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Hydrodynamic regulation of lymphatic transport and the impact of aging

Anatoliy A. Gashev and David C. Zawieja*

Department of Systems Biology and Translational Medicine, Cardiovascular Research Institute
Division of Lymphatic Biology, College of Medicine, Texas A&M Health Science Center, 702 SW
H.K. Dodgen Loop, Temple, TX 76504, USA

Abstract

To accomplish its normal roles in body fluid regulation/macromolecular homeostasis, immune function, and lipid absorption; the lymphatic system must transport lymph from the interstitial spaces, into and through the lymphatics, through the lymphatic compartment of the nodes, back into the nodal efferent lymphatics and eventually empty into the great veins. The usual net pressure gradients along this path do not normally favor the passive movement of lymph. Thus, lymph transport requires the input of energy to the lymph to propel it along this path. To do this, the lymphatic system uses a series of pumps to generate lymph flow. Thus to regulate lymph transport, both lymphatic pumping and resistance must be controlled. This review focuses on the regulation of the intrinsic lymph pump by hydrodynamic factors and how these regulatory processes are altered with age. Intrinsic lymph pumping is generated via the rapid/phasic contractions of lymphatic muscle, which are modulated by local physical factors (pressure/stretch and flow/shear). Increased lymph pressure/stretch will generally activate the intrinsic lymph pump up to a point, beyond which the lymph pump will begin to fail. The effect of increased lymph flow/shear is somewhat more complex, in that it can either activate or inhibit the intrinsic lymph pump, depending on the pattern and magnitude of the flow. The pattern and strength of the hydrodynamic regulation of the lymph transport is different in various parts of the lymphatic tree under normal conditions, depending upon the local hydrodynamic conditions. In addition, various pathophysiological processes can affect lymph transport. We have begun to evaluate the influence of the aging process on lymphatic transport characteristics in the rat thoracic duct. The pressure/stretch-dependent activation of intrinsic pumping is significantly impaired in aged rat thoracic duct (TD) and the flow/shear-dependent regulatory mechanisms are essentially completely lacking. The loss of shear-dependent modulation of lymphatic transport appears to be related to a loss of normal eNOS expression and a large rise in iNOS expression in these vessels. Therefore, aging of the lymph transport system significantly impairs its ability to transport lymph. We believe this will alter normal fluid balance as well as negatively impact immune function in the aged animals. Further studies are needed to detail the mechanisms that control and alter lymphatic transport during normal and aged conditions.

*Corresponding author. Tel.: +1 254 742 7033; fax: +1 254 742 7145. dcz@tamu.edu (D.C. Zawieja).

Keywords

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1. Introduction – lymphatic transport

To maintain overall body homeostasis and health, the lymphatic system has a number of important tasks it must achieve. Lymph transport is a critical part of processes involved with body fluid regulation, macromolecular homeostasis, lipid absorption, and immune function. Lymphatic transportation of the constituents of lymph begins when fluid and other lymph elements from the parenchymal interstitial spaces, crosses the lymphatic endothelium and moves into the network of lymphatic capillaries. These lymphatics are called by various names, including lymphatic capillaries, initial lymphatics or terminal lymphatics and are the site of the exchange of fluids and constituents between the interstitium and the lymph (i.e. lymph formation) [1]. These lymphatics are typically noted by a lack of muscle cells, a discontinuous basement membrane, endothelial cells with gaps between adjacent cells and special connections to the surrounding matrix called anchoring filaments [2–4]. The exact mechanisms by which these processes occur and are regulated are still unclear, although the preponderance of data shows that convective mechanisms governed by transient gradients in fluid pressures drives the entry of lymph through the interendothelial cell gaps and into the initial lymphatic lumen [5–8]. Once within the lymphatic lumen, this fluid and whatever constituents it may contain are properly called lymph. Clearly a better mechanistic and molecular understanding of this process and how it is regulated is needed [9,10]. The lymph must then be moved within the network of lymphatic vessels from the initial lymphatics, into and through the first lymph node where much of the learned immune response develops [11–13]. Lymph will exit the lymph node (after its cell and molecular constituents may have been altered) through the efferent lymphatics. These efferent lymphatics join with other large and small lymphatics to continue through a series of lymph nodes and more post nodal lymph ducts. The larger transport lymphatics will eventually converge into the thoracic duct (for the lower half and upper left quadrant of the body) and right lymphatic duct (for the upper right quadrant of the body) before eventually emptying their lymph into the great veins. This movement of lymph within the lymphatic network, from where it is formed in the initial lymphatics to its final exit into the venous compartment will be dictated by the inherent pressure gradients within that network. Normally the net pressure gradient from the lymphatic capillaries to the venous compartment does not favor the passive flow of lymph centrally. Thus the movement of lymph along the lymphatic network relies on forces that are generated both extrinsically and intrinsically to the lymphatic system (i.e. the extrinsic and intrinsic lymph pumps) and upon the presence of lymphatic valves to minimize backflow. Of critical importance is that both the generation of lymph flow by intrinsic forces and the regulation of flow by both intrinsic and extrinsic forces, rely on the phasic and tonic contraction of lymphatic muscle to produce a controlled net unidirectional transport of lymph. The regulated movement of lymph through this network is absolutely necessary for its transport of fluid, macromolecules, lipids, antigens, immune cells and particulate matter. Thus it is crucial to remember that principal purpose of the lymphatic system is the transport

