J Physiol Sci (2015) 65:51–66
DOI 10.1007/s12576-014-0343-6

REVIEW

Proposed new lymphology combined with lymphatic physiology, innate immunology, and oncology

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Received: 29 September 2014 / Accepted: 14 October 2014 / Published online: 7 November 2014

Abstract As one of the lymphatic functions, it is well known that the transport and drainage of hydrophilic substances including plasma protein through the lymphatic system play pivotal roles in maintaining the homeostasis of the internal environment between the cells in tissues in collaboration with the exchange of the substances through the blood capillaries and venules. The physiological functions of the lymphatic system have been studied by many investigations of microcirculation, i.e., Yoffey and Courtice, Ruszunyak et al., Földie and Casley-Smigh et al., Roddie, Schmid-Schönbein et al., and Ohhashi et al. On the other hand, it is also well known that the initial clinical signs of primary diseases such as inflammation, tumors, and circulatory disorders including infarction and thrombosis appear as functional abnormalities of the internal environment in tissues. These abnormalities of the functions are strongly related to immunological defense reactions around the internal environment and abnormal actions of the transport and drainage of the lymphatic system. Taking into consideration the current inspired findings in lymphatic physiology, innate immunology, and oncology, we have proposed a new lymphology combined with new knowledge of the three above-mentioned academic fields from a defense mechanism points of view. In this review, we would like to demonstrate comprehensively our latest studies related to the possibility of establishing a new lymphology, hoping the readers will evaluate this possibility.

Keywords Active lymph transport · Nitric oxide · Condensing albumin in lymph · Inflammatory cytokine · Sentinel lymph node · Shear stress · Micrometastasis · Acidic microenvironment

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Introduction

Current rapid progress in lymphology has been triggered by the discovery of new markers of lymphatic endothelial cells such as lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1, 1), prospero-related homeobox 1 (Prox-1, 2), and podoplanin [3], and then the discovery of vascular endothelial growth factor (VEGF) C/D and VEGF receptor (VEGFR) 3-mediated lymphangiogenesis and lymphatic metastasis of carcinoma cells [4–8]. The progress led to holding the first Gordon Research Conference on the scheme of the lymphatic system in March 2004 in Ventura, CA, USA.

As one of the lymphatic functions, it is well known that transport and drainage of hydrophilic substances including plasma protein through the lymphatic system play pivotal roles in maintaining the homeostasis of the internal environment between the cells in tissues in collaboration with the exchange of substances through blood capillaries and venules [9, 10]. The physiological functions of the lymphatic system have been studied by many investigations of the microcirculation, i.e., Yoffey and Courtice [9], Ruszunyak et al. [11], Földie and Casley-Smigh et al. [12], Roddie [13], Schmid-Schönbein et al. [14], and Ohhashi

et al. [15]. On the other hand, it is also well known that the initial clinical signs of primary diseases such as inflammation, tumors, and circulatory disorders including infarction and thrombosis appear as functional abnormalities of the internal environment in tissues. These functional abnormalities are strongly related to immunological defense reactions around the internal environment and abnormal actions of the transport and drainage of the lymphatic system. Taking into consideration the current inspired findings in lymphatic physiology, innate immunology, and oncology, we have proposed a new lymphology combined with new knowledge of the three above-mentioned academic fields from a defense mechanism points of view. In this review, we would like to demonstrate comprehensively our latest studies related to the possibility of establishing a new lymphology, hoping the readers will evaluate this possibility.

New findings in lymphatic physiology

Intrinsic lymphatic pump mechanisms

An active pump of lymphatic capillaries

An important aspect of the lymphatic pump derives from the currently discovered structural features of lymphatic capillaries [16, 17]. The two most important features are as follows: (1) The endothelial cells forming the lymphatic capillaries are not bound tightly to each other; instead, they simply overlap. (2) The endothelial cells are tightly folded to the surrounding tissues by anchoring filaments, which attach to all parts of the endothelial cells except to the internal flap of each cell where it overlaps its adjacent cell. These two structural features allow fluid to enter the lymphatic capillary whenever the pressure outside the capillary is greater than the pressure inside. The fluid merely pushes the endothelial flap toward the interior of the capillary and wends its way into the capillary lumen. Then, whenever the pressure inside the capillary becomes greater than that outside, the endothelial flap closes over the space between the endothelial cells, and the fluid cannot escape. Thus, a lymphatic pump operates at the very tips of the lymphatic capillaries, because any compression and relaxation of the tissues, or of the lymphatic capillaries themselves, will create alternating pressure differences across the capillary wall. Fluid moves out of the lymphatic capillaries and up the collecting lymph vessels during the compression cycle. Then, during the relaxation cycle, it moves into the lymphatic capillaries from the surrounding tissue spaces. Therefore, our conception regarding the function of the lymphatic system has changed from that of a passive system to one that plays not only an active, but indeed a strongly active role. In fact, the findings go so far as to demonstrate that the lymphatic capillary endothelial cells themselves have a contractile nature and that their cytoplasm actually contains actomyosin [10].

Lymphatic active pump of collecting lymph vessels

In lower animals, particularly amphibians, the flow of lymph is maintained by rhythmically beating lymph hearts. Although there are no lymph hearts in mammals, all lymphatics other than the initial lymphatic networks contain smooth muscles in their walls [9].

Currently, it is also very clear that the rhythmic activity of collecting lymph vessels combined with the presence of valves inside these vessels can create pumping pressures as great as 20–30 mmHg [18]. Therefore, the lymphatic system is, in effect, a sump pump for the tissues, always attempting to propel excess free fluid away from the tissue spaces. In some animals, including humans, sheep, cattle, rat, and mice, the collecting lymph vessels have been shown to undergo spontaneous rhythmic contractions [19–23]. The frequency of heart-like contractions seems to be determined mainly by the amount of fluid in the lymph vessels. When a segment of the vessel immediately below a valve becomes distended, it contracts, and the fluid is pushed forward beyond the valve. The excess filling on the upstream side causes the next segment of the lymphatic vessel to contract, thus propelling the fluid forward to still another segment. In other words, each segment of the lymphatic vessel operates as a separate individual pump and is responsive to the amount of lymph that fills its chamber. Thus, the lymphatic pump activity is defined as an active propulsion mechanism of lymph mediated by rhythmic spontaneous contractions of lymphatic smooth muscles.

Mechanical and electrical characteristics of the spontaneous contractions in collecting lymph vessels

Studies in isolated lymphatic preparations have been of great importance for excluding passive external forces and characterizing the contractile process. Mislin [24] first started such in vitro experiments and introduced the term "lymphangion" for an intervalve segment as the contractile functional unit. Waldeck [25] extended these observations by recording active and passive pressure-volume curves in isolated 3- to 5-valve segments of hepatic and mesenteric lymph vessels from rats. He found that the contractile strength increased to a maximum at increasing transmural pressure and then fell off. The contractions of the fine lymph vessels (volume, 0.5 μl) were able to raise the

