Morphological Studies of the Cardiac Lymphatic System*

Tatsuo SHIMADA, Tetsuo MORITA, Muneharu OYA and Hirokazu KITAMURA

Department of Anatomy, Medical College of Oita, Hazama-cho, Oita, Japan

Received November 15, 1989

Summary. The distribution and structure of the mammalian cardiac lymphatic system have been investigated by puncture injection, intra-arterial injection of silver nitrate, hydrogen peroxide immersion, and light and electron microscopy.

The cardiac lymphatic system consists of drainage vessels and lymphatic capillaries. The drainage vessels contain many valves and are mainly situated subepicardially following branches of the coronary artery. The lymphatic capillaries are composed of a thin layer of endothelial cells, and form relatively dense networks in a fishnet arrangement. These lymphatic networks are richer in the ventricles than in the atria, being present in the subepicardial myocardial and subendocardial regions. In addition, networks are found in all cusps of the atrioventricular valves, and in the sinusatrial node and atrioventricular system.

The lymphatic system maintains cardiac homeostasis by receiving proteins, electrolytes and excess fluid from the interstitial tissue and returning them to the venous system.

HISTORY

According to PATEK (1939), RUDBECK (1653) was the first to morphologically investigate the cardiac lymphatic system. He observed a few subepicardial lymphatics in the dog heart. Many years later, several other investigators succeeded in demonstrating these subepicardial lymphatics utilizing direct injection of mercury or air into the lymphatics (NUCK, 1692; MUSSCHENBROEK, 1715). Further progress was brought about by the evolution of “the indirect or puncture injection method”, which consisted of inserting a cannula into the myocardium and forcing mercury into the tissues (FOHMAN, 1833). This relatively sophisticated method made it possible to visualize small subepicardial lymphatic vessels and capillaries. Thus, a rich plexus of lymphatics was shown to be present throughout the myocardium (LUSCHKA, 1863). In spite of these studies, the detailed distribution of cardiac lymphatics was little understood until the advent of the improved “physiological injection method” (PATEK, 1939). This new technique, which consists of injection of India-ink into the cardiac muscular wall of living animals, made it possible to delineate the delicate distribution of lymphatics within the endocardial, myocardial and epicardial regions of the beating heart. PATEK (1939) showed that the subepicardial lymphatic system consisted of large and small lymphatic capillaries and drainage vessels. Moreover, he found that cardiac lymph flow originated in the subendocardial area, passed into myocardial and then epicardial plexuses, and subsequently left the heart via the main lymphatic trunks draining to the regional lymph nodes. These observations were confirmed by JOHNSON and BLAKE (1966) who used hydrogen peroxide to grossly visualize cardiac lymphatics. This procedure selectively distends the lymphatic system by the formation of oxygen in the vessels.

In 1978, LEAK et al. studied the distribution and fine structure of lymphatic capillaries and collecting vessels which drain the various regions (i.e., endocardial, myocardial and epicardial) of the mammalian heart. Although it is difficult to identify lymphatic capillaries under ordinary light microscopy, the vascular perfusion technique permits to be made a definite distinction between lymphatic and blood vessels (MARCHETTI et al., 1985). This technique demonstrates that lymphatic capillaries are filled with fine, flocculent matter (cardiac lymph). Recently, the three-dimensional architecture of the subepicardial lymphatic system has been demonstrated by scanning electron microscopy (SEM) (SHIMADA et al., 1989).

*This paper is dedicated to Professor Emeritus M. KOTANI; on the occasion of his retirement from Kumamoto University.
CURRENT METHODOLOGY

1. Puncture injection

India ink, vital dye (trypan blue), latex or radiopaque iodized oil have been used as filling substances to investigate the lymphatic system. India-ink is the most popular substance employed, being injected by needle, syringe, or glass capillary into the endocardium, myocardium, epicardium, areas of the heart conduction system and the atrioventricular valves (PATEK, 1939; ELIŠKA and ELIŠKOVÁ, 1976; GOLAB, 1977; NOGUCHI et al., 1988).

