-1 expression, although not all LYVE-1-positive vessels express VEGFR-3 [64].

In Heart Transplantation (Allorecognition and Rejection)

In transplantation, the transfer of antigen-presenting cells from vascularized allografts to secondary lymphoid organs—both spleen and lymph nodes—is critical for the priming of alloreactive T cells and the development of alloimmune responses [107, 108]. Lymphatic vessels provide easy access for inflammatory cells and their unilateral movement from peripheral tissues to secondary lymphoid organs. Thus, the lymphatic network forms a link between innate and adaptive immunity, thereby having extraordinary importance in a setting of transplantation.

A descriptive study of human patients undergoing heart transplantation shows that lymphatic endothelial markers undergo significant alterations after the transplantation, suggesting a significant change in lymphatic endothelial phenotype. Furthermore, episodes of acute allograft rejection seem to be associated with a significantly lower density of VEGFR-3-positive lymphatics after heart transplantation [106]. A recent experimental study [64] provides detailed information on lymphatic behavior in rejecting hearts: acute rejection decreases the epicardial lymphatic vessel density and chronic rejection doubles the myocardial lymphatic vessel density. Importantly, lymphangiogenesis in transplanted organs may not be only a secondary effect of chronic inflammation. Instead, lymphatic vessels also appear to have a regulatory role in the initiation of alloimmune reactions. VEGFR-3 inhibition decreases dendritic cell recruitment to the spleen and the development of the subsequent alloimmune response, thus improving cardiac allograft survival. In addition, treatment with neutralizing monoclonal VEGFR-3 antibodies decreases allograft inflammation and the development of arteriosclerosis in a chronic rejection model. According to the study, it appears possible that VEGFR-3 inhibition has direct effects on dendritic cell migration. VEGFR-3 also seems to regulate leukocyte traffic and alloimmunity through direct effects on allograft VEGFR-3-positive lymphatic vessels by upregulating allograft CCL21 production [64]. The range of currently known directions for VEGF-C signaling in a setting of transplantation is represented in Fig. 9.2.

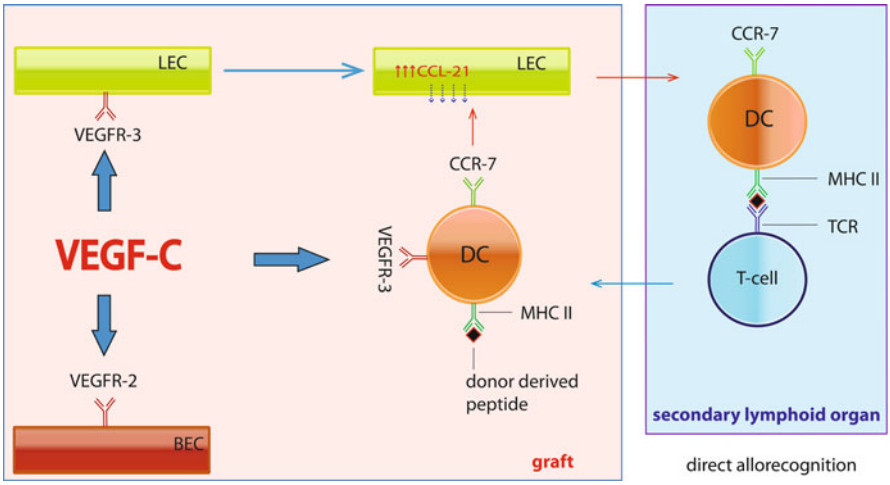

Fig. 9.2 VEGF-C–VEGFR-3 signaling in a setting of transplantation. VEGF-C modifies lymphatic vessel properties by upregulating CCL21—a chemokine that attracts CCR7+ dendritic cells. VEGF-C also has direct effects on VEGFR-3+ dendritic cells and induces their maturation and unilateral migration through the lymphatic network to secondary lymphoid organs. Thus, VEGF-C plays an important role in the initiation of direct alloimmune recognition through direct effects on lymphatic endothelial cells and antigen-presenting cells. VEGF-C may also have angiogenic effects through VEGFR-2 binding; its role in transplantation remains unclear. *BEC* blood endothelial cell; *CCL-21* chemokine ligand 21; *LEC* lymphatic endothelial cell; *CCR-7* C-C chemokine receptor type 7; *DC* dendritic cell; *MHC II* major histocompatibility complex class II; *VEGFR-2* VEGF receptor 2; *VEGFR-3* VEGF receptor 3; *TCR* T cell receptor

Thus, VEGF-C/VEGFR-3 signaling seems to have important effects on proximal events in cardiac allograft alloimmunity and arteriosclerosis. Therefore, VEGFR-3 inhibition could be used as a novel lymphatic vessel-targeted immunomodulatory therapy to regulate alloimmune activation after solid organ transplantation. Further studies that particularly describe the mechanistic role of lymphatic vessel activation in the ischemia-reperfusion injury and allograft rejection are needed.