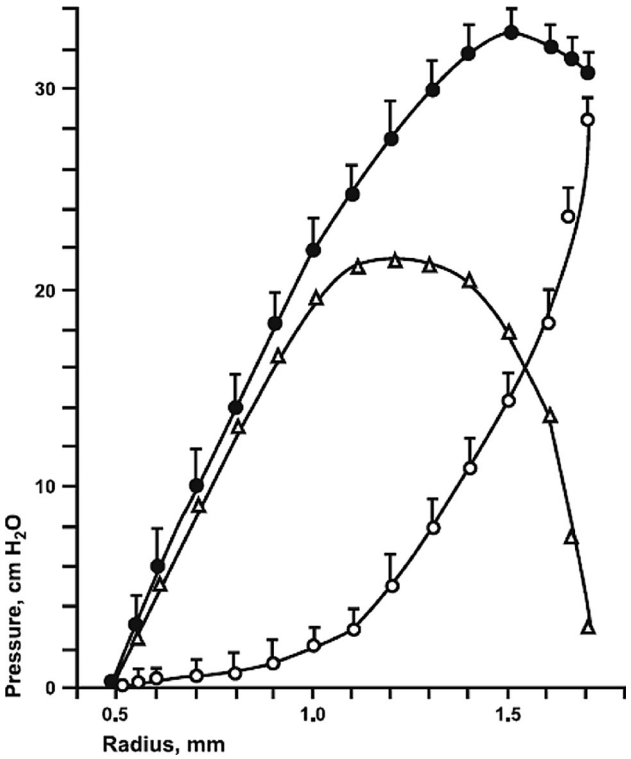

Fig. 1 Passive pressure-radius (*open circle*), total pressure-radius (*closed circle*), actively generated pressure-radius (*open triangle*) relationships in single lymphangion specimens isolated from bovine mesenteric collecting lymph vessels. *Vertical bars* stand for standard errors ($n = 10$). Data from Ohhashi et al. [21], with permission

luminal pressure by only 1–2 mmHg. A similar volume-pressure relationship was obtained by us [21] on an isolated lymphangion from bovine mesenteric lymphatics (volume, 300 µl) giving a maximal pulse pressure of 20 mmHg, as shown in Fig. 1. Even higher "systolic pressure," 60–120 mmHg, has been shown to be generated in obstructed popliteal lymph vessels in sheep [18] and in human legs [26]. An excellent characterization of the pumping properties of intact mesenteric collecting lymphatics in rats was obtained by Benoit et al. [22], combining continuous diameter and pressure measurements and analyzing the data in terms used to describe cardiac function. Under control conditions, the contraction frequency was 6.4 min^{-1}, the shortening velocity was very high compared with that of other types of smooth muscles, and the ejection fraction was 67 %. Increasing lymph formation by intravenous saline infusion increased end-diastolic volume, contraction frequency, and stroke volume, while criteria for inotropic effects, such as the ejection fraction and rate of increase of systolic pressure, showed less consistent results.

Tetrodotoxin fails to abolish the spontaneous contractions [27], suggesting a myogenic pacemaker that functions physiologically without nerve stimuli. A dominant pacemaker site seems to be located in each lymphangion immediately downstream from each valve [21], probably in the circular muscle layer. Within a given lymphangion the contraction spreads at a velocity of 4–5 mm/s [21], suggesting a cell-to-cell propagation as in cardiac or visceral smooth muscle. While a single contraction seems to be the rule during free flow, lymphatic obstruction and increasing pressure in human leg lymph vessels induce bursts of four to seven rapidly repeated contractions interspersed with silent intervals [26]. An activation threshold of 5–10 mmHg was found in these vessels. The importance of the rate of pressure change was pointed out by us [21], showing that both a rise and a rapid fall in transmural pressure may initiate contraction in isolated bovine mesenteric lymphangions.

We studied the electrical activity corresponding to the spontaneous contractions of lymphatic smooth muscles by using the sucrose gap [27] and intracellular microelectrode techniques [28, 29]. The mean resting membrane potential of the lymphatic smooth muscle cells is about −50 mV. The resting membrane potential sometimes shows rhythmic fluctuations or slow waves that resemble those in visceral smooth muscles [30]. The minimum depolarization necessary for inducing spontaneous contraction is about 6 mV in the lymphatic smooth muscle cells [29]. In potassium-free solution, the resting membrane potential is depolarized by about 9 mV, and then the lymphatic smooth muscles demonstrate a sustained contraction. Ouabain at 10^{-5} M also causes a depolarization of the membrane potential with a tetanic contraction in isolated bovine mesenteric lymph vessels. The findings suggest that changes in membrane potential seem to play a significant role in the activation of contractile proteins in lymphatic smooth muscles and that an electrogenic sodium pump exists on the plasma membrane of the smooth muscle cells. The depolarization and tension development in the potassium-free solution may be due to decreased activity of the electrogenic sodium pump in the lymphatic smooth muscle cells.

Two types of spontaneous contraction-mediated active pump

As described earlier by Baez [31], although a contraction usually appears to the observer as an instantaneous total contraction of the vessel wall, its progressive peristaltic character was clearly demonstrated by high-speed cinemicrophotography, as confirmed by us [21] and Benoit et al. [22]. However, according to Mislin and Rathenow [32], contraction waves may also spread in the upstream direction over several segments, without being elicited by an increase in local transmural pressure. Other investigators have described propagated peristaltic waves and suggested

that contraction and emptying of one segment would increase the pressure in the next lymphangion and thereby trigger its pacemaker [18, 25]. Mathematical models based on this concept led to concluding that the confluence of vessels, finally converging on the thoracic duct, may cause an irregular contraction rhythm and a very irregular flow pattern in the larger collecting lymph vessels. They also pointed out that an increase in the pressure threshold for activation from upstream to downstream segments would facilitate the coordination of contractions between the segments and increase the efficiency of lymph transport. Such a threshold gradient was indeed confirmed experimentally by Hargens and Zweifach [33]. The finest collecting lymph vessels in the rat mesentery with a diameter of 30–40 μm showed an average threshold of 4 cm H_2O, compared with ~10 cm H_2O in vessels with diameters of 220 μm.

We evaluated the reasons why regular and irregular spontaneous contractions were observed in isolated lymph vessel preparations by using pumping preparations of collecting lymph vessels and transmural electrical stimulation [34]. We demonstrated the very interesting finding that an activation of noradrenergic sympathetic nerve fibers innervated into the wall of lymph vessels [35] caused a clear movement of the pacemaker site of the regular spontaneous contractions of peristalsis type, resulting in the appearance of irregular spontaneous contractions of pendular type. Thus, collecting lymph vessels, about 5 cm long and 1–3 mm in diameter, were dissected from fresh bovine mesentery, cannulated at both ends, and set up in Krebs-bicarbonate solution in a horizontal organ bath so that spontaneous contractions of the vessel produced propulsion of intravascular fluid. The outflow pressure and outer diameter of the lymph vessel at the pacemaker site of the contractions were simultaneously measured by a pressure transducer and a hand-made new diameter gauge with an image sensor [36]. The platinum electrode was adjusted at the pacemaker site in order to selectively stimulate noradrenergic nerve fibers innervated on the pacemaker cells. Figure 2 shows representative responses of two kinds of pumping lymphatic preparations to the electrical stimulation, which are rectangular pulses of 50 V, 0.5 ms, and 2 Hz. As shown in the right panel, in most preparations the electrical stimulation of the pacemaker site, which is located in the wall in the immediate vicinity of the inlet valve, caused the pacemaker site to move to the intervalvular region of the lymphangion (P' in the panel). Spontaneous contractions with the new pacemaker site, resulting in contractions of the pendular type, produced passive distension of the outer diameter at the valvular region. About 1 min after an interruption of the stimulation, the moved pacemaker site returned to the previous one, the valvular region. On the other hand, as shown in the left panel, in some preparations (example for increasing the environmental temperature) the pacemaker site of the spontaneous contractions is located at the middle portion of

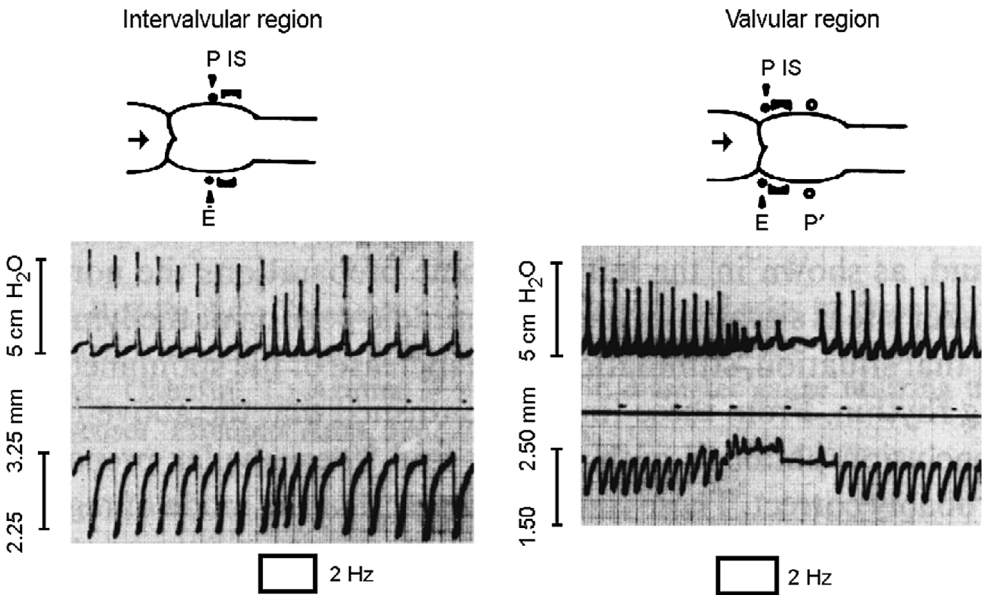

Fig. 2 Representative responses of two kinds of pumping preparations in bovine mesenteric collecting lymph vessels to electrical stimulations, which are rectangular pulses of 50 V, 0.5 ms in duration at 2 Hz. The pacemaker sites are situated in the valvular (*right panel*) and intervalvular (*left panel*) region, respectively. *P* pacemaker position of the rhythmic spontaneous contractions in each preparation, *PV* new pacemaker position of the contractions, *IS* recording position of outer diameter of the lymph vessels in each preparation, and *E* electrical stimulating position in each preparation. Data from Ohhashi [34], with permission