of lymph and that by this regulated lymph transport all the functions that the lymphatic system participates in are served [12].

2. Intrinsic and extrinsic lymph pumps

As briefly described above, both extrinsic and intrinsic driving forces can support lymph flow. The intrinsic pump relies on the spontaneous contractions of the muscle cells within the wall of the lymphangion (a section of lymphatic between adjacent valves [14–16]) to generate the pressure to drive lymph flow centrally. The extrinsic lymph pump depends on sources outside the lymphatic (blood vessel pulsations, gastrointestinal muscle contractions, heart contractions, skeletal muscle contractions, breathing movements, etc.) to generate the pressure to drive lymph centrally. Thus, lymph pressure within the lymphatic network is dependent on both the intrinsic and extrinsic forces. The pressures within the various parts of the lymphatic network will dictate the pressure gradient that open and close lymphatic valves and drive flow. There are few studies that have measured lymph pressures along significantly large sections of the lymphatic network to give a comparatively complete understanding of the pressure gradients along it. The most extensive representation of lymph pressures in the lymphatic system was reported by Szabo and Magyar [17]. These measurements of lymph pressure were made via the cannulation of some of the major lymphatic vessels in dogs. While the pressures in each vessel varied over time with various actions, the average pressures can be seen in Table 1. This study demonstrated that there was not a net pressure gradient along lymphatic system that would favor passive lymph flow. Thus the phrase “lymph drainage” is somewhat of a misnomer, since the normal average pressure gradients in the lymphatic network do not favor central lymph flow and “vis a tergo” (the force of lymph formation) is probably limited to the network of initial lymphatics and determined by the level of lymph formation [18]. Thus, under normal conditions all lymphatics in the lymphatic network beyond the collecting lymphatics must utilize additional forces to overcome the prevailing negative pressure gradients and drive central lymph flow.

An additional effect that must be considered, particularly in bipedal or tall animals is the effect of gravity on the fluid within the lymphatic network. In bipedal animals like humans, the lymphatic system must not only overcome the net negative hydraulic pressure gradient along the lymphatic network, but it must also overcome gravity to pump lymph uphill through the thoracic duct. This has been documented in studies of the pressures and flows in human leg lymphatics [19–24]. At rest, lymph pressure in human leg lymphatics rose about 6–10 mmHg when going from a supine to upright position, with basal pressures of ~1–5 mmHg (dependent on the position of the lymphatic in the leg) in the supine position to ~7–15 mmHg in the upright position, with a lymphatic contraction pulse amplitude of ~3–10 mmHg and a contraction frequency of ~2–10/min. In general, in those studies in which flow and pressure were measured, lymph flow was only observed during the phasic contractions with little or no flow in between the phasic pulses. Lymph pressure measured in human thoracic duct, which is main lymphatic outflow tract, was ~14–22 mmHg under basal conditions without any outflow obstruction [25]. Lymph pressure in human thoracic duct rose to ~30 mmHg when measured as an end-lymphatic pressure [26], i.e. without any lymph outflow, with pressure fluctuations of about 5–10 mmHg. The thoracic duct basal

pressures were higher than the basal pressures in leg lymphatics and thus represent a significant afterload that the upstream lymphatics must overcome to generate a net central lymph flow. However, during inspiration thoracic duct lymph pressure drops to near zero or slightly negative values [25], which temporarily could make the net pressure gradient favorable to lymph flow into the thoracic duct. The increase in pressure during exhalation and phasic contractions of the thoracic duct may generate a gradient conducive to lymph flow from the thoracic duct into the venous circulation. This temporal pattern of pressure changes in the thoracic duct will influence lymph flow through the thoracic cavity, but because of the valves and the resulting interrupted fluid column in lymphatic vessels distal to the thorax, these gradients do not extend all the way down to the lymphatic network of the legs [27–29].

