In Situ Lymph Dynamic Characterization through Lymph Nodes in Rabbit Hind Leg: Special Reference to Nodal Inflammation

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Abstract: In some lymph nodes, water and water-soluble substances of smaller molecular weight are known to be absorbed into blood vessels, and consequently the protein concentration of lymph within the nodes increases. In this study, we examined pressure-flow relationships of lymph nodes in situ and exchange properties of water and water-soluble substances through the nodes with special reference to inflamed lymph nodes. A lymph perfusion model through the lymph node in situ was constructed by cannulating one of the afferent lymphatics and an efferent lymphatic. Increasing infusion pressure (0 to 150 cmH2O) or decreasing outflow pressure (10 to –5 cmH2O) in the model caused a significant increase of the lymph outflow rate through the node.

This rate was also increased significantly with increases in both intranodal venous pressure (range: control, 20, 30, and 40 mmHg) and prenodal lymph albumin concentration (range: 0%, 2.6%, and 10%). When formyl-Met-Leu-Phe-OH (fMLP)-mediated acute inflammation was produced in the lymph nodes, the lymph outflow rate through the node was significantly decreased. These results indicate that colloid osmotic pressure and hydrostatic pressure within the lymph node may play important roles in the transport of water and water-soluble substances through the node. Acute fMLP-mediated inflammation of lymph nodes also produced a significant decrease of the lymph flow rate through lymph nodes.

Key words: lymphatic system, regional lymph node, pressure-flow relationship, Starling’s forces, fMLP.
an afferent lymph protein concentration-dependent manner [8]. Thus lymph dynamic properties through LNs are of pivotal importance in understanding the regulation of the immune system in mammals.

Although lymph composition and drainage preferentially affect tissue fluid exchange and control of the immune system, either no quantitative studies or only a few regarding the flow dynamics of lymph through LNs have been elucidated. Lymph dynamic analysis under a pathophysiological state, such as inflammation and tumor metastasis in the LN, also remains to be clarified. Therefore in this study we first tried to establish a model for lymph perfusion through rabbit popliteal LN in situ and then evaluated the dynamic properties of lymph flow through the LN by using the perfusion model in a physiological state and also in a fornyl-Met-Leu-Phe-OH (fMLP)-mediated acute inflammation model of the LN.

MATERIALS AND METHODS

Experimental preparations. Experiments were performed on 66 male Japan White rabbits (weight, 1.5–3.0 kg). They were anesthetized with pentobarbital sodium (Tokyo Chemical Industry, Tokyo, Japan) (40 mg/kg i.v.). The trachea was cannulated to ensure a patent airway. A catheter was inserted into the right external jugular vein (PE-90 tubing, Natsume, Tokyo, Japan) for drug administration and into the carotid artery (No. 25 tubing, Igarashi, Tokyo, Japan) to measure systemic blood pressure. The catheters were filled with 10 U/ml heparin (Shimizu, Shizuoka, Japan) in physiological saline solution (Lacte®, Laboratoire Guerbet, France) was injected into one of the popliteal afferent lymphatics, and an X-ray stereomicroscope fitted with a filter suitable for FITC to determine whether the injected microspheres appeared in the collected lymph.

X-ray lymphograms. Oily contrast medium (Lipiodol ultrafluid®, Laboratoire Guerbet, France) was injected into one of the popliteal afferent lymphatics, and an X-ray image was obtained (flow rate: 1.45 ml/h).

Corrosion casting of the lymphatic pathway in rabbit hind leg. Corrosion casts of popliteal LN were made by injecting Mercox (Vilene Hospital®, CL-2R, Dainippon Ink and Chemicals, Tokyo, Japan) through a cannula inserted retrogradely into the lymphatic. After a 30-min stabilization period, 2 µg of fluorescent-labeled microspheres (4.8 µm in diameter; yellow-green fluorescence, Polysciences, Warrington, PA, USA) were administered in the perfusion fluid at one time. The perfusion of plain artificial lymph fluid contained 2.6% bovine serum albumin (BSA; Sigma, St. Louis, MO, USA), which produced a colloid osmotic pressure similar to that in rabbit afferent lymph [16], unless otherwise described. Lymph fluid was simultaneously collected via the cannula, which had been inserted retrogradely into the lymphatic. After a 30-min stabilization period, 2 µg of fluorescent-labeled microspheres (4.8 µm in diameter; yellow-green fluorescence, Polysciences, Warrington, PA, USA) were administered in the perfusion fluid at one time. The perfusion of plain artificial lymph fluid was then continued for 1 h. Ten aliquots of 1 µl in volume from each lymph sample were observed and photographed through a Leica MZ 16FA fluorescence stereomicroscope fitted with a filter suitable for FITC to determine whether the injected microspheres appeared in the collected lymph.