the lymphangion. In that case, the electrical stimulation produced an increase of the frequency of the contractions only, but did not move the pacemaker site. The findings suggest that the regulatory action of noradrenergic nerve fibers on the lymphatic pump activity may depend on the position of the pacemaker site of the spontaneous contractions, resulting in the appearance of peristalsis- or pendular-type contractions.

What is the physiological significance of the irregular pendular-type spontaneous contractions involved in several lymphangions? One possibility may be related to absorbing fluid and protein through the lymphatic capillaries, providing an increase in lymph formation. In fact, Benoit et al. [22] demonstrated that lymphatic pumping accounted for the majority of increases of lymph formation by less than five times control.

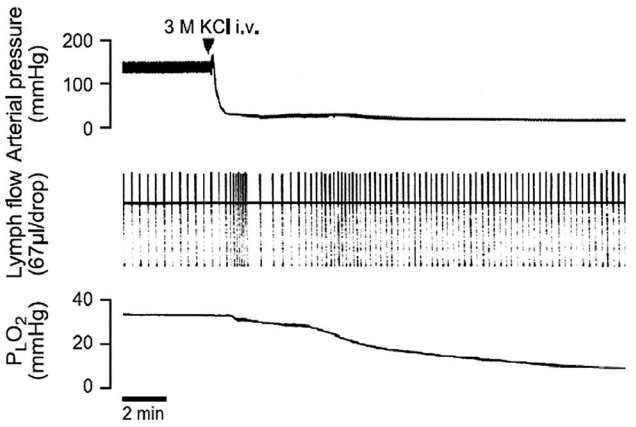

Fig. 3 Representative tracings of changes in systemic arterial pressure (*upper panel*), the flow rate of lymph (*middle panel*), and PO_2 in lymph (*lower panel*) in an anesthetized dog before and after an intravenous administration of 3 M KCl solution. The marker is a time scale of 2 min. Data from Ohhashi et al. [15], with permission

Oxygen tension in lymph

Oxygen gradients in lymph through the lymphatic system

The presence of a significant perimicrovascular oxygen gradient [37] determines that tissue PO_2 should always be significantly lower than capillary blood PO_2 and therefore also venular and venous blood PO_2. This concept has been verified by measurements of tissue PO_2 with the phosphorescence technique. It is noteworthy that this technique, which reveals significant oxygen gradients in the perimicrovascular tissue, tends to show a relatively uniform PO_2 environment [37]. By measuring of oxygen tensions (PO_2) of blood and lymph with a modified Clark needle oxygen electrode, Bergofsky et al. [38] proposed that a better estimate of tissue PO_2 could be obtained by measuring the PO_2 of excess tissue fluid (lymph) that returns to the circulation via the lymphatic system. They revealed that marked differences existed between the gaseous composition of lymph and blood; the PCO_2 of lymph was an average of 5 ± 3 mmHg higher than that of venous blood. On the other hand, the oxygen tension of lymph differed markedly from the PO_2 of blood; whereas the average PO_2 of arterial blood was 80 mmHg and that of venous blood 42 mmHg, the PO_2 of lymph averaged only 8 ± 6 mmHg.

This concept was reevaluated using polarographic oxygen electrodes by Barankay et al. [39] in the lymph vessels of the rabbit hindlimb and by Farrell et al. [40] in the mesenteric lymph vessels of dogs. The microelectrode studies were carried out in relatively large lymph vessels, yielding an average lymph PO_2 of 28 and $\sim$50 mmHg in the hindlimb and mesentery, respectively. They concluded that PO_2 values of the fluid in collecting lymph vessels and thoracic ducts were not representative of tissue PO_2. Recently, Hangai-Hoger et al. [41] attempted to measure the PO_2 of lymph in mesenteric lymph vessels (mean diameter, 43.6 μm) of anesthetized rats by using the oxygen phosphorescence quenching technique. They also confirmed that the PO_2 of the lymph and perilymphatic adipose tissue was 20.6 ± 9.1 and 34.1 ± 7.8 mmHg, respectively.

We also investigated the PO_2 of lymph through the thoracic ducts in anesthetized dogs by using an oxygen electrode. We also examined the effects of 3 M potassium chloride-mediated cardiac arrest on changes in the flow rate of lymph and PO_2 value of the lymph [42]. Thus, the mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated artificially using a respirator with room air. The thoracic duct was cannulated at the cervical position of the vessel with a polyethylene catheter equipped with a needle-type oxygen electrode. The outer end of the catheter was attached with a domestic-made drop counter flow meter that remained fixed at the same position as that of the heart at the hydrostatic pressure level. With the flow meter, the changes in the flow rate of lymph were measured continuously. The femoral artery and vein were also cannulated with polyethylene catheters to measure changes in systemic arterial pressure and administer physiological saline solution at a constant rate of 100 ml/h during the experiment. Figure 3 shows representative recordings of changes in the PO_2 of lymph, the flow rate of lymph, and systemic arterial pressure before and after the administration of 3 M KCl in an anesthetized dog. The PO_2 of lymph at physiological conditions was around 35 mmHg in the anesthetized dog. An intravenous administration of 3 M KCl produced a rapid and large reduction of the arterial pressure and then resulted in cardiac arrest of the dog. The cardiac arrest caused a transient increase of the lymph flow rate that was maintained for $\sim$15 min after the cardiac arrest. It also produced a

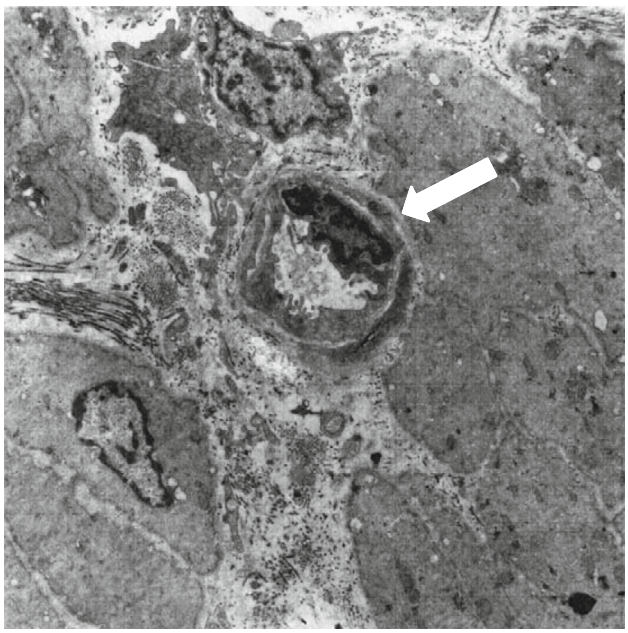

Fig. 4 Transverse section of a blood capillary found within the external longitudinal smooth layer of bovine mesenteric collecting lymph vessels (×2400). The endothelium of the blood capillary (a *white arrow*) has many pedicles that protrude into the lumen. Continuous basement membrane is found around the blood capillary. Data from Ohhashi et al. [27], with permission

gradual decrease of the PO_2 in the lymph, which became stable, ~10 mm Hg, at 15 min after the cardiac arrest.

