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Basic mechanisms controlling lymph transport in the mesenteric lymphatic net

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Abstract

This minireview summarizes an oral presentation given at the National Institute of Diabetes and Digestive and Kidney Diseases National Institutes of Health workshop “Lymphatics in the Digestive System: Physiology, Health, and Disease” in Bethesda, Maryland on November 3–4, 2009. The concepts of extrinsic and intrinsic pumps, as well as intrinsic and extrinsic flows, are discussed in relation to the lymph transport in mesenteric lymphatic vessels. Age-related alterations in the structure and regulatory mechanisms of lymph flow in mesenteric lymphatic vessels may provide the basis for their diminished ability to work during the periods of increased functional loads in them. The recent development of modern experimental tools provides the opportunity to extend the knowledge on lymph transport function of lymphatic vessels that is absolutely necessary to maintain fluid and macromolecular homeostasis and to provide a transportation route for lipids adsorbed in gut and to immune cells.

Keywords

lymphatic vessels; lymphangion; lymph pressure; lymph flow; aging

Introduction

Lymph flow is necessary for several vital life functions in mammals and humans. Neither fluid and macromolecular homeostasis nor transportation of lipids adsorbed in gut or immune cells trafficking can occur without the effective system of lymph transport. There are two groups of the driving forces that support lymph flow. These forces are separated by their relation to spontaneous contractility of lymphangions, sections of lymphatic vessels between adjacent valves,^{1–3} underlining therefore the origin of the energy source that support lymph flow at the moment. “Active” or “intrinsic” lymph pump describes the lymph driving force generated by the brisk phasic spontaneous contractions of muscle cells in walls of the lymphangions. “Passive” or “extrinsic” lymph pump summarizes together the

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Conflicts of interest

The author declares no conflicts of interest.

influences of all other forces that do not connect with active contractions of lymphatic muscle cells in lymphatic vessel wall and that may support in more or less degree lymph flow in different regions of body. For mesenteric lymphatic network these forces will include the influences of tissue filtration gradient that varies depending on level of gut adsorption and predetermines the intensity of lymph formation. Other passive lymph driving forces, which may influence the mesenteric transport of lymph, could include the vasomotions of adjacent blood vessels and periodical decreases in outflow resistance in this lymphatic net due to the inspiration, although the importance of the last two passive forces for mesenteric lymph flow still should be thoroughly evaluated in humans. In general, the lymph transport in all regions of the body represent a complicated result of the combined action of forces that support and oppose lymph flow. In the majority of mammals (excluding mouse⁴) and humans,⁵ the active phasic contractions of muscle cells in the walls of the majority of collecting lymphatic vessels are necessary for effective lymph flow. Recently, we discussed in detail the concept of the active and passive lymph pumps in Gashev *et al.*⁶

Lymphangions, main driving units of lymph flow, are extremely sensitive to the levels of transmural pressure and lymph flow inside of them. These two physical parameters reflect the degree of functional loads that lymphangions are experiencing. Historically, the stretch-dependent modulation of lymphatic contractions attracted attention of lymphatic researchers. In numerous studies performed both *in vivo*, and *in vitro*,^{7–15} it was shown that increases in transmural pressure caused positive inotropic and chronotropic effects in lymphatic vessels. This tendency reflects the fact that, during periods of high loads of lymphangions by incoming fluid, they need to contract harder, supporting the lymphangion outflow adequate to increased lymphangion inflow. Recently, we obtained new evidence to demonstrate the regional variations in pressure-induced changes in lymphatic contractility. Studies were performed on lymphatic vessels taken from four different regions of one species—rat.¹⁶ The local differences in pressure sensitivities and pumping ability were determined for thoracic duct, cervical, mesenteric, and femoral lymphatic vessels. All investigated lymphatics were able to increase their pumping during moderate increases in transmural pressure up to some pumping maximum. The largest pump productivity was observed at 3 cm H₂O transmural pressure for all lymphatics, except mesenteric lymphatics, where maximum pumping occurred at a pressure of 5 cm H₂O. Moreover, detailed analysis demonstrated that all these lymphatics had a range of transmural pressures over which there were no significant differences in pumping. Experimental data demonstrate that these ranges of pressure were: 2–4 cm H₂O for the thoracic duct, 2–8 cm H₂O for cervical lymphatics, 2–7 cm H₂O for mesenteric vessels, and 2–9 cm H₂O for femoral lymphatics. These data reveal that all selected lymphatics have their optimal pumping conditions at comparatively low levels of transmural pressure, which is comparable to typical *in situ* lymph pressures¹³ and these pressure levels have a tendency to be higher in more peripheral lymphatic vessels. Highest pumping (at the optimal pressure levels) 6–8 volumes/min was demonstrated for mesenteric lymphatics and lowest pumping (near 2 volumes/min) in thoracic duct. Last, by our opinion, underlines the fact that mesenteric lymphatic vessels must provide a greatest support to lymph flow because existence of the highest (in whole body) levels of lymph formation may occur in the wall of the small intestine and initial mesenteric lymphatic network. Recently,