2.1. Extrinsic lymph pump

The extrinsic lymph pump combines all of the forces that are generated outside of the lymphatic itself that can generate lymph pressures favorable to central lymph flow. These forces are generated in the tissues surrounding the lymphatic vessels, compressing the lymphatic vessels encased in those tissues, often through the contraction of muscle in that tissue. The extrinsic lymph pump forces include: the pressure generated by lymph formation (historically also called as “vis a tergo”), cardiac contractions, respiration motions, skeletal muscle contractions, arterial and venous pulsations and gastrointestinal peristalsis. All of these extralymphatic forces can produce pressure gradients in the lymphatic network within that tissue that can propel lymph centrally, even without local intrinsic lymphatic vessels contractions. However, lymph flow cannot occur without lymph formation. In general lymph is formed in the networks of lymphatic capillaries, typically through pressure gradients that transiently favor lymph entry into the lymphatic capillary [6,7,30]. Therefore the more favorable the conditions for lymph formation, the greater the rise in lymph pressure inside the lymphatic capillary network, and the larger the force to move lymph downstream into the collecting lymphatics. In the thoracic cavity, both cardiac and respiratory tissue activity produce rhythmic compressions and expansions of the lymphatic and thus drive lymph formation and pressure [6,7,30]. During exercise, contractions of skeletal muscles will cause compression/expansion of the lymphatics adjacent to or surrounded by those muscles. These skeletal muscle contraction/relaxation cycles produce the forces to empty and refill those lymphatics. During exercise of the skeletal muscle in human legs, lymph pressure and flow correlate with the intensity of the skeletal muscle activity [21]. Under normal conditions the majority of lymph in the body comes from the gastrointestinal lymphatics and in this tissue, intestinal peristalsis and intestinal luminal pressure are the predominant forces driving lymph formation and transport. Postprandially, increases in luminal pressure of the gut increase formation and pressure in the intestinal lymphatics. This coupled with increased gut peristalsis, leads to increases in lymph flow through the mesenteric collecting lymphatics [31].

2.2. Intrinsic lymph pump

The intrinsic pumps of the lymphatic system possess the contractile machinery within the lymphatic wall muscle cells needed to generate lymph flow in the lymphatics of most mammals, including humans. The effective unit of the intrinsic pump is the segment of

lymphatic vessel between adjacent valves, termed the lymphangion [16]. The larger collecting and transport lymphatics possess layers of smooth muscle cells in their outer walls to generate and control the movement of lymph along the lymphatic network. The lymphangions are arranged in series and separated by highly competent valves. This lymphatic muscle is responsible for the regulation of lymphatic diameter and thus its compliance and resistance to generate and modulate lymph flow through tonic and phasic contractions. Phasic contractions are initiated by action potentials that originate in pacemaker cells that reside within the muscle layer [32]. Whether the pacemaker cells are specialized cells that possess unique characteristics like the interstitial cells of Cajal in the gastrointestinal tract [33,34] or if they are lymphatic muscle cells with spontaneous depolarizations is the focus of active debate and still unclear [35–37]. The depolarization of the lymphatic muscle cell elicits increases in intracellular calcium that are dependent on both intracellular and extracellular calcium sources to produce a phasic lymphatic contraction [13,36,38–44]. The rise in intracellular calcium produces an increase in force generation leading to lymphatic vessel contraction [42–44]. The pCa–tension relationship of permeabilized rat mesenteric lymphatics was investigated and compared to those of mesenteric arteries and veins. The pCa₅₀ of the lymphatics (6.16±0.05) was similar to the veins (6.00±0.05) but was significantly lower than the arteries (6.44±0.02) [45]. Precisely how these variations in the calcium sensitivity of the lymphatic contractile apparatus play into its unique roles is the subject of current studies by a number of groups.

Lymphatic muscle can contract both tonically and phasically. Elevations of calcium appear to be necessary for both the tonic and phasic contractions. Lymphatic muscles also have a unique mixture of smooth and striated muscle contractile proteins [46]. Rat mesenteric lymphatics express only the SMB isoform of smooth muscle myosin heavy chain (SM-MHC), whereas thoracic duct expressed both SMA and SMB isoforms. Rat mesenteric and thoracic lymphatics expressed both SM1 and SM2 isoforms of SM-MHC. Uniquely the rat mesenteric lymphatics expressed the fetal cardiac/skeletal slow-twitch muscle-specific β-MHC. The mesenteric lymphatics have all four actins present, cardiac α-actin, vascular α-actin, enteric γ-actin, and skeletal α-actin; while in thoracic duct; the cardiac α-actin and vascular α-actin were predominantly found [46]. Thus lymphatic muscle has a mix of vascular, cardiac, and visceral contractile proteins, which are needed to fulfill its unique roles. Recent evidence indicates that rat mesenteric lymphatic muscle utilizes myosin light chain kinase to phosphorylate myosin light chain 20 and activate the tonic contractile process, similar to other smooth muscles [47]. However the myosin light chain 20 phosphorylation status does not appear to modulate the phasic contractile strength. Much further research is needed to evaluate the molecular mechanisms that are responsible for the tonic and phasic lymphatic contractile activity.