In Myocardial Infarction

In the human heart, myocardial remodeling after myocardial infarction (MI) results in scar formation through several sequential stages of myocardial necrosis, granulation, and fibrosis [109]. The viable cardiomyocytes around the lesion express cytoprotective proteins and cytokines which facilitate the healing of the affected lesion [110, 111]. VEGF is critical for angiogenesis in the healing area [112]: it is promptly expressed in the surviving cardiomyocytes around the infracted lesion after the onset of MI, and angiogenesis in the lesion begins at 4–5 h and continues up to day 90 [110].

A study by Ishikawa et al. [113] demonstrates that lymphatic vessels are not detected in stages with coagulation necrosis, but a few lymphatics first appear in the peripheral region adjacent to viable myocytes in the early granulation period. Lymphatic density subsequently increases in the mature granulation period and is thereafter maintained during scar formation. After the onset of myocardial infarction, lymphangiogenesis lags behind blood vessel angiogenesis, whereas VEGF-C is expressed in the cardiomyocytes around the lesion at all stages of myocardial remodeling. The results of this study suggest that during the entire process of myocardial infarction healing, blood vessels supply the blood and nutrients mainly during the granulation period, but lymphatics participate mainly in fibrosis maturation and scar formation through the drainage of excessive proteins and fluid, probably mediated by VEGF-C.

In Atherosclerosis and Degenerative Valve Disease

Recently there has been an emerging body of evidence linking lymphangiogenesis to atherosclerosis. Lymphatic vessels are found at sites of atherosclerosis, which is associated with inflammation and lipid accumulation in arterial walls [114, 115]. Arterial smooth muscle cells constitutively produce lymphangiogenic factors, and lymphatic vessels are present in the adventitia of arteries adjacent to small blood vessels, called the vasa vasorum, which are expanded in atherosclerotic plaques [116, 117]. However, according to the study of Nakano et al. [117], impaired lymphangiogenesis may contribute to plaque progression. Here, VEGF-A and VEGF-C might synergistically contribute to angiogenesis in coronary atherosclerotic plaques. So, in atherosclerotic lesions, the imbalance of angiogenesis and lymphangiogenesis in favor of angiogenesis seems to contribute to sustained inflammatory reactions during human coronary atherogenesis.

In atherosclerosis, lymphatic vessels might have an important role in the efflux of interstitial fluids, fats, and inflammatory cells from the wall of the coronary artery, slowing down the development of atherosclerotic lesions. It remains to be investigated whether therapeutic targeting of lymphangiogenesis might reveal an antiatherosclerotic tool.

Aortic valve stenosis (AS) is another degenerative disease of the heart, where the pathogenesis is linked to lymphangiogenic factors. Pathological features of AS are calcification [118], extracellular matrix remodeling, and valvular accumulation of lipids and inflammatory cells [119]. In contrast to normal avascular aortic valves, stenotic aortic valves are vascularized [120]. Importantly, neovessels may contribute to the progression of AS by facilitating the entry of inflammatory cells and lipids into the leaflets [121]. The study of Syväranta et al. [122] demonstrates that lymphangiogenesis is a part of the pathogenesis of AS and shows that myofibroblasts and endothelial cells are responsible for the valvular production of lymphangiogenic growth factors VEGF-C and VEGF-D.

Furthermore, mast-cell-derived compounds degrade VEGF-C, suggesting their anti-lymphangiogenic potential. Similar to atherosclerotic lesions, in AS, the balance between angiogenesis and lymphangiogenesis may disadvantageously favor the accumulation of inflammatory cells and lipids into the lesions, thus possibly leading to disease progression.

In Heart Failure

The two most frequent causes of terminal heart failure are dilated (DCM) and ischemic (ICM) cardiomyopathy. The investigation of microvascular structures in cardiac remodeling has mostly been limited to the sequela of myocardial ischemia and infarction rather than in terminal heart failure. Nevertheless, the role of lymphatic system in the failing myocardium might be of increased importance, since the hemodynamics, fluid, and pressure balance are severely damaged. However, there are barely any publications available about lymphatics in the failing heart, except for several descriptional reports.

The study of Aharinejad et al. [123] provides evidence that VEGF-C mRNA levels are upregulated in both forms of cardiomyopathy and that after cardiac transplantation, these mRNA levels returned to the baseline level of nonfailing cardiac tissues in DCM or decreased even below the baseline level in ICM. A further study [124] describes the distribution of several lymphatic and blood markers, including VEGFRs, in DCM and ICM, comparing them to nonfailing hearts.

Whether any therapeutic manipulation of lymphatic function could improve impaired myocardial function by draining the failing myocardium remains so far an exciting speculation.

In Inflammation

In adulthood, lymphangiogenesis and elevated VEGFR-3 expression coincide with various inflammatory conditions including cancer [125], wound healing [126], and chronic inflammatory diseases. Increased lymphatic vessel density has been documented in chronic airway infection [62], psoriasis [127], and arthritis [76]. VEGF-C and VEGF-D are elevated during inflammation, being produced by a variety of cells residing at inflamed sites, including macrophages [62, 128, 129], dendritic cells and neutrophils [62], mast cells and fibroblasts [130], and tumor cells [129].