Lymph flow rate in rabbit hind leg. To estimate the flow rate of lymph through the rabbit popliteal LN, retrograde
cannulation was performed in one of the popliteal efferent lymphatics in the groin. All other efferent lymphatics running parallel to the cannulated lymphatic were ligated. In the groin of rabbits, all efferent lymphatics ran along the femoral artery and vein and could be clearly identified by a subcutaneous injection of Evans blue dye into the dorsal skin of the rabbit paw. Lymph fluid was collected into a glass test tube containing 10 µl heparin. The lymph catheter tip was positioned inside the syringe, about 5 cm below the cannulation site. The height of the catheter tip was similar to the height previously described [17]. A rotating movement of the leg was performed passively in the sagittal plane with an electric motor [17]. The diameter of this rotating movement was 8 cm. After a 30-min stabilizing period at 0.3 Hz whole-leg rotation, the rotation frequency was shifted in steps from 0 to 0.06, 0.1, 0.3, and 1.0 Hz. At each frequency, the lymph flow rate was measured over a period of 30 min.

The relationship between lymph infusion- and outflow-rate through the LN was determined in the lymph perfusion model with a node perfused by artificial lymph fluid from the popliteal afferent lymphatics to the efferent lymphatics in the groin (Fig. 2I). After a 30-min stabilizing period at a rate of 1.47 ml/h, the infusion rate was shifted in steps from 0 to 0.74, 1.47, 3.7, and 7.4 ml/h. At each infusion rate, the lymph outflow rate was measured over a period of 30 min.

**Lymph dynamic characterization in rabbit hind leg.** The same perfusion models as described before were adopted to investigate lymph dynamic characterization through the rabbit lymphatic system in the hind leg (Fig. 2). One model was perfused from just before the popliteal LN to the groin (Fig. 2I), and the other was perfused from just after the popliteal LN to the groin (Fig. 2II). First, the relationships between infusion pressure and outflow rate were determined in these models. The diameter of this rotating movement was 8 cm. After a 30-min stabilizing period at an infusion pressure of 25 cmH2O, the infusion pressure was shifted in steps from 0 to 0.74, 1.47, 3.7, and 7.4 cmH2O. At each infusion rate, the lymph outflow rate was measured over a period of 30 min.
to 25, 50, 75, 100, 125, and 150 cmH2O at a constant outflow pressure of –5 cmH2O. In the latter model, the infusion pressure shifted in steps from 0 to 5, 10, 15, 20, 25, 30, 40, and 50 cmH2O. At each infusion pressure, the lymph flow rates were measured over a period of 10 min after a stabilizing period of 5 min.

Resistance of the perfused lymphatic circuit was calculated at an infusion pressure of 25 cmH2O in this series of experiments. Because the outflow pressure was –5 cmH2O, perfusion pressure was estimated as 30 cmH2O. This resistance in cmH2O/ml/h was calculated from the equation of (resistance of the perfusion circuit) = 30/(lymph outflow rate).

Second, the relationships between outflow pressure and outflow rate were determined in these models. In the former model, after a 15-min stabilizing period at an outflow pressure of –5 cmH2O, the outflow pressure was shifted in steps from –35 to –25, –15, –5, 0, 5, 10, and 15 cmH2O at constant infusion pressure of 25 cmH2O. In the latter model, the outflow pressure was shifted in steps from –45 to –35, –25, –15, –5, 0, 5, 10, 15, 20, and 25 cmH2O. At each outflow pressure, the lymph flow rates were measured over a period of 10 min after a stabilizing period of 5 min.