During the phasic contraction of a lymphangion, the lymphatic muscle cells increase the lymph pressure and produce a transient, positive, local pressure gradient with the lymphatic network to propel lymph centrally. The force generated by one lymphangion is not enough to propel lymph throughout the lymphatic network, partially because of the high compliance of these tissues. Thus, lymphatic vessels are organized in chains of lymphangions containing many dozens of these pumps in series in any single lymphatic internodal segment. These series-lymphangions do not contract simultaneously, but instead the phasic contraction

propagates along the chain of lymphangions electrically [48,49]. Therefore each lymphangion can be described as a local pump chamber that drives a bolus of lymph along the central direction to the next couple of few lymphangions. Working together, the chains of the lymphangions can generate effective long-distance lymph transport. The intrinsic lymph pump can be analyzed using an analogy to the cardiac cycle [50] where the phasic contractile cycle is separated into lymphatic diastole and systole. Intrinsic pump function can then be evaluated using the phasic contraction frequency, stroke volume, ejection fraction and lymph pump flow [50,51]. Extending the cardiac pump analogy, the intrinsic lymph pump can be modulated via inotropic (i.e. the strength of contraction) and/or chronotropic (i.e. changes in the contraction frequency) fashion [10,48,52–67]. But the lymphatic system must perform both conduit and pump functions. Regulation of the conduit function is achieved using local, neural and humoral factors to modulate flow by altering the outflow resistance (via tonic contraction/relaxation of lymphatic muscle). Humoral and neural agents such as adrenergic agonists, prostanoids, bradykinin, substance P, natriuretic factors and other agents modulate lymphatic tone and thus lymphatic function [13,53,55,57,62–64,68–86]. However it has long been understood that local hydrodynamic factors, such as stretch/pressure and shear/flow play the crucial role in modulating lymphatic tone and pump function and that these mechanisms are inherent to the lymphatic structure [14,15]. It has also been shown that lymphatics possess myogenic tonic activity [87] and that stretch modulates the phasic contraction strength and its temporally pattern [48,66,67,88–91] as described below.

3. Modulation of the lymphatic phasic contractility by pressure/stretch

For many years it was thought that distension is necessary to generate phasic lymphatic contractions, essentially a myogenic response for phasic activity. While stretch will clearly modulate phasic lymphatic contractions it is known that it is not absolutely necessary to initiate the phasic contractions. Lymphatics can contract in a coordinated fashion, the contractile wave can propagate in directions retrograde to flow [16,49,51,92,93] and these can occur without distension stimuli [48,66,89,94]. At low or normal levels of lymph formation, in many tissues at the end of the phasic contractions the lymphangions are often empty or close to empty [29], yet they still exhibit phasic contractile activity. Indeed it has been shown that isolated bovine and rat mesenteric lymphatics can have stable long-lasting phasic contractile activity at essentially 0 cmH₂O intraluminal pressure, and in the absence of axial distension [95–97]. Thus distension of the lymphatic wall by intraluminal/transmural pressure is an important factor to regulate the phasic contractile activity in lymphatics, but it is not a mandatory condition for pacemaking of the phasic contractions.

In the muscular collecting lymphatics, distension of the lymphatic wall activates the lymphatic contraction and vessel distensions are reached. Beyond those distention pressures, lymphatic inotropy will plateau, while chronotropy continues to rise. As the pressure/stretch rises further, inotropy will fall as the lymph pump begins to fail and chronotropy reaches a maximum. This results in a Starling-like effect for the lymphatic pump. McHale and Roddie have demonstrated [48] that isolated bovine mesenteric lymphatics respond to an increase of transmural pressure from 1 to 4 cmH₂O with increases in stroke volumes and contraction frequency. At a transmural pressure of about 5 cmH₂O, the lymphatic pumping was

maximally activated. Further elevations in pressure decreased the stroke volume, while contractions frequency continued to rise. However the increase in contraction frequency was not enough to overcome the fall in stroke volume when distension rose above 6 cmH₂O. Thus pump output fell. Similar patterns of lymphatic intrinsic pumping were later seen by others [98] in response to elevations in transmural (pressure (pumping maximums between 5 and 10 cmH₂O).

