Development of the Pharyngeal Arch System Related to the Pulmonary and Bronchial Vessels in the Avian Embryo

With a Concept on Systemic–Pulmonary Collateral Artery Formation

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Background. The literature is ambiguous as to the question of the developmental background of systemic–pulmonary collateral arteries. These are found in combination with various congenital heart malformations such as pulmonary atresia. From a clinical point of view, it is of interest to know whether we are dealing with the persistence of transient embryological vessels such as ventral segmental arteries or parts of pharyngeal arch arteries or with the prenatal or postnatal recruitment of the bronchial vasculature that normally supplies the lung. This study of the embryology of the extrapulmonary and intrapulmonary vasculature aims at a better understanding of the variations in origin, course, branching pattern, and histology of collateral arteries.

Methods and Results. Serial sections of quail embryos ranging between stage HH11 and stage HH28 were incubated with a monoclonal antibody (aMB1) against endothelial cells and their precursors. Additional series of chick embryos were injected with India ink to study the lumened vascular patterns. A splanchnic plexus consisting of endothelial cells and precursors is present around the foregut before the lung buds develop. This plexus expands and gives rise to the pharyngeal arch arteries, the ventral pharyngeal vessels, the pulmonary vessels, and the bronchial vessels, including the intrapulmonary vessel network. During two subsequent periods, the splanchnic plexus is transiently connected to the systemic arteries and veins. The bronchial arteries and veins develop in the second period from these transient vessels. The expansion and extension of the splanchnic plexus to many organs during the formation of the bronchial vessels explains the varying course and branching pattern of the bronchial vasculature.

Conclusions. These results show that we are not dealing with two or more individual vascular systems that contribute to the developing vessels of the lungs but with one vascular plexus that normally gives rise to the pulmonary and bronchial vascular network but has the potential to give rise to other systemic–pulmonary connections. (Circulation 1993;87:1306–1319)

Key Words • angiogenesis • bronchial vessels • collateral arteries • pulmonary vessels • vasculogenesis

A normal lung is supplied by a pulmonary artery branching from the pulmonary trunk and possessing an elastic wall structure. In its intrapulmonary course, it branches with the airways. At the segmental level, the wall structure becomes that of a muscular artery, ending in a capillary bed surrounding the alveoli. Furthermore, there is a varying number of nutritive bronchial arteries that, with a marked variation in their origin and course, mainly originate from the descending thoracic aorta and the upper posterior intercostal arteries.1–3 These bronchial arteries supply the wall of the bronchi and the media of the larger intrapulmonary arteries.

Although the pulmonary and the bronchial arteries basically supply different tissues, anastomoses are common. Larger connections can be found at the level of the segmental pulmonary arteries, whereas many small anastomoses are seen with the pulmonary arterioles at the lobular level.4–7 The bronchial arteries in general share their venous drainage with the pulmonary arteries by way of the central pulmonary veins. There is a description of a drainage by a separate bronchial vein directly to the left atrium.2

A basis for confusion in terminology is found as the extrapulmonary part of the bronchial arteries provides branches for the trachea, esophagus, pleura, vagus nerve, and lymph nodes in the mediastinum. The venous drainage of these tributaries is mainly to the systemic venous system and thus, to the right atrium. Marchand and coworkers2 proposed to define the extra-
pulmonary tributaries of the bronchial arteries as “pleuro-hilar vessels.”

The common venous drainage of the bronchial and pulmonary systems allows the bronchial arteries not only to have a nutritive function but enables them to serve as a collateral artery in case of general obstruction of a pulmonary artery,8 emphysema,9 or sudden pulmonary edema caused by left heart failure.7 In case of congenital obstruction,9-11 other vessels present only during normal development can be considered as sources for collateral artery supply. These can be persisting ventral splanchnic aortopulmonary vessels12 or persisting ventral segmental arteries.10,11