2. Intra-arterial injection of silver nitrate solution

Hearts were perfused via the coronary arteries, first with 3.3% sodium sulfate solution and then with a 0.7% silver nitrate solution with India-ink added (MORI, 1969; IKEDA, ICHIKAWA and UCHINO, 1987). Tissue blocks were immersed in 10% formalin, dehydrated in an ascending series of ethanol, embedded in paraffin or celloidin and serially sectioned at 30-40 μm.

3. Hydrogen peroxide topical application

Fresh or formalin fixed tissues were immersed in a 0.5-1.0% solution of hydrogen peroxide for a few minutes (PARKE and MICHELS, 1963; JOHNSON and BLAKE, 1966; NOGUCHI et al., 1988). Hydrogen peroxide initiates an oxidoreduction reaction with catalase and peroxidase in the tissue or lymph, producing oxygen and water. The released oxygen causes distention of lymphatics and sometimes blood vessels.

---

**Fig. 1.** Light micrograph of a 1 μm thick epon section from the left branch of the sheep heart. A lymphatic capillary (LC) containing flocculent material lies within the connective tissue between fascicles of specialized muscle cells. ×130

**Fig. 2.** Lymphatic capillary (LC) in the monkey myocardium (a). Lymphatic endothelial cells show an oak-leaf shape (b). AgNO₃ treatment (Micrographs courtesy of Prof. Shigeo UCHINO and Dr. Sanae ICHIKAWA).
4. LM and TEM of Epon sections

The hearts were removed and perfused in a retrograde manner through the aorta with a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Small tissue blocks of various regions were then immersed in the same fixative for 3 h at 4°C and postfixed in buffered 2% osmium tetroxide (OsO₄) or buffered 2% OsO₄-0.5% potassium ferrocyanide for 2 h. Specimens were dehydrated in a graded series of ethanol and embedded in epoxy resin.

Semi-thin sections (1.0-1.5 μm thick) were stained with 1% toluidine blue for LM (MARCHETTI et al., 1985; IKEDA, ICHIKAWA and UCHINO, 1987; NOGUCHI et al., 1988). Subsequently, ultrathin sections (80-100 nm thick) were stained with uranyl acetate and lead citrate and examined under transmission electron microscopy (TEM) (LEAK et al., 1978; MARCHETTI et al., 1985; SHIMADA et al., 1988).

5. SEM of subepicardial lymphatic system

Hearts were removed and immersed in a 1% solution of hydrogen peroxide for 10 min, then placed in 10% formalin containing 1% hydrogen peroxide, and the epicardium carefully stripped off with forceps under a dissecting microscope (SHIMADA et al., 1989). The specimens were further treated with 8N HCl for 30 min at 37°C to effectively remove connective tissue elements. They were then washed in a saline solution, postfixed in buffered 1% OsO₄, dehydrated, critical point-dried and sputter-coated with gold.

---

**Fig. 3.** TEM image of a lymphatic capillary (LC) within the atrioventricular bundle of the rabbit heart. The lymphatic capillary shows an irregular contour and consists of extremely thin endothelial cells. BC blood capillary. ×1,400

**Fig. 4.** Relationship between continuous endothelial cells of lymphatic capillaries of the rabbit heart.

a: End-to-end adhesion, b: overlapping, c: fork-like interlocking. ×22,000
MORPHOLOGY OF LYMPHATIC SYSTEM

1. Light microscopy
Perfusion fixation through blood vessels allows the distinction of blood capillaries and lymphatic capillaries. Blood capillaries uniformly exhibit a well distended wall without intraluminal contents, while lymphatic capillaries are consistently filled with fine, flocculent matter (MARCHETTI et al., 1985) (Fig. 1). The caliber of the lymphatic capillaries, which are of irregular contour, ranges from 20 to 100 μm, and is larger than that of blood capillaries (about 5-10 μm). The endothelial cells of lymphatic capillaries are extremely attenuated except in areas occupied by the nucleus (Fig. 1). Observations of heart tissue intravascularly perfused with a silver nitrate solution reveals that the endothelial cells of the lymphatics show a peculiar “oak leaf” shape (Fig. 2). Lymphatic capillaries have no valves.