The exact cellular and molecular mechanisms by which these effects occur are still unknown. However, lymphatics from different tissues and species will exhibit Starling pumpcurves at the different values of lymph pressure. It appears that the smaller lymphatics, which are located more peripherally in the lymphatic network, have peak pumping activity at higher transmural pressures. For example, the maximum pumping in prenodal popliteal lymphatics in sheep was seen at transmural pressures of ~18–26 cmH₂O [99]. Recent studies in bovine prenodal mesenteric lymphatic demonstrated increased contractility during elevations in transmural pressure that peaked at transmural pressures 6–9 cmH₂O and remained essentially constant up to 15 cmH₂O [100]. However, pumping in bovine postnodal mesenteric lymphatics is typically depressed at transmural pressures higher than 10 cmH₂O. To further evaluate this mechanism, studies of lymphatic pump function and its response to stretch were conducted in lymphatics taken from four different regions of the rat [101]; thoracic duct, cervical, mesenteric and femoral lymphatic vessels. To compare pumping ability in these different lymphatics (that have widely disparate sizes) fractional pump flow was used to determine pump effectiveness, where fractional pump flow is the product of the ejection fraction times the contraction frequency. This allowed a direct comparison of pump strength regardless of anatomical size. All of these lymphatics increased their pumping activity with moderate increases in transmural pressure up to a maximum level of pumping, where it typically reached a plateau before falling at higher levels of stretch. The maximum pump activity was found at a transmural pressure of 3 cmH₂O for all of these lymphatics except the mesenteric lymphatics, which peaked at a pressure of 5 cmH₂O. Maximum pumping in these vessels remained almost constant over different ranges of pressure: 2–4 cmH₂O for the thoracic duct, 2–8 cmH₂O for cervical lymphatics, 2–7 cmH₂O for mesenteric vessels, and 2–9 cmH₂O for femoral lymphatics. The optimal pumping in these lymphatics occurred at transmural pressures comparable to those found in situ [51]. Again there is a tendency for the optimum pressure range to be somewhat higher and the fractional pump flow to be much larger in the lymphatics that are more peripherally located. Highest pumping (at the optimal pressure levels) was seen in the mesenteric lymphatics (6–8 vol/min) while the lowest was observed in the thoracic duct (~2 vol/min). This indicates that more peripheral lymphatic vessels may develop much higher pressures to overcome the greater outflow resistance for their particular location in the lymphatic network and that the conditions for optimal pumping are shifted towards the greater values of intraluminal/transmural pressures accordingly.

4. Modulation of the lymphatic vessel contractility by flow

The second principal hydrodynamic factor in these fluid filled vessels is flow/shear. Shear has long been known to be a critical factor regulating blood flow [102]. Even though the fluid flow and shear rates observed in lymphatics are typically much lower than those seen

in either arteries or veins, flow/shear is still an important modulator of lymphatic contractile function. Lymph flow is the result of a complicated combination of lymph formation, intrinsic and extrinsic forces/pumps. Thus the lymph flow patterns can be extremely variable and bidirectional. Recently techniques were developed and implemented that allowed the reliable measurement of lymph flow velocities and shear stress in microlymphatic vessels in situ in the rat [103–105]. A high-speed video system was used to track immune cell movement throughout the lymphatic contractile cycle. The images were used to determine lymph velocity, flow rate, wall shear stress, and the patterns of orthograde/retrograde flow. The lymphatics exhibited typical phasic contractile activity. They had a mean outer diastolic diameter of $91 \pm 9.0 \mu\text{m}$ and phasic contraction amplitudes of $\sim 40\%$. Lymph velocity varied with the phasic contractions in both direction and magnitude, with an average of $0.87 \pm 0.18 \text{ mm/s}$ and peaks of $2.2\text{--}9.0 \text{ mm/s}$. The velocity was directionally almost 180° out of phase with the phasic lymphatic contractile diameter cycle. Average lymph flow was $14.0 \pm 5.3 \mu\text{l/h}$, with transient periods of reversed flow where 92.5% of the time flow was orthograde, but 7.5% of the time it was retrograde. This resulted in an average shear of $0.64 \pm 0.14 \text{ dynes/cm}^2$ with peaks of $4\text{--}12 \text{ dynes/cm}^2$. These studies confirmed that the shear rate in mesenteric lymphatics is low compared to shear in nearby blood vessels, but it had very large variations in magnitude compared to blood vessels as well as brief but common periods of flow reversal as the valves open and close. What the profiles of in situ lymph flows and shears are in lymphatics from other tissues and other species remains to be determined.

