Lymphatics of the Mitral Valve of the Dog
Demonstration and Discussion of the Possible Significance

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This report presents evidence that lymphatics are present in the mitral valve of the dog. This observation, if it can be translated to man, may be of significance in valvulitis.

Histological studies in the past have failed to solve the problem of whether or not lymphatics are present in the heart valves of man or animals. Eberth and Belajeff, in 1866, reported finding a subendocardial plexus of lymphatics which extended into the atrioventricular and semilunar valves in both human and other mammalian hearts. In 1924, Aagard described finding lymphatics entering the leaflets of the atrioventricular valves in animals, but was unable to locate any similar channels in human hearts. In 1939, Patek reported that he was unable to demonstrate lymphatics in the atrioventricular valves of dogs by injection techniques. This appeared to cast doubt upon the previous reports. None of the many other investigators studying cardiac lymphatics have reported finding lymphatics in the heart valves.

Study of the lymphatics of the heart has been relatively neglected by both pathologists and physiologists. The apparent lack of interest by pathologists probably relates to the difficulty in evaluating the lymphatics in post-mortem material. This is also a major problem for the anatomist, but has been circumvented, at least in part, by use of injection techniques. India ink has generally been employed for this purpose. Patek used India ink injections in the living heart in order to permit better filling of lymphatics. The distribution of the ink was subsequently studied both grossly and microscopically.

Methods

The wholly extrapericardial surgical approach which we used to obstruct lymph flow has been reported previously. Those animals which did not die spontaneously were reoperated at varying time intervals between 2 and 27 weeks after the initial surgery. The patency of the cardiac lymphatic system was estimated after T 1824 dye injection into the myocardium, the functioning lymphatics thus being grossly visualized. These animals were then sacrificed, either by intravenous sodium pentobarbital or by anoxia, or an attempt was made to visualize mitral valve lymphatics grossly. In addition to the animals with surgically induced obstruction of cardiac lymph drainage, gross and histological studies were made in two other groups of dogs: (1) animals in which the cardiac lymph flow was obstructed, but in which death was induced within two hours; and (2) “control” animals, the cardiac lymphatic system of which was presumed to be intact, sacrificed during the course of other laboratory experiments.

In all three groups of animals, after gross examination of the mitral valve at autopsy, sections were cut from the free edge to the valve ring. All sections were stained with hematoxylin-eosin and a combined orcein-Van Gieson stain for histological examination.

As mentioned above, in some animals a special technique was employed in an attempt to visualize grossly the mitral valve lymphatics with India ink while the heart was still beating. Preliminary observations had shown that diluted India ink injected into the anterior mitral leaflet after the animal’s death remained localized. Thus, the following procedure was employed: The inferior and superior venae cavae, the azygos vein, and the ascending aorta proximal to the right innominate artery were ligated simultaneously. Then the
main pulmonary artery was partially ligated. The left atrium was incised, and, using the index finger, the anterior mitral leaflet was quickly raised into the opening. India ink (diluted two to one or three to one with Ringer's solution) was injected through a 27-gauge needle into the leaflet, usually near the free edge, but on occasion in the midportion. The mitral valve leaflet was then permitted to fall into place, and the left atrial opening was closed with a clamp. The heart was allowed to beat for about 10 minutes after this injection before the animal was sacrificed. The spread of ink into lymphatic channels was evaluated grossly at autopsy. The mitral valves were examined both grossly and histologically.

Results

Control Dogs

Small thin-walled channels* were seen microscopically in 2 of 15 dogs studied (fig. 1). In 3 dogs in which the India ink technique was employed, the ink remained localized, and no vessels were visualized in the valve leaflet either grossly (fig. 2) or microscopically.

Dogs with Surgically Produced Acute Obstruction of Cardiac Lymph Flow

One of these dogs had evidence of significantly decreased cardiac lymph flow by the T 1824 method prior to the surgical production of acute lymphatic obstruction. The injection of diluted India ink into the anterior mitral leaflet while the heart was still beating prior to death showed an extensive network of vessels (fig. 3). At autopsy, this dog had definite whitening and thickening of the left ventricular endocardium. On microscopic examination, the India ink was found to be within large thin-walled channels, similar to the histological findings in dogs with surgically produced chronic impairment of cardiac lymph flow (see section below and fig. 7).

In 11 other dogs, T 1824 injection prior to lymph obstructive surgery revealed no impairment of cardiac lymph flow. In 6 of them, small thin-walled channels were visualized microscopically in sections from the anterior mitral leaflet. In 5 of these 6 dogs, dilute India ink injections were made into the mitral leaflet, and at autopsy small grossly visible channels were apparent in 3 of them.

Dogs on Which Surgery Was Performed to Produce Chronic Impairment of Cardiac Lymph Flow

Microscopic examination of sections from the mitral leaflets showed small thin-walled channels in 5 of 13 dogs which had died spontaneously within five weeks after surgery.

In 6 dogs, the T 1824 dye method demonstrated a normal lymph flow at reoperation. Small thin-walled channels were found in sections from the mitral leaflet in 5 of them.

