Contamination of lymph from the major prenodal cardiac lymphatic in dogs

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Contamination of lymph from the major prenodal cardiac lymphatic (MPCL) is the most common approach for the investigation of myocardial lymphatic function. However, the assumption that the MPCL drains pure cardiac lymph has been questioned. We studied variations of MPCL anatomy and investigated whether non-cardiac lymph is drained by this lymphatic. After dye was injected into the lungs and left ventricular myocardium in 21 dogs, dissection of the cardiac lymphatic system yielded 3 anatomic variations. In variations 1 and 2 (81% of dogs), a mixture of cardiac and pulmonary lymph was drained via the MPCL. In variation 3 (19% of dogs) no connection was found between MPCL and pulmonary lymphatics. In variations 1 and 2, alteration of tidal volume resulted in significant changes of lymph flow rate. The pulmonary contribution to MPCL lymph flow was estimated as 34% in variation 2. We conclude that MPCL lymph may contain not only cardiac lymph but also significant pulmonary contamination. This finding should be considered in the interpretation of lymph data from cannulation of the canine MPCL.

MYOCARDIAL LYMPHATIC FUNCTION has been widely investigated for its involvement in myocardial edema formation (10, 11, 13, 18), mediator release after myocardial reperfusion injury (4, 5, 9, 19), and noncholinergic-nonadrenergic neurotransmitter release from the heart (2, 3).

Cannulation of the canine major prenodal cardiac lymphatic (MPCL), which drains into the so-called "cardiac node of Drinker," is the most common model for the investigation of myocardial lymphatic function. However, the description of cardiac lymphatic anatomy is not uniform among investigators (12, 15, 23), and the assumption that the MPCL drains pure cardiac lymph has been questioned (12). A particular controversy exists about the anatomic connections of the left tracheobronchial node (TBN), which is located proximally to the MPCL on the anterior surface of the left main bronchus. Whereas Miller (15) and Ullal et al. (23) described a principal myocardial lymphatic draining into the TBN with the MPCL originating from the TBN, Leeds et al. (12) reported pulmonary lymphatics draining into the TBN and multiple connections between the TBN and the MPCL (Fig. 1). In the case in which the MPCL contains a mixture of cardiac and pulmonary lymph, modification of ventilation parameters should result in corresponding changes of MPCL lymph flow, because pulmonary lymph flow depends on respiratory movement (8, 22). Therefore, it was the purpose of this study to describe the anatomic relationship between the MPCL and TBN, to determine whether lymph of pulmonary origin is drained via the MPCL, and to investigate the impact of ventilation on MPCL lymph flow.

METHODS

Animal preparation. All procedures were approved by The University of Texas Animal Welfare Committee and were consistent with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Twenty-three mongrel dogs (24.9 ± 1.2 kg) of either sex were anesthetized by intravenous administration of thiopental sodium (25 mg/kg body wt), endotracheally intubated, and mechanically ventilated with room air using a volume-cycled respirator (Siemens-Elema, Sundbyberg, Sweden) at a rate and volume to maintain arterial PCO2 at 35–40 mmHg. Anesthesia was maintained with intravenous infusion of 1% thiopental sodium in Ringer solution. Fluid-filled catheters were placed into the left femoral artery and vein for arterial pressure monitoring, arterial blood sampling, and fluid administration, respectively. The pressure-monitoring catheters were connected to calibrated pressure transducers (Isotec; Healthdyne Cardiovascular, Irvine, CA), and data were recorded on a computer (MacLab; World Precision Instruments, Sarasota, FL).

Preliminary experiments. In two dogs a median sternotomy and pericardiotomy were performed. To facilitate identification of efferent cardiac lymphatics, 0.1–0.2 ml of Evans blue dye solution (T-1824, 2%) was injected into the left ventricular myocardium. The MPCL was identified between the superior vena cava and the innominate artery and was cannulated thereafter with the use of a heparinized 20-gauge (0.9 mm) cannula as previously described (11). The cannula was connected to polyethylene tubing held at heart level. After the lymph was free of visible dye residuals for at least 15 min, a second dye injection into the right and left lung was
performed. Thereafter, we noted whether dye from the pulmonary injection would appear in MPCL lymph.

