

Chapter 2

Development and Patterning of the Cardiac Lymphatic Network

Jörg Wilting and Jörg Männer

Abstract An overview of the prenatal development of the cardiac lymphatic system can be achieved only when it is seen in the general context of prenatal morphogenesis of the heart. Moreover, since it was found that the development of the cardiac lymphatic network follows, in time and place, that of the coronary blood vessels, cardiac lymphangiogenesis cannot be fully understood without knowledge about the essential steps in the development of the blood supply of the developing heart. We therefore start this chapter with short descriptions of both the morphogenesis of the human embryonic heart and the development of its blood supply, and we will then focus on the development of the cardiac lymphatic system.

Keywords Heart development • Cardiac looping • Trabecular myocardium • Proepicardium • Vasculogenesis • Subendocardial lymphatic plexus • Myocardial lymphatic plexus • Subepicardial lymphatic plexus • Jugular lymph sac • Lymphangiogenesis

Introduction

The heart is the first organ to function in vertebrate embryos. The human heart, for example, starts its pumping action around the twenty-first embryonic day. From its first until its last beat, cardiac pumping function depends on sufficient blood supply

J. Wilting (✉)

Department of Anatomy and Cell Biology, University Medicine Gottingen,
Kreuzberggring 36, Goettingen 37075, Germany
e-mail: joerg.wilting@med.uni-goettingen.de

J. Männer

Department of Anatomy and Embryology, University Medicine Gottingen,
Kreuzberggring 36, Goettingen 37075, Germany
e-mail: jmaenne@gwdg.de

to its myocardium. In the primitive embryonic heart and in the mature heart of several lower vertebrate species, myocardial blood supply comes exclusively from the endocardial lumen. The ventricular myocardium of these hearts has a spongy architecture with intertrabecular spaces, which become filled and emptied with blood during ventricular diastole and systole, respectively. The formation of a thick layer of compact ventricular myocardium, which is a characteristic feature of higher vertebrate hearts, is accompanied by the establishment of a new mode of myocardial blood supply via a system of blood vessels called the coronary vasculature. Due to the fatal consequences of coronary vessel insufficiency (e.g., myocardial infarction), this vascular system receives enormous interest, whereas the second vascular system, the cardiac lymphatics, is mostly neglected, although cardiac lymphedema has also been recognized as a clinically important problem for a long time. In the mature human heart, plexuses of initial lymphatics are found in subendocardial and subepicardial positions, connected by myocardial lymphatics, usually accompanying the coronary arterioles. The lymphatic collectors contain valves and, arranged in pairs, follow the subepicardial branches of the coronary arteries. They drain via the left coronary trunk into the right venous angle and via the right coronary trunk into the left venous angle. The coronary blood vessels are derived from a primarily extracardiac population of cardiac progenitor cells, the proepicardium (PE), which is located near the venous pole of the tubular embryonic heart. PE cells colonize the initially naked myocardial surface of the embryonic heart and give rise, first, to the epicardial mesothelium and, later, to various other cell types, including fibroblasts, endothelial cells, and vascular smooth muscle cells. Embryonic development of the cardiac lymphatics starts with the formation of subepicardial vessels, which proceeds from the base to the apex of the heart. Despite their intimate topographical relationship to coronary arteries, fate-mapping studies have shown that the cardiac lymphatic endothelium does not derive from the PE. The precise origin of the cardiac lymphatics is a current matter of research. It is likely that they derive from sprouts of the jugular lymph sacs (JLS), which are located at the confluence of the cranial and caudal cardinal veins into the common cardinal vein. The co-option of lymphangioblasts into growing cardiac lymphatics has not been studied experimentally. The embryonic origin of cardiac lymphatics and their functions in the pre- and postnatal heart is an open field for future investigations.

Short Overview on the Morphogenesis of the Human Embryonic Heart

The development of the human heart starts during the third week of prenatal development (fifth postmenstrual week). A bilaterally paired *heart-forming field* forms within the splanchnic layer of the lateral plate mesoderms of the tri-laminar embryonic disc. The paired heart-forming field harbors the progenitor cell populations for the myocardial and endocardial cell lineages of the future heart. Experimental and molecular data from nonhuman embryos have shown that each half of the paired heart-forming field can be further subdivided at least into two genetically different