2. Fine structure of lymphatic capillaries
Lymphatic capillaries in different regions of the heart show similar ultrastructural features (LEAK et al., 1978; MARCHETTI et al., 1985, 1986; SHIMADA et al., 1988). When tissue from the perfused heart is examined under TEM, lymphatic capillaries appear as irregularly shaped vessels with a continuous endothelial lining. Their lumina are filled with a fine flocculent precipitate of low density (Fig. 3). The endothelial cells are extremely attenuated over large areas of cytoplasm and the major cellular organelles are concentrated in perinuclear areas. Cytoplasmic filaments are always observed in endothelial cells. Pinocytotic vesicles are seen along both luminal and abluminal surfaces of the endothelium. Relationships between adjacent endothelial cells are variable, consisting of end-to-end adhesions with desmosome-like junctions, overlapping cytoplasmic processes or fork-like interlockings (MARCHETTI et al., 1985) (Fig. 4). There are few open junctions in the perfused heart.

Fig. 5. Mast cell situated near a lymphatic capillary in the moderator band of the sheep heart. ×9,100
Fig. 6. Nerve fibers (N) running close to a lymphatic capillary (LC) in the sinuatrial node of rabbit. ×9,200
Fig. 7. SEM image of the anterior surface of the rat heart. The anterior interventricular trunk (LT) is continuous with lymphatic capillary networks (LC), and ascends the anterior interventricular sulcus. The epicardium has been removed with a forceps following the topical application of hydrogen peroxide. RV right ventricle, LV left ventricle. ×30
tissue. The basal lamina is of variable thickness and discontinuous, unlike that of blood capillaries (Fig. 4). Numerous anchoring filaments, which are closely apposed to the abluminal endothelial cell surface, extend into the surrounding connective tissue (LEAK, et al., 1978) (Fig. 4). There are no pericytes on the stromal surface of lymphatic capillaries.

Like blood vessels, lymphatic capillaries are surrounded by loose connective tissue that also extends into the fascicular planes between bundles of myocytes (LEAK et al., 1978) (Fig. 3). Elastic fibers and collagen fibers are closely associated with the endothelial surface. Occasionally, fibroblasts, macrophages and mast cells are observed (Fig. 5) in the connective tissue, but the number of connective tissue cells are small. Bundles of nerve fibers are frequently found in close proximity to lymphatic vessels (LEAK et al., 1978; NOGUCHI et al., 1988; SHIMADA et al., 1988) (Fig. 6).

**DISTRIBUTION AND ARCHITECTURE OF LYMPHATIC VESSELS**

**1. Ventricles**

A plexus of lymphatic vessels lies in the subepicardial, myocardial and subendocardial connective tissues of both ventricles (PATEK, 1939; JOHNSON and BLAKE, 1966; LEAK et al., 1978; OKADA and SHOZAWA, 1974; MILLER, 1984; MARCHETTI et al., 1985; IKEDA et al., 1987).

**1) Subepicardium**

In the rat, the subepicardial lymphatic system consists of capillaries and drainage vessels and is located in the connective tissue between the epicardium and the myocardium. The lymphatic capillaries form a continuous plexus which covers the entire surface of each ventricle (SHIMADA et al., 1989) (Fig. 7). The capillaries vary in diameter from 25 to 100 μm, and

![](image1.png)

**Fig. 8.** Higher magnification of Fig. 7. The subepicardial lymphatic capillary network (LC) appears to be continuous with the myocardial lymphatic capillaries (arrows). M myocardium. ×120

**Fig. 9.** Gross-view of subepicardial lymphatic system in the dog heart. Lymphatic capillaries communicate with a lymphatic trunk (LT) which shows a beaded appearance, suggesting the presence of valves. Hydrogen peroxide treatment. ×8
ramify and anastomose. The networks or meshes of lymphatic capillaries thus formed are irregular in contour and are of variable size ranging from 150 to 400 μm. The subepicardial lymphatic capillaries are continuous with efferent lymphatics. Some of these subepicardial lymphatic capillaries run close to cardiac muscles cells, and appear to be continuous with myocardial lymphatic capillaries (Fig. 8).