Early indications of how lymphatics respond to flow were seen in isolated bovine mesenteric lymphangions, which were exposed to elevations in the axial pressure gradient [106,107]. The total flow through the lymphangion increased as the axial pressure gradient increased as expected. However, phasic contractile activity was only measured in the lymphangions with pressure gradients up to $+3$ to $+5 \text{ cmH}_2\text{O}$. Further increases in the axial pressure gradient caused a complete inhibition of active pumping. It was hypothesized that phasic pumping stops when the axial flow gradient exceeds $3\text{--}5 \text{ cmH}_2\text{O}$, presumably because this gradient is enough to drive lymph flow through the lymphatic segment. Thus it does not need the phasic contractions. However transmural pressure was not controlled in these experiments, thus the lymphatics were exposed to a complicated scheme of stimulation by increased stretch but inhibition by increased shear. More controlled studies of the influences of imposed flow on lymphatic contractile activity were performed in isolated rat lymphatics [54,101]. These were performed in isolated and perfused lymphatics from four different regions of body from the rat, allowing a comparison of the flow/shear modulation of lymphatic contractility. In these studies, the inflow and outflow pressures were changed simultaneously in order to maintain the mean transmural pressure constant during the periods of increased imposed flow. A flow-dependent inhibition of the active lymph pump was found in mesenteric lymphatics and thoracic duct [54] as well as femoral and cervical lymphatic vessels [101]. The imposed flow caused decreases in both the frequency and amplitude of the phasic lymphatic contractions. As a result of these effects, the active lymph pump was greatly inhibited by the imposed flow/shear. Because total lymph flow in situ is the sum of intrinsic and extrinsic pump, conditions that favor a pressure gradient for flow, similar to what was used in these studies, would only occur if lymph formation and/or extrinsic pumping were high. At high levels of lymph formation, conditions favorable to passive lymph flow could

occur, rendering the intrinsic lymph pump unnecessary and a waste of energy. Thus the imposed flow-dependent inhibition of the active lymph pump in such situations could be a mechanism to save energy, by decreasing or inhibiting contractions during the time when the lymphatic does not need it. An additional outcome of the lymph pump inhibition would be a reduction in lymph outflow resistance because of the reduction in tone and the inhibition of the phasic contractions. For example, complete inhibition of the mesenteric intrinsic lymph pump would result in an increase in the average diameter by about 23%, thereby reducing resistance by approximately 56% [54]. This reduction in the outflow resistance could ease the removal of fluid from the affected compartment that is producing the high lymph flows and facilitate the resolution of edema. In these experiments support for the influence of the total outflow resistance for any given lymphatic on the sensitivity of the active pumping has been obtained [101]. Thus even when imposed flow was high in the upstream lymphatics, i.e. the mesenteric and femoral lymphatics, these vessels still had comparatively high pumping. The opposite was seen in lymphatics from the outflow end of the network, i.e. the thoracic duct and cervical lymphatic trunk, where intrinsic pumping was dramatically inhibited by a high level of imposed flow (~92% reduction in pumping in cervical lymphatics and ~99% reduction in the thoracic duct). This provides additional support for the concept that lymphatics from upstream segments of the lymphatic network generally need a strong active pump (with high sensitivity to pressure and low sensitivity to flow) to overcome the high outflow resistance, while lymphatics from regions near the output of the lymphatic network are not as strong a pump (with low sensitivity to pressure and high sensitivity to flow) and will behave more like conduits [46,54,95,108].

The roles of the NO-synthase and cyclooxygenase pathways in the flow-mediated responses in blood vessels are well-known [109–112]. Likewise, the importance of nitric oxide in the flow-mediated modulation of lymphatic contractile cycle was demonstrated both in vitro and in vitro [36,54,56,80,113–115]. While the effects of cyclooxygenase in endothelium-mediated modulation of lymphatic contractile activity are well-known [62,116–120], the involvement of the cyclooxygenase pathway in the flow-mediated control of lymphatic pumping is somewhat controversial [121]. The involvement of NO-synthase pathway and lack of involvement of cyclooxygenase pathway in the flow-mediated regulation of the lymphatic pumping, are supported by functional and biochemical data in rat mesenteric and thoracic duct and canine thoracic duct [52,122]. These results are further supported by recent evidence that measures lymphatic NO production using NO-sensitive microelectrodes in situ in the rat mesentery [123]. These experiments demonstrated that both the valvular and tubular sections of the lymphatic increased their generation of NO during each phasic contraction (with the concomitant rise in flow/shear) and that the NO generated by the phasic contractions was additive as contraction frequency increased. Thus strongly linking the flow-mediated lymphatic pump modulation with the lymphatic endothelial production of NO. The results reported by Koller et al. [121] are somewhat different in that they concluded that prostanoids were primarily responsible for the imposed flow-mediated responses in isolated, perfused iliac lymphatics in the rat. These authors reported that an increase in flow rate across the isolated rat iliac lymphatic caused reductions in both the diastolic and systolic diameters, and a fall in the phasic contraction amplitude. It is not clear why these data differ from others, though it is possible that differences in the methodological approach resulted in

the differences observed. It is also possible that the mechanisms responsible for flow/shear mediated changes in the lymphatic contractile responses are different in different tissues, as has been shown in blood vessels [110]. Clearly more work on the mechanisms responsible for the flow/shear-dependent modulation of lymphatic contractile functions are needed.