Current data on the embryonic development of the bronchial and pulmonary vasculature cannot explain satisfactorily the possible origin of congenital systemic-pulmonary collateral arteries. In the older literature on vessel development, there are two main lines of thought that partly overlap. First of all, there is the description of a splanchnic plexus surrounding the foregut that either originates from the endocardial tube13 or even takes up contribution from surrounding systemic arteries and veins.14 Brown15 and Squier16 assume that the pulmonary vasculature develops from this splanchnic plexus. Several authors describe transient connections between the developing lung vasculature and the dorsal aorta,12,17-19 the gut vasculature,16,20 and the systemic veins.20,21 On the other hand, investigators focused on the development of the pulmonary arteries growing from the aortic sac22 or from the dorsal aorta18 into the developing lungs, in which a preexisting plexus may be present. The development of the central pulmonary veins is then described as a proliferation of the endocardium of the atrium into the splanchnic mesoderm of the foregut and the lung buds,14,19,23 which already may possess a venous splanchnic plexus.20 Literature regarding the bronchial arteries and veins is scant; however, Boyden24,25 described the growth of bronchial arteries from the descending aorta in the fetal period after establishment of the pulmonary system in one normal and one abnormal embryo, and the venous drainage of the bronchial arteries has been described as a remnant of the pulmonary plexus connected once with the systemic veins.21

Recent studies from our own group, using the monoclonal antibodies αMB126 to detect both endothelial cells and their precursors in quail embryos, explain in more detail the early development of the splanchnic plexus and its connections.27 From the 1-somite stage onward, the extraembryonic vasculature is continuous with the intraembryonic endothelial network, initially always located at the interface of endoderm and splanchnic mesoderm. This intraembryonic vascular network is remodeled during growth of the embryo, giving rise to the endocardium of the heart tube and the pharyngeal arch arteries but also to the systemic venous system: There is a splanchnic plexus of partly lumenedized, partly isolated endothelial cells (so-called precursors) that surround the foregut already contributing endothelial cells to the earliest lung buds even before a pulmonary artery can be distinguished.

The aim of the present study is to collect more specific data on the developing bronchial and pulmonary vessels from this early splanchnic plexus by using the monoclonal antibody αMB1. For the older stages, additional series of chick embryos were injected with India ink to visualize the luminedized parts of the developing vascular bed. These data are expected to shed light on the possible origin of congenital systemic-pulmonary collateral arteries as seen in a number of congenital cardiac malformations.

Methods

White leghorn chick (Gallus domesticus) and Japanese quail (Coturnix coturnix japonica) embryos of 48 hours to 6 days of incubation were used for this study. The chick embryos were staged according to the age determination criteria of Hamburger and Hamilton28 as stages HH11–HH28. This classification was also used to describe the quail embryos rather than the less detailed criteria of Zaczek.29

Immunohistochemistry

Quail embryos, stage HH11 (14 somites) to stage HH28, were fixed for 24 hours in periodate-lysine-paraformaldehyde fixative30 at 4°C. After embedding in paraplast, the embryos were serially sectioned transversely at 3 μm. The deparaffinated and rehydrated sections were incubated with the αMB1 monoclonal antibody, diluted in phosphate-buffered saline (PBS) with 0.05% Tween-20 and 0.1% bovine serum albumin. The overnight incubation at room temperature was followed by washing in PBS with 0.05% Tween-20. Subsequently, the slides were incubated for 2 hours with rabbit anti-mouse IgG conjugated to horseradish peroxidase in the same buffer as the primary antibody. After washing in PBS, the staining reaction was performed with 0.04% diamino benzidine tetrahydrochloride in 0.05 M tris-maleic acid (pH 7.6) with 0.07% imidazole and 0.06% hydrogen peroxide for 10 minutes, followed by washing in the buffer. Negative controls, omitting αMB1 or the second antibody, were also included in the staining protocols. Last, the sections were briefly counterstained (10 seconds) with Mayer’s hematoxylin.

The αMB1 antibody detects quail endothelial cells and hemopoietic cells of the white blood cell line.26 For the description of the developing vascular cell network, it was necessary to distinguish between endothelial cells that line a lumen and precursors of endothelial cells that are also αMB1 positive but do not line a lumen. These precursors can be found in isolation or may be connected to each other, forming strands of cells.