Anatomic dissections and lymph flow measurements. In 21 dogs we performed an extended right lateral thoracotomy with resection of the second to fifth rib to improve anatomic exposure of mediastinal lymphatics. After the injection of 0.6–0.8 ml of Evans blue into the subpleural tissue of the right and left lung with a 27-gauge subcutaneous needle (0.4 × 19 mm), we carefully dissected the mediastinal lymphatics among the superior vena cava, trachea, and aorta to identify the pulmonary efferent lymphatics. After the dye from the first injection had cleared from the lymphatics, we performed a second dye injection of 0.4–0.6 ml of Evans blue into the left ventricular myocardium to identify the MPCL and to determine whether cardiac lymph was drained via different or the same lymphatic pathways as pulmonary lymph. In nine dogs in which the MPCL drained cardiac and pulmonary lymph, we cannulated the MPCL with Silastic tubing (outer diameter 1.2 mm, inner diameter 0.6 mm; catalog no. 602-155; Dow Corning, Midland, MI). MPCL lymph flow rate (QL; in µl/min) was measured using a calibrated pipette held at heart level. To determine the influence of ventilation on QL, measurements were taken during ventilation with a normal tidal volume (VT; 16 ml/kg, with arterial PCO₂ kept at 35–40 mmHg) and with a low VT (30% of normal VT). During QL measurements under low VT, ventilation was switched to 100% oxygen. When VT was changed, QL measurements were taken after an equilibration period of 5 min. Arterial blood gas samples taken at the end of low-VT ventilation periods showed no hypoxia and only mild hypercapnia (45–50 mmHg). All QL measurements were performed in duplicate, and the order in which measurements were taken was randomly altered throughout the experiments. In five of the nine dogs with cannulated MPCL, we found that cardiac lymph bypassed the TBN and that pulmonary lymph drained from the TBN into the MPCL via multiple connections between the TBN and MPCL (Fig. 2). In these animals we interrupted the pulmonary contribution by ligation of the lymphatic connections between the MPCL and TBN. Additional measurements of QL and total protein concentration of lymph (CL) and plasma (CP) were taken before and after ligation of the pulmonary lymphatic contribution. CL and CP were measured using a refractometer (American Optical, Buffalo, NY).

Statistical analysis. Data are presented as means ± SD. A paired t-test was used for statistical comparison, and a P value <0.05 was considered significant.

RESULTS

Preliminary experiments. In both animals lymph drained from the MPCL was stained by dye injection...
into the lungs, indicating a mixture of cardiac and pulmonary lymph in the MPCL.

Anatomic variations. The approximate diameter of the MPCL was between 0.75 and 1.5 mm before ligation. Dissection of mediastinal lymphatics yielded three variations of MPCL and TBN anatomy (Fig. 2). 1) In 33% of dogs, cardiac and pulmonary efferent lymphatics drained into the TBN. The MPCL originated from the TBN and contained a mixture of cardiac and pulmonary lymph. 2) In 48% of dogs, the MPCL was a continuation of the principal myocardial lymphatic, and cardiac lymph bypassed the TBN. Pulmonary lymph drained from the TBN via multiple connections into the MPCL. 3) In 19% of dogs, the MPCL was a continuation of the principal myocardial lymphatic, and cardiac lymph bypassed the TBN, but in variation 3 no connection was found between MPCL and TBN. In 81% of dogs (variations 1 and 2), the MPCL drained lymph of cardiac and pulmonary origin. Figures 3 and 4 show the anatomy of variations 1 and 2. Dye injections into the lung did not result in staining of the MPCL in only 19% of the dogs (variation 3). However, the TBN was stained in all dogs after dye injections into the lung. Additional dye injections into the diaphragm and peritoneum in four dogs did not result in staining of cardiac lymphatics.