In the dog, the subepicardial lymphatic plexuses are made up of both large and small capillaries. The large capillaries overlie the interfascicular depressions formed by the most superficial layer of muscle bundles. They receive afferents from the myocardial plexus and converge to form drainage vessels. The small capillaries unite adjacent large capillaries (PATEK, 1939; OKADA and SHOZAWA, 1984). Lymphatic capillary networks show a fishnet form similar to those in the rat (Fig. 9), but each mesh of the lymphatic capillary network is apparently larger than that in the rat (about 1-2 μm) (SHIMADA et al., 1989). The subepicardial lymphatic system in human heart generally shows a similar pattern to that of the dog heart (JOHNSON and BLAKE, 1966).

The efferent (drainage) lymphatics contain many valves and are usually situated in the subepicardial layer (Figs. 7, 9), following branches of the coronary artery. These in the human heart are composed of left and right cardiac lymphatic trunks. The former divides into an anterior and a posterior interventricular trunk. The cardiac lymphatic trunks mainly end in tracheobronchial lymph nodes.

2) Myocardium

The myocardial lymphatic vessels form a loosely meshed three-dimensional plexus which is uniformly distributed throughout the entire myocardium (PATEK, 1939). Since the vessels forming the myocardial lymphatic plexus contain few valves, the entire plexus is composed of only lymphatic capillaries. The lymphatic capillaries are less extensive towards the luminal surface of the ventricular wall (MARCHETTI et al., 1985). The myocardial lymphatic plexus receives short communicating branches from the subendocardial plexus, and then is connected with the

Fig. 10. A schematic drawing illustrating the relationship between the subepicardial (Ep), myocardial (M) and subendocardial (En) lymphatic plexuses of the heart.

Fig. 11. Light micrograph of a semi-thin section from the sheep myocardium. Blood capillaries (arrows) are situated in connective tissue around each myocyte, while lymphatic capillaries (LC) lie in the connective tissue surrounding the myocyte fascicles. ×230
subepicardial lymphatic plexus (Patek, 1939) (Fig. 10).

The myocardial lymphatic capillaries lie in the interfascicular connective tissue (Figs. 2, 11), being few in number when compared with blood capillaries (Fig. 11). The ventricular myocardium in cat, rabbit and human hearts possesses an average of 1029 blood capillaries per 1000 muscle cells (Wearn, 1928), while the myocardium in dog heart contains one lymphatic capillary per 20–30 muscle cells (Okada and Shozawa, 1984).

3) Endocardium
Lymphatic capillaries are also localized in the subendocardial connective tissue of both ventricular walls. They form a well-developed loose reticular fishnet-like arrangement, and are in the interventricular septum (Fig. 12) and in the papillary muscle (Fig. 12) of both ventricles (Patek, 1939; Johnson and Blake, 1966; Uhley, Leeds and Sung, 1972; Eliška and Elišková, 1980; Ikeda, Ichikawa and Uchino, 1987).

Patek (1939) described the subendocardial lymphatics as being connected to a myocardial, and subsequently, to a subepicardial plexus which, in turn, leaves the heart via several drainage trunks.

2. Atria
There is a general agreement that the lymphatic system in the atria is less extensive than in the ventricles. Patek (1939) readily visualized a ventricular lymphatic network using India-ink injection but was unable to detect atrial lymphatics with this method. Recently, LM and TEM studies of resin-embedded sections (Marchetti et al., 1986; Ito et al., 1988) have shown that an extremely scanty network of small lymph channels extends throughout the whole subepicardial region of the right and left atrial wall. Rarely, some lymphatic capillaries exist between the outer myocardial cells. There are no lymphatic capillaries in the subendocardium.

Fig. 12. Gross view of the luminal surface of the right ventricle after treatment with hydrogen peroxide. The subendocardial plexus tends to be well-developed in the papillary muscle (a) and interventricular septum (b). Arrow right branch. ×19
3. Cardiac valves

Cardiac valves consist fundamentally of endothelium and connective tissue elements, including collagen and elastic fibers. There are no blood capillaries in cardiac valves except for the proximal portion of the atrioventricular valves (SMITH and TAYLER, 1971; NOGUCHI et al., 1988). However, lymphatic capillaries exist both in the tricuspid and mitral valves of pig, dog and human hearts. They are however missing from aortic and pulmonic valves (MILLER, PICK and KATZ, 1961; JOHNSON and BLAKE, 1966; NOGUCHI et al., 1988; ITOU et al., 1988). Lymphatic capillaries have been observed in all cusps of the atrioventricular valves, and there are delicate networks of lymphatic capillaries in the subendocardium on the atrial side of the valves (Fig. 13). The extent of their development varies among cusps, being most prominent in the anterior cusp of the mitral valve (NOGUCHI et al., 1988). The lymphatic plexus of the atrioventricular valves appears to join the lymphatic system of the atria.

