Editorial

Therapeutic lymphangiogenesis after myocardial infarction: vascular endothelial growth factor-C paves the way

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The new excellent study by Henri and colleagues published in the April 12 issue of the *Circulation* journal addresses an important issue of the involvement of lymphatic vasculature in the restoration of cardiac function after infarction (1).

The heart is permeated with an intricate network of blood and lymphatic vessels, which is indispensable for the adequate cardiac activity. While blood vessels provide oxygen and nutrients, the cardiac lymphatics are crucial for the control of intra-myocardial pressure, prevention of edema, lipid metabolism, and balanced regulation of tissue inflammation (2-4). The cardiac lymphatic system drains interstitial fluids flowing centrifugally from the sub-endocardium to the sub-epicardium. The adult cardiac lymphatic vasculature consists of a network of subepicardial and sub-endocardial vessels and a plexus of myocardial capillaries of various diameters and variable concentrations in the different regions of the heart (2,4-6). The liquids and macromolecules are absorbed via blind-ended lymphatic capillaries (a.k.a. initial lymphatics) in the myocardium that coalesce into larger collecting vessels, which channel the lymph into cardiac lymph node, before ascending to the thoracic duct and the right lymphatic trunk, ultimately joining the central venous circulation via lymphovenous valves located at the junction of the subclavian and internal jugular veins (2,4). The lymphatic system also serves as a major conduit for the trafficking of immune and other cell types, and it is a critical player in tissue inflammatory and reparatory responses (7,8).

Notably, there is a documented association between lymphatic malfunction and cardiovascular diseases, including edema and fibrosis after myocardial infarction (MI), and the evolution of congestive heart failure (3,4,9-13). Despite the recognized importance of the cardiac lymphatic vessels (CLVs) for the heart, hitherto, only limited investigations have addressed the CLV activity in health and diseases.

Furthermore, it becomes increasingly clear that the CLV network is markedly altered in pathological conditions, particularly after acute MI. In this regard, several groups independently observed a substantial increase in the numbers of intra-myocardial lymphatic capillaries and pre-collecting subepicardial lymphatics in human (11,13,14), mouse (15,16) and rat (1,17,18) cardiac tissues, predominantly in the peri-infarcted area. The time-course of the lymphatic vessel appearance conspicuously coincides with the commencement of the reparatory stages of wound healing in the infarcted heart, while post-MI lymphangiogenesis is peaking during the development of the fibrotic scar replacing the lost myocardium; and, intriguingly, the CLVs, once formed, seem to persist in the scarred tissue (14,15,18).

The biological significance of the CLV growth in the injured heart is interpreted in a somewhat contradictory manner: by removing excess fluids, cytokines, noxious antigens, cells and cell debris from the affected area, the new lymphatic vessels quench inflammation, and thus promote the maturation of fibrosis and formation of a stable scar (14,16). On the other hand, as discussed below, there are appealing evidences that by reducing inflammation via post-MI lymphangiogenesis one can avert fibrosis, improve myocardial integrity and achieve a better restoration of cardiac function (1,15,19).

Indeed, previous findings in experimental animals and human pathological specimens strongly indicate that improving cardiac lymphatic flow ameliorates the post-MI

heart (3,4,12,13). In their present study, Henri *et al.* carried out an exhaustive analysis of the impact of therapeutic lymphangiogenesis that is stimulation of lymphatic vessel growth in the infarcted heart, on the development of myocardial edema and efficacy of cardiac contractility after MI (1). To this end, the authors used rat models of temporary and permanent occlusions of the left coronary artery anterior descending branch, which recapitulate some aspects of human cardiovascular diseases and chronic heart failure. The cardiac lymphangiography in the infarcted animals after the first 4 weeks after surgery revealed an impaired lymphatic drainage at the infarcted region and non-infarcted left ventricular free wall, with partially restored transport capacity by 12 weeks post-MI. Moreover, the water content in the heart was increased in the infarcted as well as non-infarcted tissues at 4 weeks and even 12 weeks after MI, symptomatic of slowly dissipating edema. The authors argue that since at the above time points the ischemia-induced vascular permeability has long subsided, the fluid accumulation reflects insufficient cardiac lymphatic transport.