Lymph flow and \( C_L / C_P \) measurements. Table 1 summarizes the results of \( Q_L \) measurements. In variations 1 and 2, \( Q_L \) decreased substantially with \( V_T \) reduction. Ligation of pulmonary connections to the MPCL in variation 2 resulted also in a significant \( Q_L \) decrease. This decrease of \( Q_L \) had the same magnitude as the decrease we observed with \( V_T \) reduction. After ligation of the pulmonary contribution, \( Q_L \) showed no further change with \( V_T \) reduction.

We calculated the amount of pulmonary contribution to MPCL flow in variation 2 from \( Q_L \) measurements taken before and after ligation of pulmonary lymphatics draining into the MPCL. Pulmonary contribution was estimated as 34 ± 16%. In the same animals, \( C_L / C_P \) showed no difference between measurements taken before and after ligation of pulmonary contribution (0.54 ± 0.1 vs. 0.62 ± 0.26, \( P = 0.62 \)).

DISCUSSION

Our data show that lymph drained from the canine MPCL may contain not only cardiac lymph but also significant pulmonary contamination. This finding may have substantial impact on the interpretation of lymph data obtained from the canine MPCL in certain experimental models.

Cannulation of cardiac lymphatics was first reported by Drinker et al. (6) in 1940. After dye was injected into the cardiac muscle of dogs, the authors identified the stained lymph node between the superior vena cava and innominate artery as the “cardiac” lymph node. They cannulated the prenodal lymphatic draining into this node under the assumption that it would contain pure cardiac lymph. Thereafter, more extensive studies (1, 7 17, 23) led to the recognition of considerable variability in canine cardiac lymphatic anatomy. The
most commonly described anatomic configuration can be summarized as follows. Epicardial lymph vessels usually merge into a left and right efferent cardiac lymphatic, which run cephalad in the mediastinum. The right efferent either joins the left efferent or drains into the thoracic duct via other mediastinal lymphatics. The left efferent lymphatic (or principal cardiac lymphatic) drains into the tracheobronchial lymph node (TBN), sometimes referred to as the pretracheal node. From the TBN, usually one to three parallel running lymphatics drain into the cardiac node of Drinker. The largest of these lymphatics is referred to as the MPCL. Our findings differ from this anatomic configuration in two major points. First, the MPCL originated from the TBN only in approximately one-third of the dogs (variation 1), whereas the MPCL appeared as a continuation of the principal myocardial lymphatic in the majority of dogs (variations 2 and 3). Second, the TBN received pulmonary efferent lymphatics in all dogs. Mixture of cardiac and pulmonary lymph occurred in 81% of dogs, either at the level of the TBN (variation 1) or in the MPCL (variation 2). These results agree, in part, with the findings of Leeds et al. (12), who found pulmonary efferent lymphatics merging into the left efferent cardiac lymphatic in dogs. These authors described an anatomic configuration that is in great accordance with variation 2 of our study.

Most investigators have chosen the median sternotomy approach for MPCL cannulation (10, 11, 13, 14). It is important to note that neither the TBN nor the principal myocardial lymphatic is discernible from this approach because each is located dorsal to the base of the heart. Ligation of accessory lymphatics in the proximity of the MPCL and cardiac node, as performed by many investigators after MPCL cannulation (11, 13–15), would not interrupt the pulmonary lymphatic connections described in this study.

We calculated the amount of pulmonary contribution to MPCL lymph as 34%. This number can only be a crude estimate because of the extensive anatomic variability of the lymphatic system. In addition, pulmonary lymph flow depends on respiratory movement and can be modified by ventilation parameters (8, 22). Our data show the impact of ventilation on MPCL lymph flow in dogs with anatomic variations 1 and 2. When V_T was reduced to 30%, Q_L decreased by 30%. After ligation, Q_L showed no significant changes with V_T reduction. *P < 0.05 vs. 100% V_T before ligation of pulmonary lymphatic branches.