*The phrase "small thin-walled channels" refers to capillaries consisting of an endothelial lining and a minimal amount of connective tissue.
This animal, in which the cardiac lymph drainage was surgically acutely obstructed, showed evidence of significantly decreased cardiac lymph by the T 1824 method (see text) prior to the surgical procedure. Injection of diluted India ink into the mitral leaflet of the beating heart revealed an extensive lymphatic plexus.

Microscopy revealed thin-walled channels to be present in the mitral leaflets of 24 out of 28 operated animals judged to have decreased lymph flow at reoperation (fig. 4). In 8 of this last group of animals, dilute India ink was injected into the anterior mitral leaflet while the heart was beating. In 7 dogs, extensive networks of vessels were seen grossly, radiating from the injection site toward the valve ring (figs. 5 and 6). These vessels were all on the atrial surface of the leaflet. Microscopic study revealed the India ink to be within large thin-walled channels (fig. 7).

The thin-walled channels seen microscopically in this last group of operated animals generally were much larger and more numerous than those seen in the leaflets of the control animals and of those with acute obstruction of cardiac lymph drainage.
Discussion

Thin-walled channels, capillaries consisting of an endothelial lining, and a minimal amount of connective tissue, have been demonstrated in the anterior mitral leaflet of the dog. These channels are greater in number and larger in caliber after chronic impairment of cardiac lymph flow. The fact that India ink enters them promptly from an injection site in the free edge of the valve leaflet indicates that they are lymphatic rather than blood vessels.

Thus, it is apparent that the mitral valve of the dog does contain lymph vessels and that chronic interference with lymph flow enlarges their caliber and increases their number, either by opening up unrecognized pre-existing collaterals or by growth of new channels into the leaflet. Such new channels could come from budding out of pre-existing vessels in the valve, or from the adjacent heart wall.

It would be desirable to establish whether or not lymphatics are present in human heart valves. The use of improved dyes or suspensions of particulate matter might be employed to compare normal hearts with those with rheumatic valvulitis.

The increased vascularity of heart valves after acute rheumatic involvement is well established, and at times the atrial surface of the rheumatic mitral valve shows grossly visible vascularization. It is also on the atrial surface of the mitral valve that the grossly visualized lymphatic capillaries are found in dogs after chronic cardiac lymph obstruction. It is possible therefore, that some of the grossly and histologically identified vascular channels of the human heart valves are lymphatics rather than blood vessels.

If lymphatics are proven to be present in the human heart valves, our thinking concerning the development and progression of valvulitis will require revision. Thus, it is known that rheumatic fever has a tendency to involve tissues lined by endothelium, for example, blood vessels, pericardium, endocardium, and synovial membranes. Lymphatics are also lined with endothelium, and their inflammation and obstruction in heart valves could be expected to have significant pathological consequences. Rheumatic fever does involve lymphoid tissue.

Obstruction of lymphatics impairs the removal of edema fluid and predisposes to the laying down of fibrous tissue in the affected area. In addition, lymphatic obstruction predisposes to infection and inflammation. This pathological sequence is particularly well demonstrated in elephantiasis. The impairment of lymph flow results in edema and extensive fibrosis in the affected part. The infections which then occur serve further to embarrass lymph drainage, and to enhance the fibrotic process. Thus, a continuing vicious mechanism is set into motion. It appears logical to consider that the pathological sequence occurring in the heart valve affected by rheumatic fever, and the valve’s subsequent predisposition to inflammation and infection, could be related to impairment of lymph flow. The early pathological changes in heart valves affected by rheumatic fever include edema. Subsequently, fibrosis and the formation of verrucae occur. Moreover, it is known that
heart valves involved by the rheumatic process are predisposed to additional bouts of valvulitis either from recurrent rheumatic fever or from bacterial infections. It becomes of some urgency, therefore, to determine whether lymphatics can be visualized in normal or pathological human hearts.

It must be noted that we have no definitive experimental proof that impairment of cardiac lymph flow causes fibrosis of the heart valves of the dog, as it does in the endocardium. The histology of the valves, in both control and lymph-obstructed dogs, is quite variable from one animal to another. Variability in the histology of the heart valves of the dog has been described in the veterinary literature. This may require repetition of the experiment upon a pure strain of carefully controlled dogs, rather than using the stray dogs upon which our observations were based. We have been loath to make any judgment as to abnormal fibrosis of the mitral valve in our experiments because of this variability. However, from time to time, apparent increases of fibrous and/or elastic tissue in the valves of animals with chronic interference to cardiac lymph flow has tempted us to conclude that a cause and effect relationship did exist.

Summary

Thin-walled vessels have been visualized histologically in the mitral valve of the dog; these vessels were increased in number and caliber in animals with chronic impairment of cardiac lymph flow. Injection of diluted India ink into the free edge of the anterior mitral leaflet of the beating heart revealed extensive networks of vessels, grossly visible on the atrial surface of the valve. Histological study showed the India ink to be within thin-walled channels. Thus, there is compelling evidence that the visualized vessels are lymphatics and that their size and number increase when the cardiac lymphatic drainage is chronically impaired. The possible significance of the presence of lymphatic capillaries in the mitral valve is discussed.

References

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