The deficiency in the fluid balance in the injured heart was accompanied by an extensive structural remodeling of the CLV at 4–12 weeks following infarction. Consistent with prior publications on experimentally-induced MI (15,16,18), Henri *et al.* found that the density of lymphatic capillaries in the affected region of the myocardium was more than 10-fold higher compared with sham-operated controls. Additionally, the sub-epicardium of the adjacent free wall of the left ventricle was characterized by a considerable increase in the CLV number. Yet, the average diameter of the CLVs in the infarcted rats was significantly lower than in sham animals. The authors also noticed that the frequency of larger pre-collecting lymphatics was diminished after MI. Collectively, these data suggest that although MI provokes an extremely robust lymphangiogenic response, paradoxically, the newly-formed CLVs are relatively small and not sufficiently open to adequately meet the requirements for interstitial liquid drainage.

In an attempt to restore fluid homeostasis in the infarcted heart, Henri and colleagues then introduced an approach to ectopically stimulate the cardiac lymphangiogenesis after MI. They delivered into the myocardium microparticles impregnated with a modified form of vascular endothelial growth factor-C (VEGF-C), namely VEGF-C_{C152S}. The cytokine VEGF-C is a principal driver of lymphatic vessel formation (4,19,20). VEGF-C binds to VEGFR-2 and VEGFR-3 on blood and lymphatic endothelial cells, respectively, eliciting multiple signaling outcomes, which regulate blood and lymphangiogenic growth. VEGF-C_{C152S} is a point mutant of VEGF-C, generated by the replacement of the second conserved Cys by a Ser residue of the recombinant rat VEGF-C protein, rendering it unable to interact with VEGFR-2, while maintaining preferential activity towards VEGFR-3 (21). Administration of the VEGFR-3-specific VEGF-C ligand (VEGF-C_{C156S} in humans and mice) in diverse experimental settings was demonstrated to promote lymphatic vessel formation, resulting in alleviated immune reaction and reduction of edema (19,22,23). Recently it was reported that in mice, intra-myocardial provision of recombinant VEGF-C_{C156S} protein, performed acutely after permanent coronary artery ligation, activates cardiac lymphangiogenesis, followed by a significant improvement in the ejection fraction at 3 weeks post-MI (15). In the present work, a novel sustained-release platform was employed for supplying VEGF-C_{C152S} into the myocardium of rats subjected to temporary coronary artery occlusion: at the time of surgery after the reperfusion, biodegradable microparticles loaded with a lower (1.5 μg/rat) or a higher (5 μg/rat) dose of VEGF-C_{C152S} were injected into the left ventricular free wall. These treatments were compared with sham-operated animals and the control rats injected with empty microparticles. The targeted delivery of VEGF-C_{C152S} by microparticles is predicted by the authors to last for several weeks. As expected, VEGF-C_{C152S} selectively stimulated the CLV formation, primarily initial lymphatics, but not the blood vessel expansion, as assessed at 3 weeks after MI. However, at a later time point analyzed, 8 weeks post-MI, there were no significant differences in the CLV density or size between the VEGF-C_{C152S}-treated and empty particle-injected infarcted hearts. These findings suggest that although VEGF-C_{C152S} accelerated the CLV growth, the lymphangiogenic responses in the infarcted myocardium reaches its maximal extent even in the absence of exogenous VEGF-C. Also, as reflected by vessel diameter, the reduction in the frequency of open lymphatics after MI was not mitigated by VEGF-C_{C152S}. Nevertheless, the lower dose (but not the higher dose) of VEGF-C_{C152S} attenuated the remodeling of pre-collectors in the non-infarcted sub-epicardium, which might signify an increase in the total area of open lymphatics. In terms of epicardial CLVs, a greater number of large pre-collector lymphatics were observed in hearts treated with a higher dose of VEGF-C_{C152S}, whereas the lower dose of VEGF-C_{C152S} had no effect. In accordance with that, cardiac lymphangiography at 8 weeks after MI detected no major improvement in the lymphatic

transport in the animals treated with VEGF-C_{C152S}. Next, the authors measured by gravimetry the total cardiac water content, which was found significantly reduced in the low-dose VEGF-C_{C152S} group relative to infarcted control rats, thus pointing to a more efficient regulation of myocardial fluid balance in the VEGF-C-treated animals.