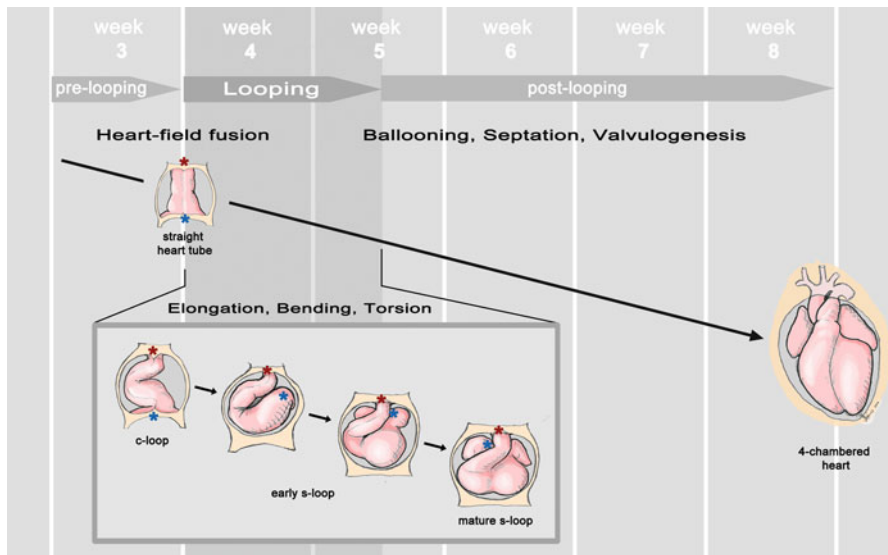


Fig. 2.1 This schematic drawing gives an overview on the morphogenesis of the human embryonic heart. Cardiac morphogenesis can be subdivided into three subsequent phases: (1) *Pre-looping morphogenesis* (third week of prenatal development). A bilaterally paired heart-forming field forms within the lateral plate mesoderm. Heart fields start fusion in the body midline and form a straight heart tube, which is connected to the veins at its caudal end (blue asterisk) and to the arteries at its cranial end (red asterisks). (2) *Looping morphogenesis* (fourth and fifth week). The tubular heart elongates by addition of new material from the heart-forming field to its cranial and caudal ends. Due to the limited space within the pericardial cavity, the elongating heart tube undergoes bending and torsion and thereby acquires the configuration of a helically wound heart loop. Looping morphogenesis brings the segments of the heart tube and the developing great vessels into an approximation of their definitive topographical relationships. (3) *Post-looping morphogenesis* (fifth to eighth week). The helically wound heart tube becomes transformed into a four-chambered heart by mainly three processes (a) ballooning, (b) septation, and (c) valvulogenesis. By the end of the embryonic period (end of the eighth week), the heart has acquired the basic four-chambered design of the mature human heart

subdomains which have been named the first and second heart fields [1]. For reasons of simplicity, however, in our short description of cardiac morphogenesis, we will not distinguish between various subdomains of the heart field and, therefore, use the term *heart-forming field* in a very general sense.

The right and left halves of the heart-forming field begin to fuse in the embryonic body midline by the end of the third week of development. Thereby, a straight heart tube is formed in front of the developing foregut (Fig. 2.1). It has a single endocardial lumen, which is connected to the veins at its caudal end (venous heart pole) and to the arteries at its cranial end (arterial heart pole). It is frequently stated in the literature that the straight heart tube contains all embryonic segments of the heart. This, however, is an incorrect statement, since the straight heart tube consists only of the future apical trabeculated regions of the ventricles (for review, see [2]).

During the fourth and fifth week of development, the tubular heart elongates by continuous addition of new segments from the heart-forming field to its cranial and

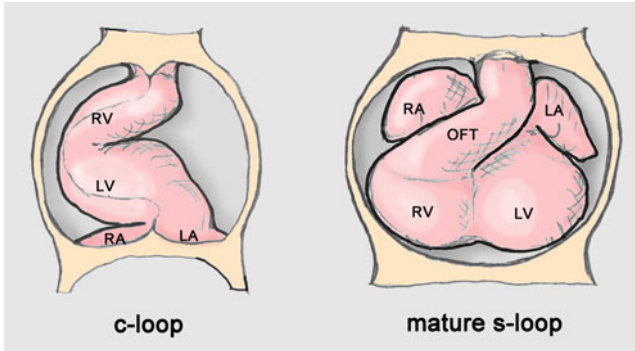


Fig. 2.2 These drawings show the normal positional changes of the embryonic ventricles caused by looping morphogenesis (ventral view into the opened pericardial cavity). (1) Caudal shift of the developing ventricles with respect to the developing atria; (2) conversion of the original craniocaudal alignment of the ventricular chambers into the definitive left–right topology of the ventricular chambers; (3) shortening of the distance between the arterial and venous heart poles. *LA* left atrium; *LV* left ventricle; *OFT* outflow tract; *RA* right atrium; *RV* right ventricle

