FAILURE OF THE CANINE PRINCIPAL ASCENDING EPICARDIAL LYMPHATIC TO REGENERATE AFTER TRANSECTION


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ABSTRACT

We transected the principal ascending anterior epicardial cardiac lymphatic in 10 dogs, and after varying time intervals re-operated to look for lymphatic regeneration using dye injection. Photographs and sketches were made to record the findings, and in six dogs serial histologic sections were also examined.

In none of the 10 dogs was regeneration of the transected principal cardiac lymphatic detected although small lymphatic collaterals from the distal side of the lymphatic developed in 2 dogs. Further studies are merited to assess the role of lymphatic insufficiency in the development of coronary vasculopathy and chronic rejection after cardiac transplantation. Global lymphatic interruption occurs with cardiac transplantation. Local interruption of a principal ascending cardiac lymphatic may also occur at the time of coronary bypass surgery or other cardiac operations.

Accordingly, we examined whether a transected cardiac lymphatic in the dog regenerates and whether lymphatic collaterals develop and function as alternative pathways for cardiac lymph transport.

MATERIAL AND METHODS

Ten mongrel dogs varying in weight from 37 to 52 pounds (6 males; 4 females) were operated upon under sterile conditions at the Center for Animal Resources at the Northwestern University Medical School. The dogs were cared for under the guidelines defined in the National Institutes of Health “Guide for the care and use of laboratory animals” (NIH Publication no. 80-23, revised 1985). Anesthesia was initiated with intravenous barbiturate, and was maintained with halothane (1.5%) administered via an endotracheal tube and standard anesthesia equipment. The heart was exposed by a median sternotomy, the pericardium was incised longitudinally in its lateral aspect and a pericardial sling was made.
Fig. 1. The principal anterior ascending cardiac lymphatic in the dog as seen after a left ventricular apical injection of T1824 blue dye (arrow). Note two large branches ascending, one on either side of the interventricular groove, which then join as they near the base of the heart.

Approximately 0.4 ml of T1824 blue dye (Evans) was diluted 1:1 with isotonic saline and injected subepicardially near the apex of the left ventricle to visualize the cardiac lymphatics. Appropriate photographs were taken to document the lymphatic anatomy (Fig. 1). Sketches were also made from Kodachrome slides. Thereafter, the principal anterior ascending cardiac lymphatic was transected in its more proximal portion with care taken to avoid injury to nearby blood vessels or adjacent tissue. Small sutures were placed in the adjacent myocardium lateral to the transection site as markers. After transection, the T1824 (Evans) blue dye was seen draining from the proximal cut end. The pericardium was closed with a continuous prolene suture (7-0). The chest incision was then closed and the dog allowed to recover.

Between 7 and 36 weeks post-operative (Table 1), the dogs were re-operated upon. We intentionally staggered the time of re-operation to allow for potential variance for regeneration and collateral formation over time of the transected principal lymphatic. Anesthesia was similar to the initial operation. The chest was re-opened via a median sternotomy. Considerable adhesions were noted at the site of pericardial incision and closure overlying the right ventricle. There were, however, only minor adhesions at the site of the transected lymphatic. These were easily lysed and did not interfere with visualization of the topographic anatomy. After dissection of the parietal pericardium from the heart surface, a small amount of Higgins India ink (approximately 0.4 ml) diluted 1:1 with isotonic saline was injected
### TABLE 1
Interval Between Transection of the Principal Cardiac Lymphatic And Repeat Thoracotomy in 10 Dogs

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<thead>
<tr>
<th>Dog #</th>
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<tr>
<td>1</td>
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<td>2</td>
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<td>22</td>
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into the apex of the left ventricle. India ink was chosen to visualize the lymphatics at re-operation because, unlike T1824 blue dye, it is not washed out during fixation and staining of tissue specimens. Similar India ink injections were made into the free wall of the left ventricle including the posterior aspect to detect collateral lymphatics that may have formed since the initial operation. Photographs were taken and sketches made of the visualized cardiac lymphatics.

The heart was harvested, placed in formalin, and prepared for histologic study. In 6 of the 10 dogs, the visible lymphatics were able to be injected directly with India ink using a 27 gauge needle. The specimens were dehydrated and cleared with methy-salicylate for two months. Serial histologic sections were then made and the course of the cardiac lymphatics was examined using a dissecting microscope. Appropriate photographs were taken. The histologic sections were studied without knowledge of the gross findings at the time of the second operation.

Photographs and sketches made at the time of the first operative procedure were then compared to the photographs and sketches made at the second operation.