we discussed in detail the stretch-dependent regulatory reactions of contracting lymphatic vessels in Gashev *et al.*⁶

As we discussed earlier, the lymph flow is a result of complicated combinations of influences of active and passive lymph driving forces or intrinsic and extrinsic pumps. Because the peaks of the actions of passive lymph pumps often are not synchronized with intrinsic contractile activity of lymphangions, flow profiles in lymphatic nets are extremely variable and bidirectional. Only the presence of valves in lymphatic vessels prevents the existence of extended periods of back flow and supports net unidirectional lymph flow. On other hand, the presence of lymphatic valves additionally complicates the lymph flow profile. New scientific data obtained during the last decade significantly extended our understanding of the mechanisms of flow-dependent regulation of contracting lymphangions.

Recent studies performed with isolated, cannulated, and pressurized rat mesenteric lymphangions demonstrated complexity of imposed-flow dependent relaxatory responses of the lymphatic vessels.^{6,17} The slowly developing imposed flow-induced inhibition of lymphatic contractility could conserve energy in lymphatic vessels when there are sufficient passive forces to move lymph without/or with minimal involvement of the active lymph pump and could decrease local outflow resistance.^{6,17} Because of the negative chronotropic and inotropic effects of imposed flow, the active pumping of lymphatics was greatly diminished. However, it is difficult to conclude that such imposed flow-dependent inhibition of the active lymph pump decreases the total lymph flow *in vivo*. Because total lymph flow is the sum of passive and active flows, it is likely that the increase in imposed (or passive) flow could overwhelm any decreases in active lymph flow. A potentially important overriding factor would be an enhanced rate of lymph formation. At high levels of lymph formation, passive lymph flow could become a greater driving force to move lymph than the active lymph pump. Imposed flow-dependent inhibition of the active lymph pump in such situations could be a reasonable physiological mechanism to save metabolic energy by temporarily decreasing or stopping contractions during the time when the lymphatic vessel does not need it. An additional outcome of the inhibition of the lymph pump under these circumstances would be a reduction in lymph outflow resistance. This reduction in the outflow resistance could ease the removal of fluid from the affected compartment, that is, producing the high lymph flows and prevents edema formation.

Recently, in addition to the influences of imposed flow that experimentally imitate the periods of dominating extrinsic flow,⁶ we investigated the importance of contraction-generated intrinsic flow in contracting lymphangions.^{6,18} We performed studies where we implemented the elegantly designed experimental conditions that allowed investigating the influences of flow and shear, generated solely by the phasic lymphatic pump, on the contractile function without any extra imposed flow.¹⁸ During this work, we obtained the first pharmacological evidences that the reduction in lymphatic tone due to the flow/shear generated by phasic contractions is a regulatory mechanism that maintains lymphatic pumping in an energy-saving efficient mode (stronger, but fewer contractions per minute).¹⁸ This novel experimental data clearly indicated that the contraction-generated reduction of lymphatic tone in the thoracic duct is mediated only by nitric oxide and phasically generated