caudal ends. Due to the limited space within the embryonic pericardial cavity, the elongating heart tube undergoes bending and torsion [3] and thereby acquires the configuration of a helically wound heart loop [4]. This phase of cardiac morphogenesis is named *cardiac looping* as a whole, but can be subdivided into the subphases of c-looping, early s-looping and late s-looping [4]. Looping morphogenesis is said to bring the embryonic segments of the heart tube and the developing great vessels into an approximation of their definitive topographical relationships. The developing ventricles, for example, are originally aligned along the craniocaudal body axis where the so-called left ventricle has a caudal position with respect to the so-called right ventricle (Fig. 2.2). As a consequence of looping morphogenesis, this positional relationship is converted into the definitive left–right topology of the ventricles (Fig. 2.2). In view of its central role in the correct positioning of the developing heart segments, cardiac looping is regarded as the key phase in the morphogenesis of higher vertebrate hearts (for review, see [4]). Having this in mind, we find it helpful to distinguish three subsequent phases of cardiac morphogenesis (Fig. 2.1): (1) *the pre-looping phase*, which is characterized by the formation of the bilaterally paired heart-forming field and the subsequent establishment of a straight heart tube (see above); (2) *the looping phase*, which is characterized by the above-mentioned morphological and positional changes of the tubular embryonic heart; and (3) *the post-looping phase*.

The post-looping phase of cardiac morphogenesis is characterized by the transformation of the helically wound heart tube into a four-chambered heart with separate pulmonary and systemic flow pathways. This transformation is accomplished mainly by three processes: (1) ballooning, (2) septation, and (3) valvulogenesis. The term *ballooning* characterizes local growth processes leading to the outward bulging of the heart wall at circumscribed areas, which represent the developing

atrial appendages and the apical trabeculated regions of the left and right ventricle [5]. The term *septation* summarizes all morphogenetic processes leading to the formation of septa, which divide the originally single cardiac flow pathway into systemic and pulmonary flow pathways. The term *valvulogenesis* defines all morphogenetic processes involved in the formation of the semilunar and atrioventricular valves.

By the end of the embryonic period (end of the eighth week of development), the developing heart has acquired the basic four-chambered design of the mature human heart. The stems of the coronary blood vessels and their main branches are present [6], but cardiac lymph vessels just appear at the base of the ventricles [7].

Development of the Epi- and Myocardial Blood Supply

The development of the coronary blood vessels precedes that of the cardiac lymphatic vessels, both in time and place [7, 8]. It, therefore, seems reasonable to start with a description of the development of the blood supply of the embryonic heart before we will focus on the formation of the cardiac lymphatic system.

The human embryonic heart starts its pumping action around the twenty-first day of development [9, 10]. At this time point, it is seen as a tubular peristaltic pump whose outer wall is formed by naked myocardium that is in direct contact with the pericardial fluid. The naked embryonic heart muscle is a two-layered epithelium that not only lacks an epicardial covering but, additionally, lacks coronary blood vessels. Its blood supply, therefore, comes exclusively from the heart lumen whose endocardial lining is connected with the myocardium by a layer of cell-free extracellular matrix, called the *cardiac jelly* (Fig. 2.3a). The cardiac jelly forms a relatively thick diffusion barrier between the heart lumen and the myocardium. Its presence seems to conflict with the need for an efficient blood supply of a steadily working myocardium. It has been found, however, that its presence is needed for efficient peristaltic pumping of the tubular heart [11]. The design of the early embryonic heart, therefore, seems to be optimized for the purpose of peristaltic pumping but, on the other hand, seems to neglect the need for an efficient blood supply of its contracting muscle layer.

The situation changes during the mid-phase of cardiac looping when pouch-like invaginations of the endocardium start to remove the cardiac jelly from the apical portions of the developing ventricles (Fig. 2.3b) and, thereby, reduces the thickness of the diffusion barrier between blood and myocardium [12, 13]. The latter is, for the most part, still a naked myocardium forming the outer surface of the tubular heart. It is only at the dorsal wall of the embryonic ventricles where patches of epicardial mesothelium appear in the early s-shaped heart loop (fifth week; Carnegie stage 12; [14]). These patches of primitive epicardium are derived from a primarily extracardiac population of cardiac progenitor cells called the *proepicardium* (PE). The latter structure is a cauliflower-shaped accumulation of villous or vesicular protrusions of the pericardial mesothelium covering the ventral wall of the *sinus venosus* and the *septum transversum* [15].