Further, due to the critical role of the lymphatic vasculature in the control of inflammatory processes, the investigators studied the influence of lymphangiogenic therapy on the abundance of immune cells, i.e., macrophages and dendritic cells, and inflammatory cytokine CCL21. At 3 weeks after MI, in the hearts injected with VEGF-C_{C152S}, there was a seemingly dose-dependent decrease in the presence of CD68-positive macrophages. Concomitantly, a higher density of CLVs with elevated CCL21 expression was registered, which inversely correlated with the frequency of CD11c-positive dendritic cells in the tissue. Additionally, the authors reported a substantial reduction in the interstitial collagen histological labeling in the hearts of animals treated with VEGF-C_{C152S}, which they argue is a direct result of diminished inflammation and edema that fuel fibrosis.

Lastly, the authors examined the impact of VEGF-C_{C152S}-induced lymphangiogenesis on the cardiac function. At 6 weeks after MI, no significant differences in cardiac perfusion were observed by MRI comparing to control animals. Likewise, echocardiography measurements showed no substantial amelioration of left ventricular dilatation or fractional shortening. In contrast, invasive hemodynamic assessment by left ventricular catheterization, performed at 8 weeks post-MI, documented a better recovered diastolic and systolic function of the VEGF-C_{C152S}-treated hearts. Therefore, an improvement in cardiac performance following infarction was achieved by the lymphangiogenic therapy with VEGF-C_{C152S}.

A compelling conclusion from the work of Henri *et al.* is that the potentiation of lymphatic vessel growth in the heart might be of considerable benefit to the patients with signs of chronic myocardial edema. While much remains to be understood regarding the endogenous processes resulting in lymphatic hyperplasia after MI, the significance of newly-formed lymphatic capillaries for the development and maintenance of myocardial cicatrix, and the reasons the new CLVs do not sufficiently contribute to the drainage capacity of the heart, the present paper validates the necessity for future explorations of the use of exogenous VEGF-C as a therapeutic modality for cardiac diseases. Based on cumulative data, including the present research, it is evident that the induction of cardiac lymphangiogenesis and better preservation of the existing pre-collecting vessels are attainable by intra-myocardial protein delivery or, possibly, gene therapy with VEGF-C; more specifically, a VEGF-C analogue that has a greater ability to signal via VEGFR-3. Concerning therapeutic benefit and safety, the optimal dosage and duration of such an approach have to be carefully evaluated, as a persistently high VEGF-C signaling will likely interfere with the CLV maturation and transport function. Also, the impact of exogenous VEGF-C on other types of VEGFR-3-expressing cells in the heart (4,20), such as macrophages and cardiomyocytes, is currently unknown and might constitute a risk factor. Furthermore, whereas in experimental animal models the modified VEGF-C attenuates fibrosis when administered acutely after MI (1,15), in the hearts with pre-existing scar the pro-lymphangiogenic methodologies might elicit different and even opposing effects.

Hence, a deeper understanding of the mechanisms mediating endogenous and ectopic VEGF-C-induced lymphangiogenesis in the heart, as well as myocardial and systemic responses involved in the development and remodeling of the scar, is necessary for establishing therapies with VEGF-C or other agents augmenting the CLV growth. Finally, as discussed by Henri and colleagues, their findings bring forth considerations for the protocols combining pro-lymphangiogenic factors with additional substances known to modulate angiogenesis, inflammatory pathways, or other biological activities, thus extending the opportunities of discovering novel therapies for patients with MI or chronic heart failure.

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Footnote

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