The flow/shear mediated actions on lymphatic contractile function are somewhat more complicated than just those depicted above. This may be due to the fact that the flow within any given lymphangion can be derived from both intrinsic and/or extrinsic forces. Thus flow through the lymphatic will feed back upon the vessel to modulate its tonic and phasic contractile activity and thus alter its ability to generate and regulate lymph flow. Recently [52] it has been shown that the response of the rat thoracic duct to the flow generated by its intrinsic phasic contractions is somewhat different than the response to a high-flow imposed upon the vessels extrinsically [52]. In this study, the thoracic duct was chosen since it is very sensitive to an imposed flow and it has a somewhat variable phasic contractile behavior [25,101,124–129]. In many cases, contractions occur in one part of thoracic duct but they do not always propagate along the rest of the vessel [25,101,126–129]. Thus, many times the phasic contractions develop locally, while adjacent parts of the duct are not always phasically contracting, although they still exhibit tonic contractions [46]. This feature of the thoracic duct was utilized to evaluate the importance of flow and shear generated by lymphatic phasic contractions in the regulation of the lymphatic contractile cycle. Two different types of thoracic duct segments were experimentally studied: (1) Thoracic duct segments that were phasically active. (2) Thoracic duct segments that were not phasically active. In the phasically active sections, flow and shear only occurred as a result of their intrinsic phasic contractions. In phasically inactive segments, flow and shear did not occur since great care was used to prevent any axial pressure gradients. Thus the only influences of flow and shear on the contractile function were those generated solely by the phasic lymphatic pump, without any extrinsic flows. Lymphatic tone in the vessels that were phasically active was 2–2.6 times lower than that measured in the phasically inactive lymphatic segments [52]. Inhibition of NO-synthase in these lymphatics by L-NAME completely abolished the difference in tone between the phasically active and inactive segments. This indicated that the phasic contractions resulted in a reduction of resting tone that was exclusively associated with the phasic pumping and was mediated by NO. This reduction of basal tone enhances lusitropy (diastolic filling) of the lymph pump, which resulted in an increased inotropy (stronger phasic contraction amplitudes). This increased the ejection fraction of the lymph pump. However the NO resulting from the phasic pumping also decreased the phasic contraction frequency (reduced chronotropy). Thus NO-synthase blockade resulted in a lymphatic that must contract at a higher contraction frequency in order to maintain the fractional pump flow because of the impairment of filling and phasic contraction strength. Thus the flow/shear generated by the phasic contractions produces a (NO-dependent) decrease in lymphatic tone that maintains an effective and efficient lymph pumping.

Together the available data support the concept that the pumping/resistive activity of the lymphatics is regulated by flow/shear to adapt to the local needs to transport lymph through a continuous modulation of the extrinsic and intrinsic flows. At low levels of lymph inflow in the transporting lymphatics (i.e. low extrinsic flows), the influences of intrinsic pump will

dominate, with periodic NO release due to the phasic flow/shear patterns of the lymph pump to maintain efficient lymph transport. When the levels of lymph formation and inflow to the transporting lymphatics rise (i.e. high extrinsic flows), the influences of extrinsic forces will dominate, leading to a high NO release that inhibit the intrinsic pumping and basal tone of the transport lymphatics to optimize the conduit function of the vessel.

5. Impact of aging on the hydrodynamic regulation of lymph transport

It is well-known that aging has adverse effects on many body functions, including numerous effects on the function of blood vessels. Thus aging is associated with increased susceptibility to edema formation and impaired immune function. Given that the lymphatic transport system plays critical roles in both fluid homeostasis and immune cell trafficking, it is important to understand what role age-dependent lymphatic dysfunction may play in these problems. Currently, very little is known about the impact of the aging process on lymphatic transport function.

One seminal study [130], published two decades ago demonstrated that the human thoracic duct exhibits its maximum number of lymphatic muscle cells per unit area at about 30 years of age. In older humans (>65–70 years of age), atrophy of the lymphatic muscle cells in the thoracic duct wall occurs. Accompanying this loss of muscle, was a destruction of the lymphatic elastic matrix. This resulted in the development of sclerosis of the thoracic duct and in some collecting lymphatics, the appearance of aneurism-like formations, just downstream of the lymphatic valves. These structures contained only endothelial cells in their walls. Also after 65 years of age, the numbers of lymphatics (initial and collecting vessels) and the number of connections (anastomoses/collateralization) between lymphatics in the human mesentery were all greatly diminished. They also noted considerable signs of degradation of the blood capillaries within the wall of hepatic and mesenteric collecting lymphatics in this age group. Thus aging clearly alters the normal lymphatic anatomy, what is not clear from these studies is how that affected function.