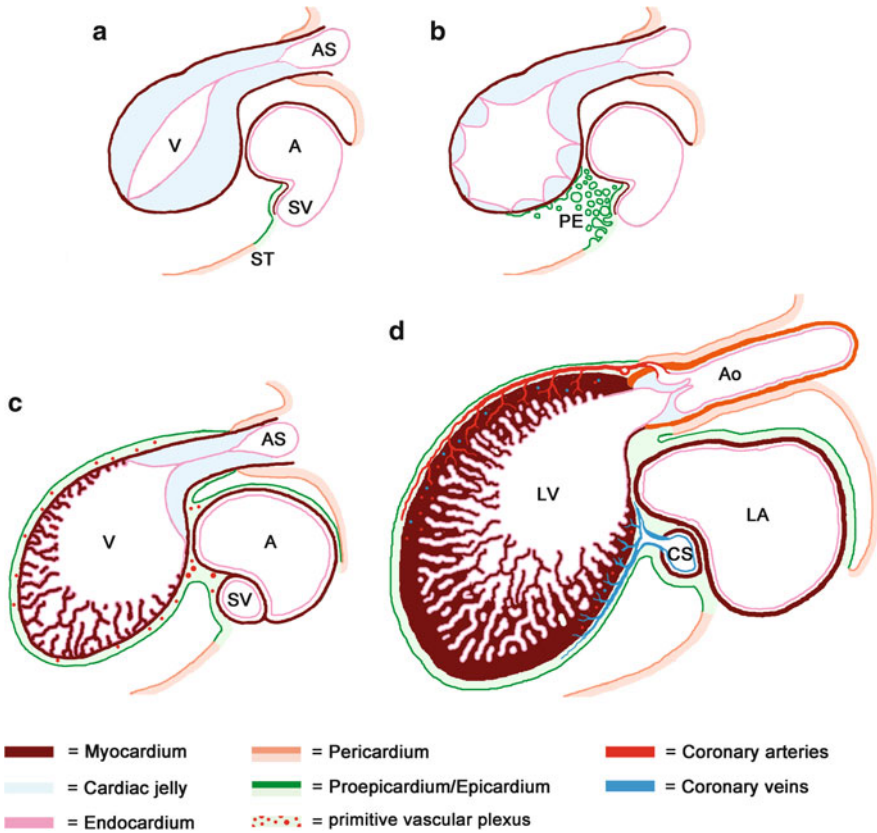


Fig. 2.3 These schematic drawings show the main steps in the development of the myocardial blood supply. (a) *Initial phase of blood supply from the endocardial lumen.* The wall of the early embryonic heart consists of three layers: (1) naked myocardium, (2) cardiac jelly, and (3) endocardium. The cardiac jelly is needed for efficient peristaltic pumping but forms a relatively thick diffusion barrier between the heart lumen and the myocardium. (b) *Transition to blood supply via myocardial sinusoids.* During the mid-phase of cardiac looping, pouch-like outgrowths of the endocardium start to remove the cardiac jelly from the apical portions of the developing ventricles, thereby reducing the thickness of the diffusion barrier between blood and myocardium. The myocardium is, for the most part, still a naked myocardium. Formation of the epicardium starts at the dorsal wall of the embryonic ventricles. It derives from a primarily extracardiac progenitor cell population called the proepicardium (PE). (c) *Blood supply via myocardial sinusoids.* During late stages of cardiac looping, endocardial pouches invade the inner layer of the ventricular myocardium. Centrifugal growth of the endocardial pouches and inner myocardium create the spongy/trabecular myocardium. The blood supply of the spongy myocardium comes from the endocardial pouches, which are now named the myocardial sinusoids. The outer myocardial layer is completely covered with PE-derived epicardium. Epicardium-derived cells colonize the subepicardial, myocardial and subendocardial layers of the heart. Epicardium-derived endothelial cells form a primitive vascular plexus within the subepicardial mesenchyme, which is the anlage of the coronary blood vessels. (d) *Blood supply via coronary blood vessels.* The subepicardial vascular plexus establishes open connections with the sinus venosus and the ascending aorta. Remodeling of the plexus forms a hierarchically arranged vascular system of coronary arteries, capillaries and veins. Myocardial blood supply via coronary blood vessels gradually replaces blood supply via myocardial sinusoids. A undivided embryonic atrium; Ao aorta; AS aortic sac; CS coronary sinus; LA left atrium; LV left ventricle; PE proepicardium; ST septum transversum; SV sinus venosus; V undivided embryonic ventricle

During advanced stages of cardiac looping, endocardial pouches reach the inner layer of the ventricular myocardium and start to invade the heart muscle [12]. Signals from the invading endocardial pouches stimulate proliferation of the surrounding myocardium [16]. Consequently, both the endocardial pouches and inner myocardium grow together in a centrifugal pattern, thereby, creating an inner myocardial layer of spongy architecture (Fig. 2.3c). The spongy myocardium is frequently named the *trabecular myocardium* and the process of its formation is called *trabeculation*. The spongy myocardium still lacks coronary blood vessels but appears like a highly vascularized tissue since it is in intimate contact with the blood-filled lumina of the endocardial pouches, which are now named the *myocardial sinusoids* or *intertrabecular spaces*. The blind-ending myocardial sinusoids become filled and emptied with blood during the cardiac cycle and, thereby, facilitate a relatively efficient blood supply of the avascular embryonic myocardium.