In conclusion, there is a significant longitudinal gradient of PO_2. Thus, mean PO_2 levels in lymphatic capillaries, collecting lymph vessels, and thoracic ducts rose sequentially from ~8, ~20, and ~35 mmHg, respectively. Therefore, it should be emphasized that the lymphatic endothelial cells seem to have physiological functions under the specific environment of lower oxygen tension of 8–35 mmHg.

Relationship between oxygen tension in lymph and spontaneous contraction-mediated active pump

It is noteworthy that lots of vasa vasorum exist within the media of collecting lymph vessels with spontaneous contractions [43], which may be essential for maintaining the vigorous contractions of lymphatic smooth muscles because of the above-mentioned lower oxygen tension in lymph. Thus, smooth muscles in bovine mesenteric collecting lymph vessels are well developed and arranged in three layers, namely, the internal longitudinal, intermediate circumferential, and external longitudinal. The outer longitudinal layer is much thicker than the other two layers. There are few elastic fibers but a large number of collagen fibers underneath the endothelial cell lining and among the smooth muscle layers. A large number of mitochondria, gathered in a cluster, are seen on both sides of the nucleus along the longitudinal cell axis of the smooth muscle cells. Numerous glycogen granules were found among and around the mitochondria. These structural features might be a morphological manifestation of the high metabolic activity required for spontaneous contractions of the lymphatic smooth muscles [43].

Figure 4 shows a transverse section of blood capillary found within the external longitudinal smooth muscle layer. It was identified as a blood capillary because of the presence of a complete basement membrane and the configuration of the endothelium. Occasionally, erythrocytes were found in the lumen. The presence of vasa vasorum within the media may reflect a relatively high oxygen requirement of the lymphatic smooth muscle cells and the relatively low oxygen supply from the lymph flowing through the lymph vessel. An ample supply of oxygen is required to maintain the spontaneous contractions in the collecting lymph vessel.

Nitric oxide and oxygen radicals in the lymphatic system

Oxygen is an important regulator of microvascular tone throughout most vascular beds in many species [44]. The study of the role of oxygen in microvascular regulation has been greatly impacted by the finding that reduced oxygen availability can increase the release of endothelium-derived nitric oxide (NO) [45, 46]. Manevich et al. [47] and Wei et al. [48] also demonstrated an 80 % increase in NO generation during acute oxygen deprivation, and the NO response was evident within 15 s after decreased oxygen availability. Various investigators have also demonstrated that hyperpolarizing factors [49], cyclo-oxygenase products [50], and adenosine [51, 52] can stimulate endothelial cells during oxygen deprivation. Recently, Nase et al. [44] demonstrated that in rat intestine, reduced oxygen availability increased both arteriolar and venular NO and that the main site of NO release under these conditions was from endothelial cells.

Taking into consideration the above-mentioned backgrounds of NO and reactive oxygen radicals (ROS), it may be reasonable to expect that NO and ROS may play important roles in the regulation of the spontaneous contractions-mediated lymph transport in physiological or pathophysiological conditions. In fact, the dense immunoreactivities of ecNOS and iNOS were confirmed in cultured canine lymphatic endothelial cells [53] and in bovine mesenteric lymph vessels in vivo [54].

Nitric oxide inhibits spontaneous contractions of lymphatic smooth muscles

Lymphatic endothelial cells, as well as arterial and venous endothelial cells, have the potential to generate endogenous NO [55–57].

In precontracted canine thoracic ducts with intact endothelium, acetylcholine (ACh) produced dose-related relaxations. The relaxations seemed to be mediated via the high-affinity muscarinic receptor subtype, because they were competitively antagonized by atropine, demonstrating a pA_2 value of 10.4 in the Arunlakshana and Schild [58] analysis. In contrast, in isolated rings of rabbit thoracic aorta and canine femoral artery, ACh-mediated endothelium-dependent relaxations were produced by a low-affinity muscarinic receptor subtype only (pA_2: 8.4–8.8, 59). Thus, it may be a characteristic feature of lymph vessels that the high-affinity muscarinic subtype is related to ACh-induced endothelium-dependent relaxation. Mechanical rubbing of the endothelium significantly reduced the ACh-induced relaxation. Pretreatment with aspirin, an inhibitor of cyclooxygenase, did not affect the ACh-mediated relaxation, suggesting that prostacyclin and the other vasodilative prostaglandins did not play a pivotal role in the ACh-mediated relaxation of the thoracic ducts. On the other hand, oxyhemoglobin (an inhibitor of NO, 60), L-NMMA (an inhibitor of NO biosynthesis, 61), and methylene blue (an inhibitor of guanylate cyclase, 62) markedly suppressed the ACh-mediated relaxation in canine thoracic ducts with intact endothelium. ACh also produced a marked relaxation in the so-called sandwich-mounted preparation, demonstrating that the lymphatic endothelial cells of the longitudinal strip in response to ACh must release some transferable substance(s) that, on diffusion into the ring segment, activated relaxation of the precontracted lymphatic smooth muscle cells. The findings strongly suggest that the ACh-induced relaxations are mainly mediated through the release of NO or its related compound(s) from the lymphatic endothelial cells and diffusion of the substance(s) in the wall of canine thoracic ducts. The substance(s) diffuse into the smooth muscle cells and then produce the accumulation of cellular guanosine 3′, 5′-cyclic mono-phosphate (GMP), which results in the relaxation of canine thoracic ducts [55].

Mizuno et al. [56] also elucidated the nature of endothelium-derived factors, produced in basal conditions and in response to agonists, that affect the smooth muscle tone of cannulated with glass micro-pipettes and pressurized rat iliac lymph vessels. They concluded that endothelium NO and prostaglandins are important mediators of lymphatic vasomotion.

NO release from lymphatic endothelial cells is also known to be able to inhibit the rhythm and amplitude of the rhythmic pump activity of isolated bovine mesenteric collecting lymph vessels [63]. Regular rhythmic pump activity at a constant rate of 2–4 beats/min were observed. ACh at concentrations between 10^{-7} and 10^{-6} M caused both negative chrono- and inotropic effects on the rhythmic pump activity. The ACh-induced negative chrono- and ino-tropic effects were significantly reduced when the intact endothelium of the lymph vessels was removed mechanically. The ACh-induced negative chrono- and ino-tropic effects were significantly reduced by pretreatment with 3×10^{-5} M L-NMMA. An additional treatment with 10^{-4} M L-arginine caused a complete reversal of the L-NMMA-mediated reduction of the ACh-induced both negative effects on the rhythmic pump activity (Fig. 5). Endogenous NO liberating from the lymphatic endothelial cells seems to inhibit pacemaker activity of the rhythmic pump activity and reduce the myogenic conduction and/or the contractile ability of lymphatic smooth muscles. A marked increase of cytosolic 3′, 5′ cyclic GMP content in the lymphatic smooth muscle cells may also contribute to the NO-mediated negative chrono- and ino-tropic effects on the rhythmic pump activity in isolated bovine mesenteric collecting lymph vessels.