**RESULTS**

Comparative photographs and sketches taken before and after transection of the principal anterior ascending epicardial lymphatic revealed that the pattern of lymphatic drainage on the surface of the heart had significantly changed in 9 of the 10 dogs studied. The lymphatics that passed from the apex of the heart to its base became more circuitous and the previously divided principal cardiac lymphatic ascending adjacent to the interventricular groove was no longer seen, or if seen ended below the site where previously it had been transected. Collateral epicardial collecting lymphatics were detected that had not been seen at the first operation. In 6 dogs, gross scarring was seen at the site where the principal cardiac lymphatic had been severed.

*Fig. 2* shows comparison sketches of the photographic patterns of the principal anterior ascending epicardial lymphatic in the 10 dogs after the first and the second operations. Of note are newly visualized collateral lymphatics at the second operation. In one dog (#5) at 15 weeks, we were uncertain whether the photographs showed that the transected ascending lymphatic had measurably changed at the second operation. Histology, however, unequivocally showed obliteration of the ascending cardiac lymphatic at the site of transection.

Histologic studies were completed in 6 of the 10 dogs. Under the dissecting microscope, the transected lymphatics in each of the six dogs terminated in a fibrotic reaction at the site of interruption. In two of the dogs, tiny collateral branches arose from the distal end.
Fig. 2. Paired drawings (made from Kodachrome slides) taken before the principal anterior ascending cardiac lymphatic was transected and at varying time intervals thereafter. Nine of 10 of the photographic studies show notable changes in the pattern of the epicardial lymphatics (see text for details). None showed regeneration of the principal cardiac lymphatic itself.

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of the transected principal cardiac lymphatic and in two of its histologic sections, a lymphatic “twig” from the distal side of the transected cardiac lymphatic coursed to a deeper lying lymphatic before reaching the site of principal cardiac lymphatic transection.

Fig. 3 shows, in a cleared specimen, the site of termination of the previously transected principal lymphatic, the adjacent marking sutures, and the nearby interventricular coronary artery and cardiac vein.

DISCUSSION

Our experimental results demonstrate that a major lymphatic collector of the heart, namely the principal anterior epicardial lymphatic ascending adjacent to the interventricular groove, does not regenerate after it is transected. This valved major collecting lymphatic has a muscular wall, and it is not unexpected that the severed ends would retract after interruption. Moreover, the continuous motion of the underlying heart muscle probably serves to separate the divided ends further, making lymphatic regeneration unlikely. Rudimentary attempts at new lymphatic formation were seen in two of the dogs, but these efforts appear inadequate to maintain lymph flow to the base of the heart. Instead, fairly large epicardial collateral collecting lymphatics open up to perform this task.

Impairment of lymph flow from an organ or bodily part predisposes to inflammation, infection, edema, and the formation of interstitial fibrosis (2-5). Myocardial edema occurs when the regional lymphatics are

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extensively obstructed (3,6,7) and impaired myocardial function has been reported with acute lymphatic obstruction (8). Microscopy after myocardial biopsies in animals and man have revealed edema, as well as interstitial and perivascular fibrosis, in transplant rejected hearts (9-12). These findings, along with more recent work suggesting that infection with a cytomegalic inclusion virus (13) or with Chlamydia pneumoniae bacteria (14-18) plays a role in the development of coronary artery atherosclerotic plaques, makes the issue of the integrity of the cardiac lymphatic system particularly pertinent.

We suggest that cardiac lymph drainage is permanently impaired in the orthotopically transplanted heart where lymphatics are completely interrupted at time of allotransplantation. Such lymphatic impairment would predispose to inflammation, infection, edema, and the laying down of fibrous tissue in the transplanted heart. It is noteworthy in this regard that the intramural coronary arteries ordinarily spared from inflammatory and atherosclerotic disease are markedly affected in post-cardiac transplant vasculopathy. We previously suggested (19) that interference with cardiac lymph drainage predisposes to accelerated coronary vasculopathy (atherosclerosis?) seen after cardiac allotransplantation and also suggested that cardiac lymph flow impairment predisposes to the histopathologic appearance of chronic rejection.

Clark (20) has emphasized that initial peripheral lymphatics (capillaries) readily regenerate but that larger lymphatic vessels with valves have not been shown to do so. Our study was directed specifically at whether the principal canine anterior ascending epicardial lymphatic regenerated after transection.

Failure of the principal anterior cardiac lymphatic to regenerate after transection is probably indicative of what happens to the large epicardial collecting lymphatic when transected more proximally as occurs when a recipient heart is removed and a donor heart is transplanted. The long-term effects of impaired cardiac lymphatic drainage after heart transplantation awaits further investigation.

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REFERENCES


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