While the inner myocardial layer of the developing ventricles transforms into a blood-filled sponge, the outer myocardial layer of the human embryonic heart remains compact and becomes completely covered with epicardial mesothelium derived from the PE (fifth+ sixth week; Carnegie stages 12–16; [14]). The PE and primitive epicardium play a central role in the formation of the coronary blood vessels since they provide not only the mesothelial covering of the heart but, additionally, provide mesenchymal cells that colonize the subepicardial, myocardial, and subendocardial layers where they differentiate into various cell types such as fibroblasts, coronary endothelial cells, and coronary smooth muscle cells [15]. The formation of the coronary blood vessels starts within the subepicardial mesenchyme from where it progresses into the myocardium. Epicardium-derived endothelial cells form a primitive coronary vascular plexus by vasculogenesis (sixth week; Carnegie stages 14/15; [17]). Initially, this plexus is neither connected with the *sinus venosus* nor with the developing great arterial trunks and, therefore, does not contribute to myocardial blood supply during this developmental period.

The situation changes, however, when the subepicardial part of the coronary vascular plexus establishes open connections firstly with the *sinus venosus* (sixth week; Carnegie stages 15–17) and later with the ascending aorta (end of sixth week; Carnegie stage 18; [17]). Now, blood starts to flow through the plexus, and the hemodynamic stimuli trigger the remodeling of the plexus into a hierarchically arranged vascular system of coronary arteries, capillaries, and veins (Fig. 2.3d). The remodeling process starts at the base of the ventricles from where it progresses towards the cardiac apex. As a consequence, the stems of the coronary blood vessels as well as their main branches are present at the end of the embryonic period (end of eighth week; Carnegie stage 23; [6]). Myocardial blood supply via coronary blood vessels gradually replaces blood supply via myocardial sinusoids during the late embryonic and early fetal periods. This is accompanied by expansion of the outer compact layer of the ventricular myocardium and reduction of the inner spongy layer of the ventricular myocardium.

Mature Pattern of Cardiac Lymphatics

The lymphatics of the heart were first described by Rudbeck [18] and were also noted early on by Mascagni [19] (cited from [20]). Cardiac lymphatics are formed by three interconnected parts: subendocardial, myocardial, and subepicardial plexuses. The subendocardial plexus is regularly present in the cardiac walls of the human heart, but rarely found in the atrioventricular and semilunar valves, and absent in the *Chordae tendineae* and the intima of the large arteries [21]. Lymph of the subendocardial plexus is drained by lymphatics that traverse the myocardium along branches of the coronary arteries into the subepicardial plexus [7, 22, 23]. The subepicardial plexus forms a rhomboid pattern of initial lymphatics, which, however, do not cross the interventricular sulci. Thereby a left and a right drainage territory are formed. The lymphatic capillaries discharge into paired collectors, largely following the branches of the coronary arteries. The left and the right coronary lymphatic trunks only form sparse interconnections, mainly at the base of the ascending aorta. The right coronary lymphatic trunk runs ventrally along the *aortic arch* and the *left subclavian artery* and drains into the left venous angle. The left coronary lymphatic trunk runs dorsally along the pulmonary arterial trunk, ascends to tracheobronchial lymph nodes, and finally drains into the right venous angle [7, 23].

Development of Cardiac Lymphatics

It may be a general rule of prenatal lymphangiogenesis that the development of the lymphatics starts considerably later than that of blood vessels. The earliest morphological signs of the lymphatic vascular system are the JLS, which develop at the confluence of the cranial and caudal cardinal veins. In the human, JLS were found in 6- to 7-week-old embryos of 10–14 mm total length [24], which is 3–4 weeks after the development of the first blood vessels. In the mouse, blood vascular development starts at embryonic day (ED) 7.5 [25] whereas the anlagen of the lymphatics are visible at ED 10 [26].

Corresponding to the general timing of prenatal lymphangiogenesis, it has been found that the development of the cardiac lymphatics follows, in time and space, the development of the coronary blood vessels [7, 8, 27–30]. An analysis of the developmental patterning of the cardiac parasympathetic nerves in chick embryos, as shown by Kuratani and Tanaka [31] and Verberne et al. [32], also discloses a striking spatial and temporal correlation with cardiac lymphangiogenesis, whose functional significance remains to be determined. The development of human cardiac lymphatics was studied in detail by Kampmeier [7]. At the end of the embryonic period (eighth week), he found two plexiform extensions from the mediastinal lymphatics at the base of the heart of a 30 mm embryo. The first plexus was located ventral from the left carotid artery and the left aortic arch and extended from the left

JLS to the area of the right coronary artery. The second plexus was located dorsal to the pulmonary arterial trunk and extended from the right JLS to the area of the left coronary artery. Obviously, the early anlagen represent the future efferent lymphatic conduits. In 40 mm fetuses, the lymphatics extended in the subepicardium further along the coronary arteries towards the ventricular apex. By the end of the third and beginning of the fourth prenatal month, most of the surface of the human heart was covered by lymphatics, much more densely over the ventricles than the atria. Only then, ingrowth of lymphatics into the myocardium was noticed. Corresponding observations were made in the developing heart of pigs [27], arguing for an outside-in development of lymphatics in the heart. A similar pattern of cardiac lymphangiogenesis can be observed in mice [33, 34]. Development of lymphatic valves starts in the fourth month of human prenatal development in the main channels of the cardiac lymphatics, and they are much more abundant in the region of the ventricles compared to the atria [7].