Resistance of the perfused lymphatic circuit was calculated as described by Drake et al. (1982) [18]. In this series of experiments, the lymph outflow rate varied linearly with lymphatic outflow pressure ranging from –5 to 5 cmH2O. Decreased lymph outflow pressure in this range might increase lymph perfusion pressure. Thus the change in the lymph outflow rate was produced by a change in lymph perfusion pressure of 10 cmH2O. The resistance of the perfusion circuit was therefore calculated from the equation of (resistance of the perfusion circuit) = –(change of perfusion pressure)/(change of lymph outflow rate). This equation means that resistance (cmH2O/ml/h) is a minus reciprocal number on the gradient of the graph that shows a relationship between lymph outflow pressure (cmH2O) and outflow rate (ml/h).

Third, the effect of venous pressure on the outflow rate of lymph was examined in these models. After a 15-min stabilizing period at an infusion rate of 1.47 ml/h, venous pressure in the hind leg was elevated in steps from intact venous pressure to 20, 30, and 40 mmHg at a constant infusion rate of 1.47 ml/h. Even if the venous pressure was elevated up to 40 mmHg, no increase or only a slight increase in afferent lymph flow was observed when the leg did not move [17]. Leg venous pressure was elevated by means of a blood pressure cuff placed around the abdomen of rabbits. In this series of experiments, a polyethyl-
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e tube (PE-20 tubing, Natsume) was inserted into a small vein running parallel to the tendo calcaneus and connected to a pressure transducer to continuously monitor venous pressure. At each venous pressure, lymph flow rates were measured over a period of 15 min after a stabilizing period of 5 min.

Fourth, the effect of arterial pressure on the outflow rate of lymph was examined in these models. After a 15-min stabilizing period at an infusion rate of 1.47 ml/h, the experiments were performed. The lymph collection was continued for 80 min, and 30 minutes after it was started, the femoral artery in the ipsilateral hind leg was ligated, which took about 5 min. Lymph flow rates were measured at 15-min intervals. In this series of experiments, a polyethylene tube (PE-20 tubing, Natsume) was inserted into a small artery running parallel to the tendo calcaneus and connected to a pressure transducer to continuously monitor arterial pressure.

Fifth, the effect of lymph colloid osmotic pressure on the outflow rate of lymph was examined in these models. After a 30-min stabilizing period at an infusion rate of 1.47 ml/h by 2.6% BSA containing artificial lymph fluid, the BSA concentration was increased in steps from 0% to 2.6% and 10% at a constant infusion rate of 1.47 ml/h. At each concentration, the lymph flow rates were measured over a period of 15 min after a stabilizing period of 30 min.

Acute inflammatory model of rabbit popliteal LNs. The same perfusion model from just before the popliteal LN to the groin, as described previously, was adopted to investigate lymph pressure-flow relationships through inflamed LNs. After the lymphatic cannulations had been completed, formyl-Met-Leu-Phe-OH (fMLP, 10–5 M, Sigma) dissolved in artificial lymph fluid was perfused for 1 h. This concentration of fMLP is known to activate polymorphonuclear leukocytes (PMNs) and produce acute inflammation [12]. Infusion pressure-outflow rate relationships were determined in this inflammatory model. The infusion pressure was shifted in steps from 0 to 25, 50, 75, 100, 125, and 150 cmH2O at a constant outflow pressure of –5 cmH2O. In other experiments, outflow pressure-outflow rate relationships were determined in this inflammatory model. The outflow pressure was shifted in steps from –35 to –25, –15, –5, 0, 5, 10, and 15 cmH2O at a constant infusion pressure of 25 cmH2O. At each infusion or outflow pressure, the lymph flow rate was measured over a period of 10 min after a stabilizing period of 5 min.

fMLP (10–5 M) was contained in the artificial lymph fluid throughout the experimental period.

Histological studies. The histology of the intact and fMLP (10–5 M)-perfused LNs was delineated to evaluate acute inflammation. For light microscopic observation, specimens including the lymph pathways were fixed with 10% formalin solution for 24 h. They were dehydrated through a graded series of ethanol and embedded in paraffin in a routine manner. Sections of 3–4 µm were processed by hematoxylin and eosin stain or PAS (periodic acid-Schiff base) stain. PAS stain was performed to confirm the existence of PMNs in the sections. The sections were viewed and photographed through a light microscope (DIAPHOT, Nikon, Japan).