India Ink Injections

Chick embryos of stage HH11 to stage HH28 were carefully removed from the yolk. It was important that the vitelline vessels were not damaged. The embryos were placed in a small dish with an agar bottom and covered by Locke solution (0.94% NaCl, 0.045% KCl, and 0.004% CaCl2) at 37°C. India ink, diluted in a 0.9% NaCl solution and filtered through a 0.45-μm millipore filter, was injected into a vitelline vein with help of a glass needle with a tip diameter of 10–16 μm. Young embryos of stage HH11 were injected directly into the heart. The glass needle was connected via a pressure-insensitive, oil-filled tube to a Hamilton syringe, allowing for careful and controlled administration of minute amounts of ink. To avoid leakage or expansion of the vascular system, only slight manual pressure was exerted on the microinjection system. The heart pulsations
appeared to exert enough primary suction to fill the vascular system. In this way, an adequate visualization of the vascular system in toto was obtained. After injection of HH11-12-staged embryos, these were immediately fixed in 4% phosphate-buffered paraformaldehyde. To obtain an optimal adhesion of India ink to the endothelial cells, older embryos with a wider vascular lumen were fixed 15 minutes after completion of injection. Fixation lasted at least 24 hours. Before the embryos were embedded, the yolk sac was either dissected or the embryos were sectioned midsagittally with iridectomy scissors. The specimens were dehydrated in a graded series of ethanol. They were placed in two changes of styrene (C6H5CH=CH2) for 30 minutes at 21°C. The styrene was replaced by a graded series of styrene-polyester resin (crystal clear polyester resin; Bayer, Germany) mixture: 2:1, 1:1, and 1:2. Each step lasted for at least 30 minutes at 37°C. Finally, the specimens were left overnight in pure polyester resin and the next day were embedded in fresh polyester resin, to which 2% initiator was added. Polymerization at room temperature lasted at least 3 days. The injected parts of the vascular system could not be photographed entirely because of their three-dimensional aspects; instead, they were drawn by the medical illustrator.

Results

For clarity, the description of the results has been subdivided into the splanchnic plexus, the pharyngeal, the pulmonary, and the bronchial systems. Both the arterial component and the ultimate venous drainage are described. The constant remodeling in time of the vascular bed does not facilitate the understanding of this process in its three-dimensional extension. We hope to elucidate this by schematic pictures, transverse immunohistochemically stained sections, and drawings of the India ink-injected embryos.

The Splanchnic Plexus

At stage HH11, the splanchnic plexus, situated in the splanchnic mesoderm, flanks the ventral and lateral sides of the foregut. This plexus merges fluently with the area of the dorsal aortas and the head mesenchyme. The endothelial cells and their precursors of this plexus are intricately involved in the formation of both the arterial and venous vascular systems in the head, thoracic, and upper abdominal region. For the present study, this is the area that will be evaluated.

It is remarkable that at stage HH11–13, the endothelial cells and precursors of the splanchnic plexus are not evenly distributed. Ventrally, more endothelial cells are present, whereas in the transition zone of the lateral splanchnic mesoderm and the head mesenchyme, only precursors are visible (Figure 1A). At stages HH12 and HH13, the splanchnic plexus ventrally to the foregut mainly consists of endothelial cells, whereas the number of endothelial precursors in the transition zone has increased. Some of these endothelial precursors form strands around the foregut.

During these stages, there is the formation of the endocardium-lined heart tube, recruiting its endocardial cells from the endothelial cells of the splanchnic plexus. With the formation of the dorsal mesocardium, a strand of endothelial cells formerly in contact with the endocardium remains situated ventral to the foregut.

The Pharyngeal Arch System

Stage HH11 to stage HH13. During these stages, the first pair of pharyngeal arch arteries has a large circular lumen connecting the endocardial heart tube to the dorsal aorta (see Figure 2A). These arteries are con-

Figure 1. Panel A: Transverse section of HH11 embryo (11 somites). The splanchnic plexus consists ventrally of endothelial cells (arrowhead), but in the transition zone (large arrow) of the lateral mesoderm and the head mesenchyme (H) it consists mainly of precursors (arrows). Bar, 50 μm. Panel B: Transverse section of HH12-staged embryo (15 somites) shows the midpharyngeal endodermal strand (mpes), a remnant of the endocardium (E). Bar, 50 μm. CI, cardiac jelly; cc, coelomic cavity; Dao, dorsal aorta; En, endoderm; F, foregut; M, myocardium; nt, neural tube; Sp, splanchnic mesoderm. Dorsal mesocardium is between the dots.
FIGURE 2. Schematic representations of four stages of development (panel A, HH13; panel B, HH18; panel C, HH21; panel D, HH28) of the pharyngeal arch arteries (1,2,3,4,6), ventral pharyngeal vein (VPV), pulmonary artery (pa), pulmonary vein (pv), and bronchial artery (ba) from the splanchnic plexus around the foregut (F) and lungs (L). The omphalomesenteric vein arising from the sinus venosus is not drawn but has comparable connections with the splanchnic plexus as the vitelline vessels on the yolk sac (YS). The splanchnic plexus around the developing lung buds is indicated as pulmonary plexus (pp). ACV, anterior cardinal vein; Dao, dorsal aorta; m pes, midpharyngeal endothelial strand; PCV, posterior cardinal vein.
nected in a caudal direction with the splanchnic endodermal plexus flanking the foregut. At the level of the otic placode (HH11), this splanchnic plexus contains (left and right of the foregut) a strand of precursors that connect the ventrally situated endothelial cells in the region of the aortic sac with the dorsal aortas. At stage HH12, these strands are lumenzized at the site of the aortic sac and the dorsal aortas. The endothelial precursors in between lumenzize subsequently, by which the second pair of pharyngeal arch arteries is completely lumenzized at stage HH13.