The descriptive data from Kampmeier [7] as well as recent experimental data from our labs [30] argue for a predominant origin of the cardiac lymphatics from the JLSs. We have addressed the question on the origin of the cardiac lymphatic system by studying avian embryos, which are well-suited model organisms for clarifying the embryonic origin of cell lineages and organ systems [35]. Like mammals, birds possess a completely septated four-chambered heart and the pattern of formation of the lymphatic system of the chick heart is similar to the pattern observed in the human embryonic and fetal heart [8, 28]. At incubation day (ID) 9, corresponding to late stages of human embryonic development, subepicardial lymphatics are present at the base of the heart from where they grow towards the apex (Fig. 2.4a). By ID 17, corresponding to early fetal stages of human development, they cover the whole surface of the ventricles [8]. At ID 15, lymphatics are found in the myocardium, adjacent to the branches of the coronary arteries (Fig. 2.4b).

Cardiac lymph is drained by lymphatic trunks that accompany the pulmonary arterial trunk and the aorta (Fig. 2.4c). The lymphatics are often in close association with the coronary arteries and it has been found that the development of the coronary blood vessels precedes that of the cardiac lymphatic vessels, both in time and place [7, 8]. It was, therefore, tempting to speculate that the two vascular systems of the heart may share a common origin. However, whereas the coronary blood vessels originate from the proepicardium (see above), lymphatics were not formed by quail cells, when quail PE was grafted homotopically into chick embryos, indicating a non-PE origin of cardiac lymphatics (Fig. 2.5). In these quail-chick chimeras, we only observed a single exception to this rule. At the base of the heart we found one lymphatic trunk that regularly contained quail endothelial cells (Fig. 2.5c). This implies that (1) there exists a lymphovenous anastomosis at the base of the heart, and (2) this anastomosis may develop by ingrowth of a sprout from subepicardial blood vessels—similar to the homing of the subepicardial vascular plexus into the aorta and the coronary venous sinus (see above). Lymphovenous anastomoses have been found in various organ systems. In the subepicardium of human and pig hearts they occur frequently [36], but their functional significance remains to be determined.