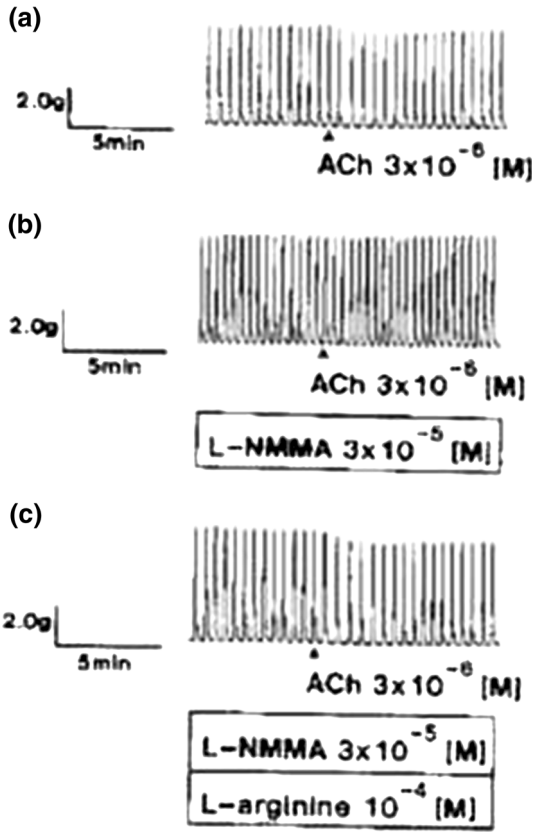

Fig. 5 Representative recordings of the effects of L-NMMA (**b**) and an additional treatment with L-arginine in the presence of L-NMMA (**c**) on the 3×10^6 M acetylcholine-induced negative chronotropic and inotropic effects on spontaneous contractions (**a**) in the same lymphatic preparation isolated from bovine mesenteric lymphatics. Data from Ohhashi et al. [116], with permission

Atrial natriuretic peptides (ANP) also caused negative chrono- and ino-tropic effects on rhythmic pump activity in the isolated bovine mesenteric collecting lymph vessels

through synthesis of 3′, 5′ cyclic GMP in the walls, independent of the lymphatic endothelial cells [64].

Nitric oxide inhibits the spontaneous contraction-mediated active pump in vivo

Shirasawa et al. [65] attempted to evaluate the physiological roles of endogenous NO in lymphatic pump activities of rat mesenteries in vivo by using an intravital videomicroscope system. Changes in the pumping frequency (F), end-diastolic diameter (EDD), and end-systolic diameter (ESD) of the mesenteric lymph microvessels were measured with the microscope system, and then the pump flow index (PFI) was calculated. A 15-min superfusion of 30 μM L-NAME in the mesenteries caused a significant increase of F and PFI and a significant decrease of the EDD and ESD. Simultaneous superfusion of 1 mM L-arginine with 30 μM L-NAME produced a significant reversal of the L-NAME-mediated increase of F and decrease of ESD. A 15-min superfusion of 100 μM aminoguanidine caused no significant effect on the F, EDD, and ESD of the mesenteric lymph vessels in vivo. They concluded that endogenous NO physiologically modulated the lymphatic pump activity in rat mesentery in vivo and that the production and release of NO may be mediated by endothelial constitutive NOS but not by inducible NOS. The conclusion may be compatible with the studies obtained with anesthetized sheep [66].

Lymph flow-induced generation of nitric oxide from lymphatic endothelial cells

We also studied what physiological factor(s) contribute to the NO-dependent inhibition of lymphatic pump activity in vivo. Many previous studies showed that an increase in flow rate (in the presence of constant intraluminal pressure) increased the diameters of arterioles [67] and venules [68, 69] in an endothelium-dependent manner. Therefore, we examined the effects of flow on lymphatic endothelial cells by using cascade arterial preparations without intact endothelium. The pressurized canine thoracic ducts were intraluminally perfused at a constant flow rate ranging from 0.5 to 2 ml/min. The flow rate of 2.0 ml/min produced ~30 % of sodium nitroprusside (SNP)-induced maximal relaxation of the cascade bioassay preparations. The flow-mediated relaxation of the bioassay preparations was completely reduced by the mechanical rubbing of the lymphatic endothelial cells. Pretreatment with 5×10^{-5} M L-NAME on the lymphatic endothelial cells caused a significant reduction of the flow-mediated relaxation of the bioassay preparations. Pretreatment with 10^{-5} M indomethacin on the endothelial cells produced no significant effect on the flow-mediated relaxation. The authors suggested that the lymphatic endothelial cells can produce and release endogenous NO, but not vasodilative prostaglandin (PG), by the stimulation of flow (~2.0 ml/min). In addition, a linear relationship was observed between the flow rate and the normalized amount of endogenous NO released from the lymphatic endothelial cells [70].

Gashev et al. [71] also studied the effects of imposed flow on active lymph pumping under conditions of controlled intraluminal pressure. Rat mesenteric lymph vessels were isolated, cannulated, and pressurized. Input and output pressures were adjusted to respond to various flows in the lymphatic endothelial cells. Lymphatic systolic and diastolic diameters were measured and used to determine contraction frequency and pump flow indices. Imposed flow inhibited the active lymph pumping in the mesenteric lymph vessels. Thus, the imposed flow reduced the frequency and amplitude of the rhythmic pumping. NO was partly but not completely responsible for the inhibitory action of flow on the mesenteric lymph pumping. Exposure to NO mimicked the effects of flow, and inhibition of the ecNOS by L-NMMA attenuated but did not completely reduce the inhibitory effects of flow.

New findings in new lymphology-related innate immunology

The lymphatic system has been known to cooperate with lymph nodes and then support the biological defense mechanisms, but it has not fully emerged to the forefront of immunology or inflammatology. This is partly related to the evidence that molecular biology and molecular genetics primarily dealing with lymphocytes, cytokines, and immunoglobulins have been regarded as the mainstay of immunological research, but the dynamics of the movement of lymphocytes and lymph through the lymph vessels and lymph nodes have been overlooked in immunology. In addition, the technical difficulty of relative evaluation of the dynamic movement of lymphocytes, the lymph flow rate, and the concentration of protein in lymph has contributed to the lack of attention to the interaction with innate immunology and lymph flow dynamics.

Recirculation of filtered plasma protein from venules through the lymphatic system

Since the 1950s, the lymphatic system has been known to be vital for fluid homeostasis, recirculation of plasma protein, and immune surveillance under physiological conditions [72]. Thus, most of plasma protein, especially albumin, is known to be filtered through the walls of venules into interstitial spaces and is returned through the lymphatic system to the venous circulation via the thoracic

duct. In addition, chemical and electrophoretic analyses of blood plasma and lymph have shown that all of the proteins are found in the lymph, although in lower concentrations in most instances [9]. However, the precise mechanisms by which proteins are transferred across the blood capillary and venular walls to the tissue spaces and ultimately to the lymph vessels are still unknown. In addition, the physiological and pathophysiological meanings of the recirculation of plasma protein through the lymphatic system are still unsolved.

Condensing mechanisms of plasma protein in lymph

It is well known that lymph protein increases during the transport of the lymph from peripheral lymph vessels to thoracic ducts [73–75]. Heterogeneity in the concentration of lymph protein between the afferent and efferent lymph vessels of the regional lymph nodes has also been reported [76–79]. These results suggest that the concentration of lymph protein is actively and/or passively modulated while lymph returns to the systemic blood circulation. These results on the changes in the concentration of lymph protein are based on in vivo studies and the collection of lymph fluids. However, no finding to demonstrate the possibility of a single lymph vessel condensing the concentration of albumin in lymph is available. Therefore, we attempted to evaluate whether or not isolated small lymph vessel walls are able to condense the concentration of albumin in lymph. According to the regression analyses of the relationship between concentrations of the dyes and the intensities of the digitized images in the glass pipettes, we could determine the concentration of the dyes in the intraluminal space of the lymph vessels and could calculate the net flux of the dyes through the lymph vessel wall. Thus, the study demonstrated that NaFl and 4 kD significantly (>5 % of the initial concentration) passed through the wall of lymph vessels, and 12 kD slightly ($\sim$5 % of the initial concentration) penetrated the wall. On the other hand, 70 kD did not permeate the lymphatic walls ($\sim$0 % of the initial concentration). Macromolecules with a molecular weight as high as or higher than 6,000 remain in the lymph vessels and are returned to the systemic circulation via the regional lymph nodes and thoracic ducts. On the other hand, smaller molecules including water, sodium, and urea leave from the intraluminal space to the matrix tissues around the lymph vessel. Thus, the limit of permeability through the walls of the lymph vessels is considered to be between 2,300 and 6,000 mol wt [80, 81]. These results strongly support the findings that NaFl and 4 kD but not 12 and 70 kD could significantly permeate from the intraluminal to the extraluminal space of the lymph vessels. The study is the first demonstration of the transport of small molecular hydrophilic substances through the walls of a single isolated lymph vessel. In addition, the nonpermeability of 70 kD in the walls of the lymph vessels may reflect the nature of albumin (69,000 mol wt) collection in the lymphatic system. In conclusion, the walls of small-sized lymph vessels may play a crucial role in condensing the concentration of lymph protein.