Statistics. Experimental data in the text, figures, and tables were expressed as the means ± standard error of the mean (SEM) unless otherwise indicated. Comparisons were performed by repeated measures analysis of variance (ANOVA), followed by Fisher’s protected least significant differences multiple-range (PLSD) test, one-way ANOVA, followed by Fisher’s PLSD test or paired or unpaired Student’s t-tests. A value of p < 0.05 was considered significant.

RESULTS

Connection of the afferent lymphatic, popliteal LN and efferent lymphatics in rabbits, and lymph flow rate through the LN

The afferent lymphatic, popliteal LN and efferent lymphatics were clearly observed by X-ray lymphography (n = 3) (Fig. 1, A–C). In all rabbits, neither a bypass route of the popliteal LN nor lympho-venous communication was observed on the image. A cast of the same lymphatic pathway demonstrated that the afferent lymphatic separated into small branches, and all these branches connected with the marginal sinus of the popliteal LN (n = 2) (Fig. 1, D and E).

No microsphere of 4.8 µm in diameter could be observed in lymph fluid collected from efferent lymphatics in the groin when microspheres had been administered into the afferent lymphatic (n = 3) (Fig. 1, F and G). On the other hand, microspheres were clearly demonstrated in the collected lymph fluid when they had been injected into the efferent lymphatic running just after the popliteal LN (n = 3) (Fig. 1H).

Lymph flow rates without leg rotation were 0.063 ± 0.017 ml/h (n = 4), which is about one order of magnitude lower than those in the period with a relatively slow leg rotation at 0.06 Hz, at popliteal efferent lymphatics in the groin. The lymph flow rate during whole-leg passive rotation increased monotonically with frequency. Especially between 0.06 and 1.0 Hz, the lymph flow rate increased linearly with log frequency (0.64 ± 0.15, 0.76 ± 0.15, 1.08 ± 0.15, and 1.42 ± 0.15 ml at 0.06, 0.1, 0.3, and 1.0 Hz, respectively; r2 = 0.99, p < 0.01, n = 4).

In the lymph perfusion model, which included the LN in the perfusion circuit (Fig. 2I), lymph outflow rates increased monotonically with an infusion rate ranging from 0 to 7.35 ml/h. Significant regression was observed between infusion rates and outflow rates (r2 = 1.00, p < 0.01, n = 4).
When infusion pressure was shifted in steps from 0 to 150 cmH2O in the lymph perfusion model, which included the LN in the perfusion circuit (Fig. 2I), lymph outflow rates increased monotonically with infusion pressure (n = 4) (Fig. 3Ia). Table 1 shows perfusion resistance calculated at an infusion pressure of 25 cmH2O during a constant outflow pressure of –5 cmH2O. On the other hand, in the same lymph perfusion model at constant infusion pressure of 25 cmH2O, lymph outflow ceased at an outflow pressure of 25 cmH2O, and outflow rates increased linearly while outflow pressure decreased up to about –5 cmH2O (n = 4) (Fig. 3Ib). The outflow rate reached a constant level at about 1.8 ml/h. Because linear correlation between outflow pressure and outflow rate was observed in this model, perfusion resistance was calculated in the range between –5 and 5 cmH2O, as described in Materials and Methods (Table 1). No significant differences were observed between resistances calculated from these two different methods.

The model that did not include the LN in the perfusion circuit (Fig. 2II) was also used to examine lymph pressure-flow relationships. Relationships similar to those obtained in the former model were observed (each animal number n = 4) (Fig. 3, Iia and IIb). Lymph flow rates, how-