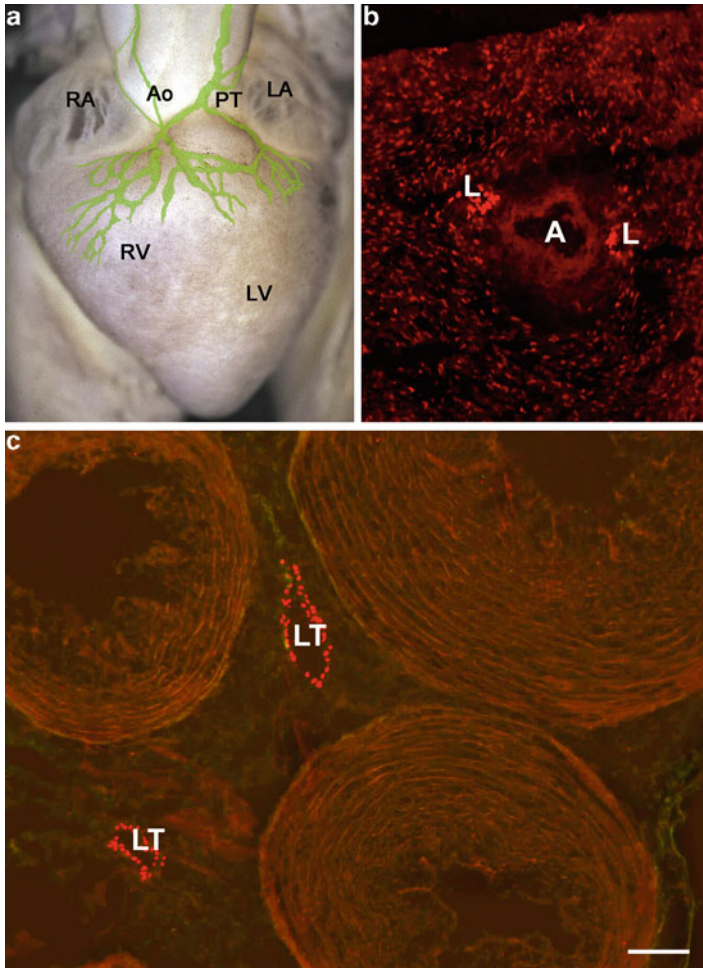


Fig. 2.4 (a) Chick heart at incubation day 9. Whole mount staining of subepicardial lymphatics with anti-Prox1 antibodies. For the sake of clarity, lymphatics were colorized in *green*. Lymphatics are found at the base of the heart and along the great arterial trunks. *Ao* aorta; *LA/RA* left and right atrium; *LV/RV* left and right ventricle; *PT* pulmonary trunk. (b) Chick heart at incubation day 15. In the myocardium Prox1-positive lymphatics (L) are found adjacent to an artery (A) (c) Chick heart at incubation day 15. Prox1-positive lymphatic trunks (LT) are found along the arterial out-flow tracts. Bar = 100 μ m

Direct connections of the developing cardiac lymphatics with the JLS were noted by Kampmeier [7], and centrifugal growth of the lymph sacs, as proposed by Sabin [37], may be the main mechanism for their development. Transdifferentiation of venous endothelium into lymphatic endothelium takes place in the jugular region, which is characterized by the confluence of the cranial and caudal cardinal veins into the common cardinal vein (Fig. 2.6). Transient expression of the transcription factor Sox18 and permanent expression of the transcription factor Prox1 are essential for the development of lymphatic endothelial cells [26, 38]. In all species studied

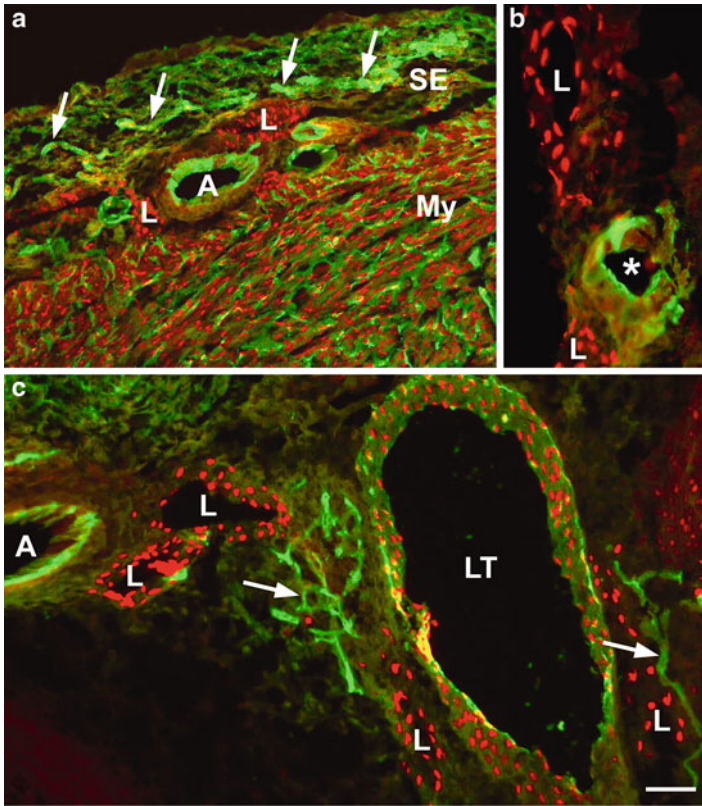


Fig. 2.5 Homotopical grafting of quail proepicardium at incubation day 2.5 into chick embryos and reincubation until incubation day 15. Staining of quail endothelial cells with QH1 antibody (green) and lymphatics (L) with anti-Prox1 antibody (red). (a) Blood capillaries in the myocardium (My) are of proepicardial (quail) origin. The same holds true for arteries (A), veins and capillaries (arrows) in the subepicardium (SE). Lymphatics (L) are not derived from the proepicardium. (b) Higher magnification showing QH1-positive blood vessel and QH1-negative lymphatics (L). (c) At the base of the heart, arteries (A) and subepicardial blood capillaries (arrows) are QH1-positive. Lymphatics (L) are QH1-negative, except for a lymphatic trunk (LT), which contains some quail endothelial cells. Bar = 70 μ m

so far, Prox1 is expressed in a subpopulation of jugular venous endothelial cells and in the JLS (Fig. 2.7). Macrophages are in close association with the developing lymphatics (Fig. 2.7a) and may promote their growth by the secretion of the lymphangiogenic growth factor VEGF-C [39]. Cells with an intermediate macrophage-lymphendothelial phenotype have also been noted [30, 40]. Their integration into embryonic lymphatics has not been proven, but there are human pathologies where the participation of circulating cells in lymphangiogenesis is highly likely, such as Kaposi's sarcoma [41] and inflammatory kidney rejection [42]. In summary, the origin of the cardiac lymphatic system has not been clarified in detail. Fate-mapping studies on avian embryos have excluded the possibility that the cardiac lymphatics derive from the PE. The JLS are the most likely source of origin and sprouting