Effects of inflammatory cytokines on the condensing effect of plasma protein in lymph

To address the physiological and pathophysiological meanings of the condensing effect of albumin in lymph through the small lymph vessel walls, we established human lymphatic endothelial cells (LEC) and evaluated the size-dependent regulation of the permeability of such layers to hydrophilic substances. We also investigated the effects of tumor necrosis factor (TNF)-α or interleukin (IL)-1β such as one of the inflammatory cytokines on the permeability and on the morphology of human LEC [82]. Significant amounts of 4 kDa dextran, but not 12 or 66 kDa dextran, passed through the layers. TNF-α or IL-1β induced significant increases in the permeability to 4- and 12-kDa dextrans (Fig. 6). TNF-α or IL-1β also produced a significant redistribution of the cytoskeletal F-actin in the LEC, which resulted in changes in their shape. Pretreatment with Y-27632, a Rho kinase inhibitor, or PD98059, an extracellular signal-regulated kinase (ERK) phosphorylation inhibitor, significantly abolished the TNF-α- or IL-1β-induced increases in the permeability of the layers to 4- and 12-kDa dextrans. Y-27632 and PD98059 significantly inhibited the changes in the F-actin distribution of the LEC produced by TNF-α or IL-1β. TNF-α or IL-1β caused significant increases in ERK 1/2 phosphorylation in the LEC, which were significantly inhibited by Y-27632 or PD98059. These findings suggest that the human LEC layer plays key roles in the transport of hydrophilic substances through collecting lymph vessel walls and that TNF-α or IL-1β significantly increases the permeability of the layers to 4- and 12-kDa dextrans via Rho kinase activation and the ERK 1/2 phosphorylation-mediated reorganization of F-actin in the LEC.

Plasma protein in lymph-mediated excretion of lymphocytes from regional lymph nodes

It is well established that lymph nodes and small lymph vessel walls have a condensing effect of albumin in lymph. In addition, a significant amount of albumin in lymph may accompany the migration of lymphocytes across the endothelium of postcapillary venules within the lymph nodes, and the mechanisms of protein transfer in the lymph nodes may in part explain the higher

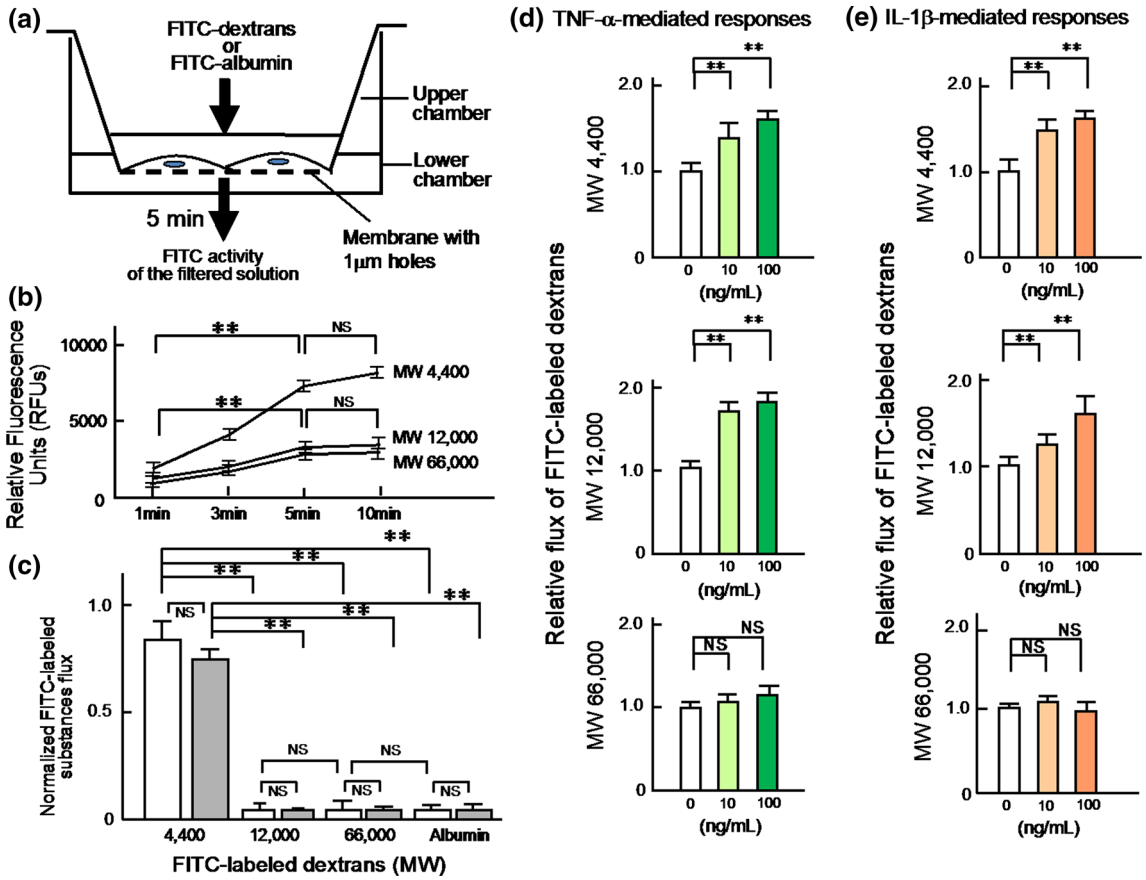

Fig. 6 Molecular weight-dependent permeability of the human LEC layers to hydrophilic substances and effects of TNF-α or IL-1β on the permeability of the LEC layers to hydrophilic substances. **a** A schematic diagram of an in vitro cell barrier permeability kit contained a 24-well cell culture plate insert and polyethylene terephthalate filters with a 1-μm pore size. The fluorescent activity of the solution that had filtered into the lower chamber was determined using a fluorescence plate reader 5 min after the addition of 1 mg/ml of the FITC-labeled dextran or albumin into the upper chamber. **b** The time-dependent changes in the relative fluorescence units (RFUs) into the lower chamber of the permeability kit evaluated using the fluorescent plate reader with the FITC-labeled 4,400, 12,000, and 66,000 Da dextrans. The ordinate shows the RFUs. The abscissa is the time after the addition of 1 mg/mLlof the FITC-labeled dextrans into the upper chamber. $^{**}p < 0.01$, a significant difference exists between these columns ($n = 8$). *NS* not significant ($n = 8$). **c** The permeability of human LEC layers to FITC-labeled 4,400, 12,000, and 66,000 Da dextrans and FITC-labeled albumin. The ordinate shows the normalized FITC-labeled substance flux, which is defined as the ratio of the level of fluorescent activity in the solution at the lower chamber, which was assessed 5 min after the addition of the FITC-labeled hydrophilic substance in the upper chamber to that of a solution containing the same FITC-labeled hydrophilic substance that had only been passed through a polyethylene filter (pore size: 1 μm). $^{**}p < 0.01$, a significant difference exists between these columns ($n = 9$). *NS* not significant ($n = 9$). **d** The effects of TNF-α [10 (*light green*) or 100 (*green*) ng/mL] on the permeability of human LEC layers to FITC-labeled 4- (upper panel), 12- (*middle panel*), and 66-kDa (*lower panel*) dextrans. The ordinate shows the relative flux of FITC-labeled dextrans, which is defined as the ratio of the level of fluorescent activity in the solution in the lower chamber at 5 min after the addition of the FITC-labeled hydrophilic substance in the upper chamber to the fluorescent activity of the original test solution. $^{**}p < 0.01$, significantly different from no treatment with TNF-α ($n = 9$). NS, not significant ($n = 9$). **e** The effect of IL-1β [10 (*light orange*) or 100 (*orange*) ng/ml] on the permeability of human LEC layers to FITC-labeled 4- (*upper panel*), 12- (*middle panel*), and 66-kDa (*lower panel*) dextrans. The ordinate is the same item as shown in panel D. $^{**}p < 0.01$, significantly different from no treatment with IL-1β ($n = 9$). *NS* not significant ($n = 9$). Data from Kawai et al. [82], with permission

concentration of albumin observed in the efferent lymph of the regional node compared with that in the afferent lymph [76, 79, 82].