### Table 1. Estimated perfusion resistance of the lymphatic system in rabbit lower extremities.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Method for estimating perfusion resistance</th>
<th>Varying infusion pressure</th>
<th>Varying outflow pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>From just before popliteal lymph node to groin</td>
<td>Varying infusion pressure</td>
<td>11.5 ± 0.9 (n = 4)</td>
<td>12.8 ± 2.8 (n = 4)</td>
</tr>
<tr>
<td>From just after popliteal lymph node to groin</td>
<td>Varying infusion pressure</td>
<td>3.0 ± 0.6 (n = 4)</td>
<td>2.8 ± 0.5 (n = 5)</td>
</tr>
<tr>
<td>Popliteal lymph node</td>
<td>Varying infusion pressure</td>
<td>8.5</td>
<td>10.0</td>
</tr>
<tr>
<td>From just before popliteal lymph node to groin (perfused with fMLP 10^{-5} m)</td>
<td>Varying infusion pressure</td>
<td>26.0 ± 4.8 (n = 4)*†</td>
<td>11.7 ± 0.6 (n = 4)</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM (cmH2O/ml/h). n, number of preparations. a) Perfusion resistance was calculated at an infusion pressure of 25 cmH2O and outflow pressure of –5 cmH2O. b) Perfusion resistance was calculated at an infusion pressure of 25 cmH2O, and outflow pressure of –5–5 or 15 cmH2O. c) Perfusion resistance of popliteal lymph nodes was described as the difference between the top two values. *p < 0.05, varying infusion pressure vs. outflow pressure. †p < 0.05, absence vs. presence of fMLP.
ever, were much larger than those in the former model at the same infusion and outflow pressures. No significant differences were observed between resistances calculated from the two different methods described above (Table 1). Resistance in the former model was significantly larger than that obtained in the latter one. Table 1 shows the estimated resistance of rabbit popliteal LN, which was calculated as the difference between data from these two models.

**Effects of venous pressure, arterial pressure, and lymph colloid osmotic pressure on outflow rate of the lymphatic system in rabbit hind leg**

To evaluate the effects of venous pressure in the LN on lymph flow, leg venous pressure was shifted in steps up to 40 mmHg. Venous pressure-dependent increments of the lymph outflow rate were observed in the perfusion model, which included the LN in the perfusion circuit ($p < 0.01$, $n = 4$) (Fig. 4Ic). In this model, control leg venous pressure was $7.0 \pm 1.2$ (n = 4).

After ligation of the femoral artery, a marked decrease in arterial pressure in the ipsilateral leg was observed (former model: $130 \pm 14/73 \pm 6$ mmHg before ligation, $26 \pm 2/26 \pm 2$ mmHg after ligation, $n = 4$; latter model: $129 \pm 9/74 \pm 4$ mmHg before ligation, $36 \pm 3/34 \pm 1$ mmHg after ligation, $n = 4$). Lymph outflow rates in these two types of perfusion models were not significantly affected by the decrease of leg arterial pressure (Fig. 4, Id and IId).

To modify the colloid osmotic pressure (COP) of perfusion fluid through the LN, the concentration of BSA was increased from 0%, 2.6%, and 10%. **$p < 0.01$ vs. 0% BSA.**

**Fig. 4.** Effects of Starling's forces on fluid exchange in the lymph perfusion model of rabbit hind leg. In these models, centripetal cannulation was performed in the lymphatic just before (Ic, $n = 4$; Id, $n = 4$; Ie, $n = 4$) or just after (Ilc, $n = 4$; Ilid, $n = 4$; IIe, $n = 4$) the popliteal lymph node. Retrograde cannulation was performed in one of the popliteal efferent lymphatics in the groin. All other efferent lymphatics running parallel to the cannulated lymphatic were ligated. Two cannulation points in the models were adjusted to the same height. c: Lymph outflow rates were measured without (control) and with leg venous pressure elevation of 20, 30, and 40 mmHg. *$p < 0.05$, **$p < 0.01$ vs. control. d: Lymph outflow rates were measured before and after ligation of the ipsilateral femoral artery. Ligation was performed at time 0. e: Lymph outflow rates were measured with an elevation of BSA (bovine serum albumin) concentration in perfusion fluid from 0%, 2.6%, and 10%. **$p < 0.01$ vs. 0% BSA.
Effects of LN acute inflammation on pressure-flow relationships in the lymphatic system of rabbit hind leg

After a 1-h perfusion of $10^{-5}$ M fMLP through rabbit popliteal LN, marked PMN infiltration in the LN was observed by HE staining (Fig. 5, A and B). PAS staining, which demonstrates PMNs in red, also confirmed the accumulation of PMNs in the LN (Fig. 5, C and D).