4. Heart conduction system

Using a variety of different morphological techniques, lymphatic capillary networks have been demonstrated in the sinuatrial node of man, dog and rabbit (ELIŠKA and ELIŠKOVÁ, 1976; GOLAB, 1977; MARCHETTI et al., 1986; ITOU et al., 1988; SHIMADA et al., 1988), in the atrioventricular node of man, dog and rabbit (GOLAB, 1977; ELIŠKA and ELIŠKOVÁ, 1980; ITOU et al., 1988; SHIMADA et al., 1988) (Fig. 14), in the His bundle of man and rabbit (GOLAB, 1977; SHIMADA et al., 1988) (Fig. 3), in the right branch of dog (UHLEY et al., 1972) (Fig. 12), in the left branch of sheep (Fig. 1), and in the Purkinje fibers of the sheep moderator band (SMOLICH et al., 1989) (Fig. 15).

In human and dog hearts, the lymphatics from the sinuatrial node drain directly or indirectly to the

Fig. 13. Lymphatic capillary network within the atrial surface of the anterior cusp of the dog mitral valve. 

a. India-ink injection, b. hydrogen peroxide treatment. a: ×11, b: ×17 (From NOGUCHI et al., 1988)
right main trunk of the heart, and those from the atrioventricular node and the His bundle drain directly to the left main trunk (ELIŠKA and ELIŠKOVÁ; 1976, 1980; GOLAB, 1977). TAWARA (1906) described the atrioventricular system as possessing a wide lymph space in the connective tissue surrounding the fascicles of specialized muscle cells.

**DRAINAGE OF CARDIAC LYMPH**

Working cardiac muscles and the conduction system are irrigated by the coronary circulation, and the exchange of gases, nutrients and metabolites are carried out exclusively via blood capillaries. In addition to blood vascular system, the mammalian heart is also provided with a well-developed lymphatic system. Essential functions of the lymphatic system are to pick up large molecules, particles and excess fluid and to transport them into the venous system (CASELEY-SMITH, 1976). According to MILLER et al. (1984), the flow rate of cardiac lymph in the dog is on average 3.2 ml/h, and cardiac lymph contains proteins (albumin and globulin), chloride and sodium potassium. In addition, the mean lactate concentrations have been found to be significantly higher in the cardiac lymph than in the coronary sinus blood (ULLAL, 1972). The cardiac lymph is therefore expected to reflect closely the changes occurring in the heart in health and disease. Several lines of evidence have demonstrated that mechanical or reactive obstruction of cardiac lymphatics causes pathological changes in the myocardium, endocardium, cardiac valves and conduction system (MILLER et al., 1961; ROSSI, 1965; RUSZNYÁK, FÖLDI and SZABÓ, 1967; KLEINE, 1969; SYMBAS and SCHLANT, 1969; UHLEY, 1972).

*Fig. 14.* Lymphatic capillary (LC) and fenestrated blood capillary (BC) running between strands of nodal cells (NC) in the rabbit atrioventricular node. ×2,600. *Inset* shows enlargement of area in rectangle. ×3,800 (From SHIMADA et al., 1988)
Thus, the cardiac lymphatic system maintains the homeostatic balance of heart, and interruption of the cardiac lymph drainage may produce pathological lesions of heart tissue.

Acknowledgments. The authors are deeply grateful to Professor M. Nakamura (Medical College of Oita) and Dr. Gordon R. Campbell (University of Melbourne) for critical advice. We also wish to thank Mr. T. Kajiwara and Miss M. Taniguchi for experimental assistance.

REFERENCES


Fig. 15. Lymphatic capillaries (LC) located near the Purkinje fibers (P) in the sheep moderator band. A small artery, V small vein, BC blood capillary. ×1,800