Table 1. Influence of tidal volume on MPCL lymph flow

<table>
<thead>
<tr>
<th>Anatomic Variation</th>
<th>n</th>
<th>V_T, %</th>
<th>Q_L, µl/min</th>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>100</td>
<td>43 ± 42</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>100</td>
<td>42 ± 29</td>
</tr>
<tr>
<td>Before ligation</td>
<td>5</td>
<td>30</td>
<td>23 ± 14*</td>
</tr>
<tr>
<td>After ligation</td>
<td>5</td>
<td>100</td>
<td>24 ± 9*</td>
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Values for major prenodal cardiac lymphatic (MPCL) lymph flow rate (Q_L) are means ± SD, n = no. of dogs. In anatomic variations 1 and 2, tidal volume (V_T) reduction to 30% resulted in substantially lower Q_L. In variation 2, interruption of pulmonary contribution to MPCL lymph by ligation of pulmonary lymphatics resulted in a Q_L decrease that had the same magnitude as V_T reduction. After ligation, Q_L showed no significant changes with V_T reduction. *P < 0.05 vs. 100% V_T before ligation of pulmonary lymphatic branches.
during intracoronary administration of isoproterenol the coronary blood flow and cardiac lymph flow rate decreased significantly. After interruption of pulmonary contamination to MPCL lymph flow, the effect of V_T reduction on MPCL lymph flow was abolished. Therefore, we conclude that the amount of pulmonary contamination may not only differ between individuals due to anatomic variations but also may change in the same individual due to alterations in ventilation. Furthermore, pulmonary lymph flow rate, and therefore the amount of pulmonary contribution to MPCL lymph flow, may change due to alterations in pulmonary microvascular permeability.

Our findings may contribute to the interpretation of discordant cardiac lymph data reported in the literature. Investigators have recorded baseline myocardial lymph flow rates ranging from 0.8 to 3.6 ml/h (6, 13, 16, 23). Our data show that MPCL lymph flow rate may change with tidal volume alteration. Therefore, among other factors such as parenteral fluid substitution and anatomic variations, differences in ventilation parameters may account at least partially for these discordant findings. Takenaka and Araki (21) investigated the coronary blood flow and cardiac lymph flow rate during intracoronary administration of isoproterenol and norepinephrine. They found a weak correlation between the two parameters of r = 0.53 by using MPCL cannulation for lymph flow measurements. This is in contrast to the results of Saito et al. (20), who cannulated epicardial lymphatics and found a strong correlation between coronary blood flow and cardiac lymph flow rate (r = 0.9). Saito et al. (20) speculated that the different cannulation sites (epicardial versus MPCL) may have caused the discrepancy of results due to variations in mediastinal lymphatic anatomy. The results of our anatomic dissections support this assumption by demonstrating pulmonary contribution to MPCL lymph flow.

Although dye injections into the peritoneum and diaphragm failed to stain the TBN or MPCL, we cannot exclude the possibility that lymph of an origin other than pulmonary or cardiac was drained into the TBN and MPCL. In a number of dogs, we observed lymphatics draining into TBN in which staining did not occur after pulmonary or cardiac dye injections. These lymphatics could have been the efferent lymphatics of pulmonary sections that were not stained by dye injections or that may have derived from other mediastinal structures.

Lymph obtained from MPCL cannulation in the dog may be of limited use for the study of myocardial lymphatics in experimental models investigating absolute changes of myocardial lymph flow rate or lymph elements that are not exclusively found in the heart. In the presence of factors that influence not only cardiac but also pulmonary lymph flow, such as hemodilution or systemic application of substances increasing capillary permeability, the impact of pulmonary contamination on MPCL lymph flow rate may be substantial. However, experimental models investigating relative changes of lymph elements that are strictly of cardiac origin or that result only from heart perturbation may not be affected by pulmonary contribution to MPCL lymph flow. In dogs, cannulation of the principal myocardial lymphatic from a right lateral thoracicotomy (12) or cannulation of epicardial lymphatics (20) may be an alternative for the investigation of cardiac lymphatic function in acute open-chest experiments.

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REFERENCES