In the perfusion model with fMLP-mediated acute inflammation of the LN, a similar lymph pressure-flow relationship to that obtained in the intact perfusion model was observed (Fig. 5, Ia and Ib). Lymph flow rates, however, were significantly lower in the model with acute inflammation than in the intact model at the same infusion and outflow pressure (animal $n = 4$, $p < 0.01$).

As described in Materials and Methods, not only in the intact model, but also in the inflammation model, perfusion resistance was calculated from data obtained from two different protocols; a protocol in which infusion pressures were changed, and one in which outflow pressures were changed in the perfusion model. Perfusion resistance calculated from data in the former protocol was significantly greater than resistance calculated from data in the latter one in the inflammation model ($p < 0.05$) (Table 1). Perfusion resistance calculated from data of the former protocol in the inflammation model was significantly greater than resistance in the intact model ($p < 0.05$) (Ta-
able 1). On the other hand, no significant difference was observed between perfusion resistance calculated from data of the latter protocol in the inflammation and intact models (Table 1).

**DISCUSSION**

To evaluate perfusion properties in the LNs, first an *in situ* LN perfusion model was developed in the rabbit hind leg. At least the present model, morphological study, such as X-ray lymphography and corrosion cast analysis, demonstrated no bypass route that directly connects popliteal afferent and efferent lymphatics. Moreover, microspheres that had been injected into the afferent lymphatics did not appear in efferent lymphatics in the groin. When microspheres were injected just after the LN, they could be collected in the groin. These observations indicate that all microspheres injected into afferent lymphatics were trapped in the LN and that no bypass route existed. Thus this perfusion model should be useful to examine perfusion properties, which may be caused by fluid exchange through the LNs.

First, to determine the physiological lymph flow rate through rabbit popliteal LN, we measured the lymph outflow rate from popliteal efferent lymphatics with or without leg rotation. The logarithm of rotation frequency is linearly correlated with the lymph flow rate in the range from 0.06 to 1.0 Hz. A similar relation was observed in rabbit popliteal afferent lymphatics, but the rate was one order of magnitude lower than that obtained in efferent lymphatics [17]. In the rabbit hind leg, no spontaneous contraction of the collecting lymphatics was demonstrated [19]. Thus it could be described that the lymph is passively transported through the lymphatics.

The infusion pressure-outflow rate relationship shows linear regression, meaning that increasing perfusion pressure produced a higher rate of lymph flow through the model perfused with and without the LN. The resistance of popliteal LN was calculated from the pressure-flow rate relationships. The resistance of the LN per se (8.5 cmH₂O/ml/h) was about 3 times higher than the resistance of the lymphatics that perfused from just after popliteal LN to the groin. Thus LN constitutes a relatively significant resistance in the lymphatic system. In an earlier study, resistance in canine periportal and popliteal LNs was reported to be 2.6 and 0.79 cmH₂O/ml/h, respectively [20]. Some reasons for the discrepancy of these resistances are thought to be species and regional differences. Furthermore, Brow et al. (1984) [21] demonstrated that the lymph flow rate, intranodal arterial pressure, and intranodal venous pressure affect the resistance of the LNs.

When lymph outflow pressure decreased under constant infusion pressure, the lymph outflow rate increased linearly in the range below ~5 cmH₂O/ml/h of outflow pressure. In this case, the change in outflow pressure could be considered a change of the driving force of the lymph flow, and the outflow rate varied in a driving force-dependent manner. Thus the resistance of the perfused lymphatic system could be calculated from the slope of linear regression between outflow pressure and outflow rate. A similar method for determining perfusion resistance was applied to canine lung lymphatics [18] and the rabbit lumbar trunk [22]. In this study, we used two different methods to estimate the resistance of the lymphatic system. Similar values of resistance could be obtained in each segment of the lymphatic system, supporting the accuracy of the data.