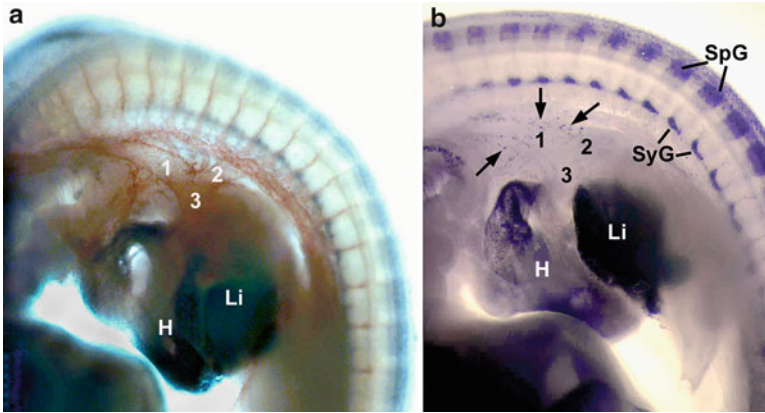


Fig. 2.6 Whole mount in situ hybridization of chick embryos at incubation day 4. (a) Double staining with a Tie2 probe (red-brown) and a Prox1 probe (blue). The jugular region is shown, characterized by the confluence of the cranial cardinal vein (1) with the caudal cardinal vein (2) into the common cardinal vein (3). *H* heart; *Li* liver. (b) Staining with a Prox1 probe reveals scattered positive cells in the jugular region (arrows), and signals in the spinal ganglia (SpG) as well as sympathetic ganglia (SyG). *H* heart; *Li* liver

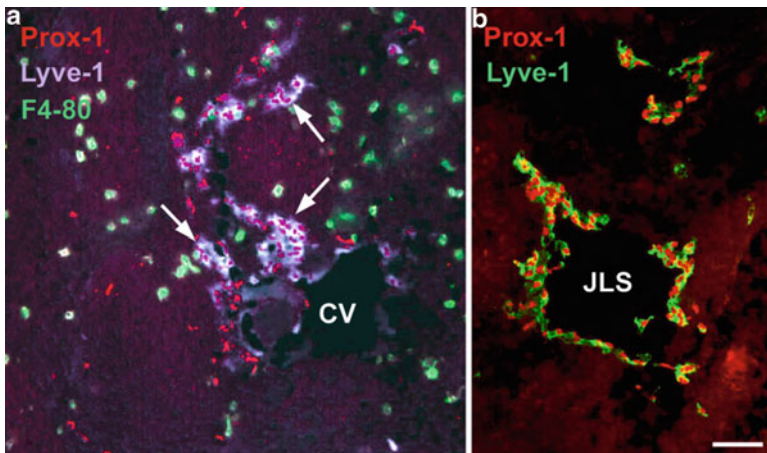


Fig. 2.7 Immunofluorescence studies of mouse embryos. (a) Triple staining with antibodies against Prox1 (red), Lyve-1 (blue) and F4-80 (macrophages, green). At embryonic day 10, Prox1 and Lyve-1 double positive lymphatic anlagen are seen, which obviously originate from the cardinal vein (CV). Some macrophages are in immediate contact with the lymphatics. (b) At embryonic day 11, Prox1 and Lyve-1 double positive jugular lymph sacs (JLS) have developed. Bar=60 μm (Specimens provided by Dr. K. Buttler, Göttingen, Germany)

angiogenesis may be the dominant mechanism in cardiac lymphangiogenesis. The possible contribution of scattered mesenchymal cells to the developing cardiac lymphatics, as suggested by descriptive data [28, 43–45], needs further investigation.

Conclusions and Outlook

As compared to the coronary blood vessels, the development and function of the cardiac lymphatics are much less understood. The origin of cardiac lymphatics has not been established unequivocally, and the mechanisms of their formation (lymphangiogenesis, lymphvasculogenesis) remain to be clarified. Recent progress in the development of transgenic animals carrying reporter genes to visualize the lymphatics will bring more light into the darkness.

The functional significance of the lymphatics in the heart was only recognized by a few specialists in the field. Myocardial edema decreases cardiac output and, when chronic, causes interstitial fibrosis [46]. In general, the impairment of the cardiac lymph flow, induced by myocardial injury or various surgical procedures, was never connected with recovery and patient outcome [47]. With water-sensitive MRI the visualization of myocardial edema is possible *in vivo* and can be used as a diagnostic tool. In combination with scar imaging it differentiates between reversible and irreversible damage and provides prognostic information [48].

Furthermore, the cross-talk between the lymphatics and the adjacent cardiac cells has never been studied. This is therefore an open field for regenerative medicine.

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