However, the detailed physiological meaning of such increases in the albumin concentration of lymph through lymph nodes and small lymph vessel walls is still unknown. One possibility is that the protein concentration of lymph may be an important regulator of innate immunity. Thus, the excretion of non-selective T- and B-cell lymphocytes and natural killer (NK) cells into the efferent lymph vessels of lymph nodes was confirmed to be positively correlated with the protein concentration of lymph in the afferent collecting lymph vessels [76, 77, 79, 82, 83, 84].

New findings in new lymphology-related oncology

It is well known that carcinoma, which is an epithelial-origin tumor, is likely to metastasize lymphogenously and that sarcoma, a non-epithelial-origin tumor, is likely to metastasize hematogenously. However, there has been little systemic research to clarify the mechanisms of lymphatic metastasis of carcinoma cells focusing on the functional properties of tissue spaces (internal environment), relationship between the expression of molecular markers on the carcinoma cells and lymphatic metastasis of the cells, lymph flow-mediated interactions between lymphatic endothelial cells and carcinoma cells, or lymph dynamic analysis of carcinoma cells through the lymph vessels and lymph nodes. On the other hand, the clinical impact of the sentinel lymph node (SLN) concept has become one of the most important topics in surgical oncology in patients with breast cancer and melanoma [85].

The sentinel lymph node (SLN) is constantly subjected to a high lymph flow rate?

Recently, gastric cancer has also been identified as a target for SN navigation surgery (SNNS). We attempted to evaluate the usefulness of the contrast-enhanced ultrasound (CEUS)-guided method with Sonazoid for imaging of the lymphatic channels and the SLN of stomach in a porcine model. Contrast imaging using the intragastric or transcutaneous CEUS-guided method with Sonazoid enabled us to produce clear images of the afferent lymph vessel and SLN of the stomach until 2 h after the injection of Sonazoid. Intranodal flow of the microbubble agent could be also be clearly identified using tissue linear harmonic images of the SLN [86].

However, it remains unclear which factors play pivotal roles in deciding which lymph node becomes the SLN. We have considered the SLN to be constantly subjected to a high lymph flow rate. We reached this conclusion in the above-mentioned ultrasound study [86], in which a high lymph flow rate was detected within the SLN of the stomach using the CEUS-guided method with Sonazoid.

Shear stress-induced ATP-mediated ICAM-1-dependent micrometastasis of carcinoma cells in SLN

The SLNs are the most common and earliest site of malignant tumor metastasis. The clinical success of sentinel node navigation surgery [87] suggests that SLNs are an effective mechanical barrier against migrating cancer cells. The SLN also contains marginal endothelial cells, which might be constantly loaded with high shear stress. It is known that primary tumors influence the microenvironments of distant organs during the development of metastasis [88, 89]. However, it is unclear which molecules in premetastatic SLN loaded with high shear stress produce a suitable environment for micrometastasis within the node. Thus, we examined the hypothesis that the high shear stress generated by increased lymph flow through the SLN and its afferent lymph vessels contributes to the development of a premetastatic environment that is suitable for carcinoma micrometastases within the node.

Therefore, we attempted to investigate the effects of shear stress (1) on the expression of adhesion molecules on cultured human lymphatic EC isolated from the afferent lymph vessels nearest to the SLN and (2) on the release of ATP from human lymphatic EC (LEC) and (3) to study whether shear stress-mediated increases in adhesion molecule expression accelerate the attachment of carcinoma cells to cultured human LEC. Finally, in in vivo rat experiments we (4) evaluated whether the ATP released from lymphatic endothelial cells in response to shear stress stimulation facilitates the expression of carcinoma cell-ligated adhesion molecules within rat SLNs.

In conclusion, shear stress stimulation induced ATP release by activating cell surface F_1/F_0 ATP synthase, which resulted in the overexpression of ICAM-1 on human LEC and hence facilitated the ICAM-1-mediated attachment of carcinoma cells to human LEC in the afferent lymph vessels of SLN from breast cancer patients (90, Fig. 7).

Crucial roles of ICAM-1 in micrometastasis of carcinoma cells

ICAM-1 expression by tumor cells has been reported to be a major contributor to the facilitation of metastatic progression [91, 92]. Recently, we also observed strong ICAM-1 expression in human breast cancer SLN tissue that had been subjected to carcinoma cell micrometastasis, but weak or no ICAM-1 expression in SLN tissue that had not been subjected to metastasis [93]. On the other hand, studies of leukocyte-endothelial cell adhesion in tumor microvessels have demonstrated diminished adhesive interactions under both basal and cytokine-stimulated conditions [94]. It has been suggested that the proposed downregulation of endothelial ICAM-1 expression facilitates tumor progression by allowing tumor cells to avoid immunosurveillance by circulating lymphocytes. However, there have been several other immunohistochemical studies of the tumor vasculature in which the enhanced expression of endothelial ICAM-1, which resembles an inflammatory phenotype, was detected in breast cancer [95]. Thus, the adhesion molecule expression profile of human LEC remains unclear. Thus, except for that obtained in this study, no information exists regarding the effects of shear stress stimulation on human LEC located near and/or

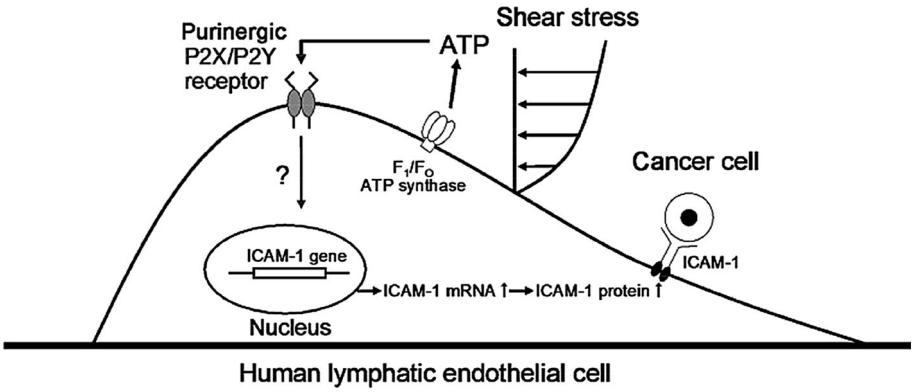

Fig. 7 Schematic diagram outlining shear stress stimulation inducing ATP release by activating F_1/F_0 ATP synthase, which results in the overexpression of ICAM-1 on human lymphatic endothelial cells. Data from Kawai et al. [90], with permission

within the SLN, particularly with regard to their expression of adhesion molecules, their interactions with carcinoma cells, and their role in the development of a premetastatic microenvironment that encourages carcinoma micrometastasis. Therefore, this study suggests that shear stress stimulation plays crucial roles in the establishment of a premetastatic environment within SLN [90].

Cell surface F_1/F_0 ATP synthase contributes to interstitial flow-mediated development of the acidic microenvironment in tumor tissues

In the past, most cancer research was based on genetics or biochemistry. In such studies, it was found that genes turn stimulatory chemical signals and protein cascades on or off in carcinoma cells [96]. However, the crucial roles played by physical factors in the development of the tumor tissue microenvironment were largely ignored. Recently, it was clearly demonstrated that primary tumors influence the tumor tissue microenvironment and microcirculation prior to carcinoma cell metastasis [88, 89, 97]. One of the pathophysiological changes observed in the primary tumor microenvironment is the development of acidic tumor tissue. The high glucose consumption and lactic acid production rates of carcinoma cells are known to be key factors for the development of acidic tumor microenvironments [98]. On the other hand, several sophisticated molecular mechanisms are responsible for maintaining the alkaline pHi and the acidic pHe in tumor cells [99]. These include proteins that import weak bases such as the HCO_3^- ion into the cells and proteins that export weak acids generated during metabolism such as carbonic acid or lactic acid out of the cells [100]. H^+ ions are also directly extruded from the cells by means of the vacuolar ATPase (V-ATPase) [101, 102]. Thus, previous studies demonstrated that the V-ATPase was functionally expressed in cell surfaces of MDA-MB-231 human breast cancer cells and then contributed to the invasion of carcinoma cells in tumor tissues [103]. However, as far as we know, no study has evaluated the effects of mechanical forces, such as shear stress, on the development of an acidic tumor microenvironment.