On the other hand, in the range up to ~15 cmH₂O/ml/h of outflow pressure, the lymph outflow rate maintained a constant level when lymph outflow pressure varied under constant infusion pressure. A similar phenomenon is known to occur in the canine lung [18], heart, liver, and skeletal muscle lymphatics [23], and rabbit lumbar trunk [22]. Holt (1969) [24] demonstrated that the flow rate in the collapsible tube is independent of downstream pressure when downstream transmural pressure is negative. Because when inflow pressure (Pᵢ) is above critical closing pressure (Pᵢₑ), but outflow pressure (Pₒₑ) is below Pᵢ in a collapsible tube, the proper driving force is indicated as Pᵢₑ – Pₒₑ rather than Pᵢ – Pₒₑ under constant resistance. The present data suggest that the lymphatic system, at least part of it, collapses when lymph fluid is strongly sucked from downstream of the lymphatics.

The lymphatic system, especially in the LNs, has the potential to alter the composition of lymph [1]. Jacobsson and Kjellmer (1964) [2] perfused the canine popliteal LN with diluted plasma and found that the protein concentration of efferent lymph was always greater than that of afferent lymph. More recently, Adair and Guyton (1982, 1983) [3, 4] demonstrated that the LN functions as a fluid exchange chamber in which protein-free fluid is transferred between the blood and lymph compartment in the direction required to establish an equilibrium of Starling forces across the blood-lymph barrier. Our data of lymph perfusion with varied lymph COP and intranodal venous pressure is compatible with this theory. When the venous pressure was elevated, outflow from the circuit of the efferent lymphatic tended to decrease (Fig. 4Hc). This may be involved with a compression of the lymphatic caused by a swelling of the surrounding tissue. On the other hand, no significant change in the lymph outflow rate was observed when intranodal arterial pressure decreased, suggesting that suppressed blood flow in the LN minimized fluid exchange between the blood and lymph. Even if the blood circulation of the LN could not be completely stopped with the ligation of the femoral artery, significantly decreased arterial pressure in the leg might disturb blood circulation. A tridimensional architecture of blood vascular network in LNs was studied by Bélisle and Sainte-Marie (1990) [25].
Acute inflammation is known to involve the initial release of proinflammatory mediators, activation of PMNs, and mobilization of PMNs to the sites. Although inflammatory reaction is the first line in protecting the host from invading pathogens, it also causes host tissue injury and microvascular dysfunction [12]. To our knowledge, the effects of acute inflammation in the LN on lymphodynamics through the LN have not been elucidated. Thus in this study, fMLP-mediated acute inflammation was formed in the LN. The concentration of fMLP used in this study is reported to produce activation and to augment the mobilization of PMLs, and the PMLs increased hydraulic conductivity in rat mesenteric microvessels [12]. The present data show that fMLP administration decreased the lymph outflow rate even if perfusion pressure was similar to the control without fMLP perfusion. This result might indicate that acute inflammation in the LN increased perfusion resistance or fluid movement from the lymph compartment to the blood compartment and to outside the capsule. However this LN perfusion model is useful for evaluating the effects of changes in Starling’s forces, permeability change in nodal microvessels or capsules is difficult to examine. The mechanisms of inflammation-induced permeability change in the vessels and capsules should be clarified. Moreover, the effects of inflammation on particle filtration through the LNs should also be clarified in the future.

Browse et al. (1984) [21] suggested that the space available for lymph flow in LNs is closely related to lymph flow resistance of the nodes. If flow resistance of the LN increased by treatment with fMLP, possible reasons why this phenomenon occurred are as follows: (i) A swelling of the capsule induced by inflammatory reaction may prevent a flow ofafferent lymph from passing through the capsule; (ii) An increased cell number associated with PMLN infiltration may decrease the intranodal space available for lymph flow; and (iii) An inflammation-induced hyperemia may reduce the intranodal space available for lymph flow. Moreover, the spread of small branches of the afferent lymphatics running between the cannulation point and the LN may have a role in inflammation-induced elevation of the resistance (Fig. 1B).

In conclusion, we developed a useful lymph perfusion model for collecting lymphatics and for studying lymph flow dynamics and fluid exchange in the LNs. Using this model, we confirmed that colloid osmotic pressure and hydrostatic pressure had an important role in the penetration of water and small molecular substances in the LNs. Furthermore, the acute inflammation of the LN is suggested to attenuate lymph flow through the LN. The present study clearly demonstrated that the method employed could be applied to analyzing the pathophysiological state in the LNs. The use of this method in the future should clarify the effects of tumor metastasis in LNs on lymph flow dynamics.

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