spike-like release of nitric oxide, synchronous with the lymphatic contractile cycle that exists in lymphatic vessels. The following studies performed during the next couple of years, with direct measurements of nitric oxide concentrations in a close proximity to lymphatic wall,¹⁹ confirmed our initial findings.¹⁸ Importantly, this new data¹⁹ validated that phasic contraction-generated release of nitric oxide discovered in studies, published in Gasheva *et al.*,¹⁸ exists in a lymphatic network other than the thoracic duct, underlining a high probability of the general distribution of this mechanism throughout all regions of the body. Additionally these new studies¹⁹ demonstrated a high level of complexity in the reactions of mesenteric lymphatic vessels on increases in lymph flow in them. Together, with our currently unpublished data from ongoing projects, this leads us to conclude that the shear-dependent regulatory mechanisms in different regional lymphatic networks are not identical.

The same complex levels of heterogeneity were observed during our ongoing investigations of the age-related alterations of the lymphatic contractility and its regulatory mechanisms. Recently, we demonstrated the severity of aging-induced changes in the lymphatic transport function of the rat thoracic duct,²⁰ where the stretch- and imposed-flow-dependent regulatory mechanisms were greatly altered by aging. These alterations of active pumping mechanisms in thoracic duct appear to be related with age-related disturbances in NO-dependent regulatory pathways and may reflect diminished lymphatic muscle contractility as well as altered lymphatic endothelium function. During our ongoing studies, we already observed a profound weakening of the aged mesenteric lymphatic pump (unpublished), which, we believe, at least in part were related to the diminished number of muscle cells in the wall of mesenteric lymphatic vessels in senile (24-month-old) animals (Fig. 1). However, the age-related changes in flow-dependent regulation in mesenteric vessels currently look similar not only as a result of the age-related disturbances in function of the nitric oxide synthases (unpublished). Age-related alterations in the structure and regulatory mechanisms of lymph flow in mesenteric lymphatic vessels may provide the ground of their diminished ability to work during the periods of increased functional loads in them.

Excitingly, our current developments in the methodology on lymphatic research have also provided promising results. To develop the techniques needed for the specific gene/protein targeting transfection experiments in isolated lymphatic vessels, we recently completed⁴ a series of experiments to optimize the experimental conditions that maintain the viability of isolated rat lymphatic vessels in culture for sufficiently long periods of time. This was done in order to permit knockdown or overexpression of selected proteins/genes and to develop effective transfection protocols for lymphatic muscle and endothelial cells in intact lymphatic vessels without nonspecific impairment of lymphatic contractile function because of the transfection protocol itself. Our new data demonstrated the effectiveness of the newly developed experimental protocols for the maintenance of isolated rat mesenteric lymphatic vessels and thoracic duct in culture up to 3–12 days without significant impairment of the parameters of their pumping and effective adenoviral/GFP transfection of lymphatic endothelial and muscle cells in isolated rat mesenteric lymphatic vessels.⁴ We believe that these experimental techniques will extend the set of the modern experimental tools available to researchers investigating the physiology of lymphatic function.

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Figure 1.

Age-induced alterations in muscle cell density in rat mesenteric lymphatic vessels. Lymphatic wall in 9-month-old (*Top*: max lymphatic diameter ~130 μm) and 24-month-old (*Bottom*: max lymphatic diameter ~120 μm) Fisher-344 rats. Live isolated rat mesenteric lymphatic vessels underwent 30 min loading by CellTracker™ Green CMFDA (Invitrogen Corp. Carlsbad, CA). The segments were observed at 50 $\times$ magnification, using a Leica AOBs SP2 Confocal-Multiphoton Microscope System (Leica Microsystems Heidelberg GmbH, Mannheim, Germany). Confocal images were acquired at 0.3- μm intervals via 489

nm of peak excitation wavelength and 508 nm peak emission wavelength in order to completely image the entire lymphatic vessel. Three-dimensional projections were generated from a series of images using confocal images processing software, provided by microscope manufacturer. Transmural pressure is ~7 cm H₂O.

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