Shear stress is the mechanical force that is physiologically or pathophysiologically generated by the flow of blood, lymph, or interstitial fluid through the cardiovascular system. Upon detecting shear stress, endothelial cells in blood or lymph vessels transmit signals to their interiors, where they trigger responses, including changes in a variety of cell functions [104–106]. Thus, initial mechanotransduction responses to shear stress appear to involve calcium influx [15], production of prostaglandins [107], and nitric oxide (NO) [108], and regulation of matrix metalloproteinases (MMPs) [109]. In addition, it is also well established that blood vessel and lymphatic endothelial cells release endogenous ATP via the activation of cell surface F_1/F_0 ATP synthase [106, 110].

However, the concept has been widely accepted that carcinoma cells in tumor tissues are most responsive to interstitial pressure and rigid matrix-generated stretch; the influences of shear stress would seem to be of little concern in the cells [111]. In contrast, if one considers the forces produced by the flow of interstitial fluid outside of the tumor tissues, then shear stress forces may in fact be an important factor in the development of tumor microenvironments.

To address pivotal roles of cell surface F_1/F_0 ATP synthase in the development of acidic microenvironments in tumor tissues, we investigated the effects of shear stress stimulation on the cultured human breast cancer cells, MDA-MB-231 and MDA-MB-157, or human melanoma cells, SK-Mel-1. Shear stress stimulation (0.5–5.0 dyn/cm^2), the levels of which are similar to those produced by the interstitial flow, induced strength-dependent co-release of ATP and H^+ from the cells, which triggered CO_2 gas excretion. In contrast, stimulation at the same shear stress stimulation did not induce significant ATP release or CO_2 gas excretion from the control human mammary epithelial cells (HMECs). Marked immunocytochemical and mRNA

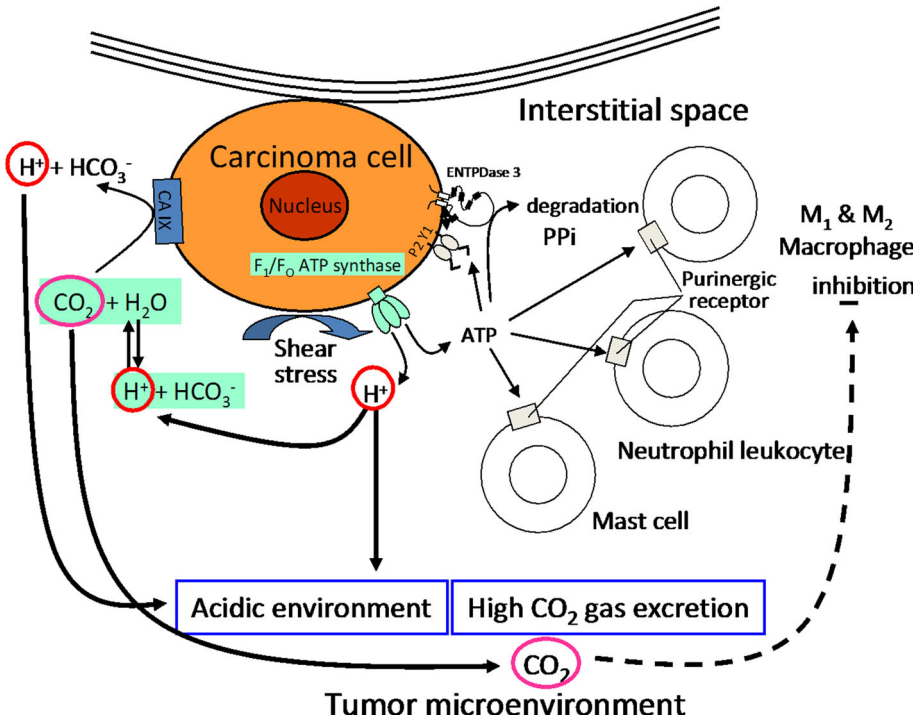

Fig. 8 Proposed hypothesis of interstitial flow-mediated development of acidic microenvironments in tumor tissues. Proposed hypothesis that shear stress, the mechanical force produced by interstitial fluid flow, in tumor tissues plays a key role in producing an acidic microenvironment for carcinoma cells by the cell surface F_1/F_0 ATP synthase-activated extracellular secretion of large amounts of ATP and high CO_2 gas excretion from the carcinoma cells. Data from Kawai et al. [110], with permission

expression of cell surface F_1/F_0 ATP synthase, vacuolar-ATPase (V-ATPase), carbonic anhydrase type IX, and ectonucleoside triphosphate diphosphohydrolase (ENTPDase) 3 were detected in MDA-MB-231 cells, but there was little or no expression on the HMEC. Pretreatment with cell surface F_1/F_0 ATP synthase inhibitors, but not cell surface V-ATPase inhibitors caused a significant reduction of the shear stress stimulation-mediated ATP release and CO_2 gas excretion from MDA-MB-231 cells. The ENTPDase activity in the shear stress-loaded MDA-MB-231 cell culture medium supernatant increased significantly in a time-dependent manner. In addition, MDA-MB-231 cells displayed strong staining for purinergic 2Y1 (P2Y1) receptors on their surfaces, and the receptors partially co-localized with ENTPDase 3. These findings suggest that cell surface F_1/F_0 ATP synthase, but not V-ATPase, may play key roles in the development of interstitial flow-mediated acidic microenvironments in tumor tissues through the shear stress stimulation-induced ATP and H^+ co-release and CO_2 gas production.

It is worth investigating how shear stress stimulation-induced ATP release from carcinoma cells contributes to the development of the tumor microenvironment. Previous studies of the roles of ATP or its metabolites in macrophage polarization [112], inflammasome formation [113], neutrophil function [114], or the secretory responses of mast cells [115] in the tumor microenvironment offer answers to this question. Namely, macrophage priming might be rapidly affected by signals from the surrounding microenvironment. Recently, novel macrophage 2-associated markers were characterized and identified as genes that control the extracellular metabolism of ATP to generate pyrophosphates (PPi). Extracellular ATP induces the expression of nucleotide-binding domain and leucine-rich repeat-containing receptors on macrophages, which are emerging as key regulators of innate immunity and are involved in inflammasome formation via the activation of purinergic 2X7 (P2X7) receptors [112, 113]. In addition, extracellular ATP enhances the respiratory burst responses of neutrophils [114] and amplifies the secretory responses of mast cells [115] during antigen stimulation-released ATP from carcinoma cells and then might contribute to the development of the tumor microenvironment by controlling the functions of macrophages, neutrophils, and leukocytes, as well as the migration of mast cells into tumor tissues.

Therefore, taking all of the findings obtained in the present study and previous reports into consideration, we propose that shear stress stimulation-mediated activation of the cell surface F_1/F_0 ATP synthase on carcinoma cells plays key roles in the development of the tumor microenvironment (Fig. 8). In conclusion, shear stress, the mechanical force produced by interstitial fluid flow, in tumor tissues plays pathophysiological roles in producing a suitable microenvironment for carcinoma cells by adjusting the functions of attacking macrophages, activated neutrophil leukocytes, and mast cells by inducing the extracellular secretion of large amounts of ATP and/or high CO_2 gas excretion in tumor tissues.

Conclusion

Taken into consideration of the above-mentioned research, we have proposed a new lymphology combined with lymphatic physiology, innate immunology, and oncology from the lymph-dynamics points of view. We believe that these lymphatic research subjects combined with the functional properties of lymph circulation can be included in the lymphatic research themes that will contribute to the establishment of a new lymphology.

Acknowledgments This study was financially supported, in part, by Grants-in-Aid for Scientific Research (19209044, 22249052, 24659098, 24590272) from the Japanese Ministry of Education, Science, Sports, and Culture and the Intelligent Surgical Instruments Project of the METI (Japan) (2007–2012).

Conflict of interest No conflicts of interest, financial or otherwise, are declared by the authors.

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