Generally, the role of lymphatic activation and lymphangiogenesis in inflammatory settings is considered to be positive. It facilitates the resolution of tissue edema and enhances immune responses by promoting macrophage and dendritic cell mobilization [56, 62]. The lymphatic vascular system and the molecular

Table 9.1 Overview of lymphatic-specific VEGF expression and signaling in the heart during embryogenesis, in healthy adult, and in various disease states of the heart

	VEGF member	Expression and signaling pattern
Embryogenesis	VEGF-C	• Induces the differentiation of first LECs from venous endothelial cells [66, 98] • Crucial for further dorsolateral sprouting, migration and survival of the first LECs, and the formation of lymph sacs [66]
	VEGFR-2	Expressed already on the first LECs [103]
	VEGFR-3	Expressed already on the first LECs, as well as on cardiac blood vessels during the first trimester [103]
Healthy adult	VEGFR-2	Expressed in a small number of lymphatic vessels (no precise data available)
	VEGFR-3	Expressed in a considerable number of lymphatic vessels (no precise data available) [64, 105, 106]
Heart transplantation	VEGF-C	• Modifies lymphatic vessel properties by upregulating CCL21 [64] • Induces maturation and migration of dendritic cells [78]
	VEGFR-3	• Important downstream target for VEGF-C effects [64] • Changes in densities of VEGFR-3+ vessels accompany episodes of allograft rejection [106]
Myocardial infarction	VEGF-C	Mediates lymphatic participation in fibrosis maturation and scar formation through the drainage of excessive proteins and fluids [113]
Atherosclerosis	VEGF-C	Contributes with VEGF-A to angiogenesis in coronary plaque, leading to imbalance of angio- and lymphangiogenesis, thus sustaining inflammatory reaction during atherogenesis [117]
Aortic stenosis	VEGF-C	Is degraded in diseased valve leaflets by mast-cell compounds, which leads to imbalance of angio- and lymphangiogenesis and favors the accumulation of inflammatory cells and lipids (disease progression) [122]
Inflammation		Although lymphatic growth accompanies infective heart diseases, chronic inflammation, infarction, etc., the role of lymphatic-specific VEGF signaling in these processes has not been studied
Heart failure		Only controversial purely descriptive data is available [123, 124]

pathways regulating inflammatory responses are intimately associated. Lymphatic vessels react to tissue inflammation with morphological changes in lymphatic endothelial cell phenotype (such as overexpression of adhesion molecules [131] or VEGFR-3 [96]), induction of proinflammatory cytokines production, as well as chronologically delayed increase of lymphatic vessel density (lymphangiogenesis) [96].

LECs at least in some tissues constitutively express NF-κB [132]. Activation of the NF-κB pathway in LECs upregulates Prox1 and VEGFR-3, which renders the lymphatic vessels more sensitive to VEGF-C and VEGF-D produced by leukocytes [96]. VEGFR-3 signaling is activated upon binding of vascular endothelial growth

factor-C (VEGF-C) or the related factor, VEGF-D [133]. NF-κB is activated, for example, downstream of Toll-like receptor 4 binding to lipopolysaccharide in the LECs, thus inducing the activation and production of leukocyte chemoattractants such as CCL2, CCL5, and CX3CL1, which in turn promotes leukocyte homing to the lymphatic vessels and eventually to the draining lymph node [134].

Inflamed lymphatic endothelium promotes the exit of leukocytes from tissue to afferent lymph through newly induced expression of the adhesion molecules ICAM-1 and VCAM-1, which were previously thought to be specific for blood vessel transmigration [131].

Furthermore, the studies of anatomical distribution for the cardiac lymphatics in diseased heart demonstrate increased lymphatic densities in infective endocarditis, myocarditis, and progressive atherosclerosis. Thus, lymphatic growth accompanies chronic inflammation, tissue degeneration, infarction, calcification, and formation of connective tissue [115]. Although not investigated yet for these disease states, the role of lymphatic-specific VEGF signaling seems to be crucial and warrants further analysis and a search for potential therapeutic targeting. The properties of lymphatic-specific VEGF expression and signaling in the heart are briefly summarized in Table 9.1.

Summary

While the critical role of angiogenic VEGF family members in cardiovascular development and disease is already appreciated, the involvement of lymphangiogenic VEGF ligands and receptors, and cardiac lymphatics, in cardiac physiology and pathology is only starting to unfold. With the rapid development of lymphatic vessel markers, better understanding of basic lymphatic vessel biology, and the use of novel genetic and pharmacological tools to activate or inhibit lymphangiogenic VEGF-C/D/R3 signaling, future studies may reveal novel lymphatic-targeted therapeutic strategies in ischemic, degenerative, and inflammatory heart disease.

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