To see if we could evaluate the potential impact of the aging process on lymphatic transport, we utilized isolated lymphatic vessels from the Fisher-344 aged rat model and studied their hydrodynamic regulation of lymphatic contractile activity [131]. We measured lymphatic diameters, phasic contraction amplitude and frequency, ejection fraction and fractional pump flow in thoracic ducts isolated from 9- to 24-month-old rats. The pressure-dependent regulation of the phasic lymphatic pump was impaired in aged thoracic ducts, especially at higher pressure levels. The alterations in stretch-related mechanisms in thoracic ducts from the aged rats implicated that both the pacemaking and contractile machinery are altered. The phasic lymphatic contraction frequency was decreased, particularly at higher levels of transmural pressure. In addition, the lymphatic tone and phasic contraction amplitude were decreased in the aged thoracic ducts. This could be a sign of an age-dependent weakening of lymphatic muscle resulting in a diminished ability to create the force needed to maintain the appropriate tone and contractile force. The imposed flow-dependent modulation of the lymph pump was completely abolished in the thoracic ducts from old rats. Western blot analyses revealed that the eNOS expression in the 24-month-old thoracic duct was greatly decreased when compared to the thoracic ducts of the 9-month-old rat. However, NO-

synthase blockade stimulated phasic pumping in a flow-independent manner. This indicated a flow-independent, but NO-dependent inhibition of lymphatic contractility occurred in the aged thoracic duct. We found that this inhibition was correlated to the activation of iNOS, since Western blot analyses of iNOS showed tremendous increases in expression in the 24-month-old thoracic duct tissues when compared to the 9-month-old animal. These data provided the first evidence that the transmural pressure and imposed flow-dependent regulatory mechanisms of lymphatic function were greatly altered in aged TD. These alterations of active pumping mechanisms in TD appear to be related with age-related disturbances in NO-dependent regulatory pathways. The impaired chronotropy and inotropy in aged rat thoracic duct led to a greatly reduced lymphatic pump flow, indicating a diminished pumping ability. The differences between adult and aged animals in the stretch-induced responses at higher levels of transmural pressure demonstrate a diminished ability of the aged thoracic duct to adapt its function to increases in preload. This results in a loss of the lymphatic functional reserve to adapt its pumping to increased levels of lymph inflow in the aged thoracic duct. Similarly the loss of the flow-mediated modulation of function leads to a reduced efficiency of the lymph pump. Together, these result in a partial or complete failure of the lymphatic system to provide adequate transport of lymph. This impairment of lymph transport function may adversely affect the fluid balance and immune function in many organs of the elderly. Further investigations are needed into the mechanisms that modulate the lymphatic hydrodynamics during aging so as to address these problems and develop potential therapeutic schemes to treat them.

6. Summary

While important advances in our knowledge of the basic principles of the physiological regulation of lymph transport have been made in the last few decades, our understanding is still far from complete. Lymphatic function is constantly influenced by the complex factors of lymph hydrodynamics; pressure/stretch of the lymphatics, active and passive lymph pumps, and the subsequent combination of intrinsic and extrinsic flows/shears. Additionally there are important species and regional variations in the transport capacities and hydrodynamic sensitivities of lymphatics. Lastly many normal and pathologic conditions can adversely affect lymphatic transport function leading to a wide array of biological and clinical problems. A good example of this is seen in the negative impact that the aging process has on lymph transport. Thus understanding the principle mechanisms that drive and regulate lymphatic transport function will promote our comprehension of the impact of lymphatic dysfunction on various disease processes, as well as provide the basis for the development of therapies to counteract those dysfunctions and more effectively treat those disease processes.

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Table 1

Depiction of the pressures found in different sections of the lymphatic network. Lymph flow begins in the most peripheral portions of the network architecture and continues through the network of lymphatics and nodes towards the final junction of the main lymphatic trunks with the venous circulation (noted by arrows).

Lymphatic network position/flow direction	Lymph/blood vessel	Pressure (mmHg)
Outflow compartment	Left jugular vein	5.8
Most central lower left 3/4	Thoracic duct	5.1
Most central upper right 1/4	Right lymphatic duct	2.1
↑	Left jugular trunk	0.8
↑	Heart efferent trunk	2.9
↑	Hepatic trunk	3.4
↑	Intestinal trunk	3.6
↑	Left lumbar trunk	2.7
Most peripheral	Femoral trunk	0.5