During the formation of the second pair, the third pharyngeal arch arteries are outlined in the splanchnic plexus. Their development will resemble that of the second pair. During lumenzization of the ventrally situated endothelial precursors, the precursors in front of the dorsal aortas align and lumenzize, forming a complex of sprouts for the developing third to sixth pairs of pharyngeal arch arteries at stage HH13 (Figure 3B).

Where the second pair of pharyngeal arch arteries develops from the splanchnic plexus, the proximal parts of the first pair of pharyngeal arch arteries remain connected to this plexus by strands of endothelial precursors (Figure 3A). The number of these precursors increases, and many small vessels with an irregular lumen in the mandibular arches are formed at stage HH13. These vessels will give rise to the left and right ventral pharyngeal veins, the definitive venous drainage of the facial region in the embryo.

Stage HH14 to stage HH18. During stages HH14 and HH15, endothelial precursors continue to arise in the transition zone of the lateral mesoderm and head mesenchyme and interconnect the lumenzized ventral and dorsal anlagen of the third pair of pharyngeal arch arteries (see Figure 2B). These precursor strands start to lumenzize at stage HH16, forming the third pair of pharyngeal arch arteries with a wide lumen at stage HH18 (Figures 4A and 4B).

The fourth and sixth pairs of pharyngeal arch arteries develop in a similar way as the third ones from stage HH15 onward. At stage HH17, two pairs of precursor strands connect the ventral sprouts with the dorsal aortas (Figures 4A and 4B), reflecting the fourth and sixth pharyngeal arch arteries (Figure 5B). At stage HH18, these parts of the fourth and sixth pairs start to lumenzize.

As in the mandibular arches, the ventral pharyngeal veins also develop within the other pharyngeal arches. Many endothelial precursors from the ventrolateral sides of the splanchnic plexus initially stay isolated and do not join in the formation of the pharyngeal arch arteries. At stage HH17, these precursors lumenzize and form a complex of small vessels at both sides of the embryo, forming a connection between the pharyngeal arch arteries, the ventral pharyngeal veins, and the common cardinal veins (Figures 4A and 5B).

The cardinal veins develop from a plexus of endothelial cells more laterally situated, which is continuous with the more ventrally positioned splanchnic plexus. The dorsolaterally situated common cardinal veins, which consist of irregular and anastomosing endothelial tubes (Figures 4A and 4B), run from the anterior cardinal veins in the head mesenchyme along the transition zone of splanchnopleure and somatopleure to enter the sinus venosus.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Facing page. Panel A: Transverse section of an HH12 embryo (15 somites) through the mandibular arch. The first pharyngeal arch artery (I) is connected to the developing ventral pharyngeal vein (VPV). Bar, 50 µm. Panel B: Transverse section through an HH13 embryo (20 somites). At the ventral side of the splanchnic plexus, the lumenzized anlagen of the fourth and sixth pharyngeal arch arteries (IV, VI) are present. The dots indicate the connections of the splanchnic plexus with the common cardinal vein (CCV) and the ventral pharyngeal veins in adjacent sections. Bar, 50 µm. Panel C: Transverse section through the caudal aspect of the lung region of an HH13 embryo (20 somites). At this level, the splanchnic plexus (arrows) is situated between the dorsal aorta (Daa) and the sinus venosus (sv). Bar, 50 µm. Panel D: Transverse section through the cranial aspect of the lung region. Near the venous pole, the midpharyngeal endothelial strand (mpes) is lumenzized, forming the cranial tributary of the central pulmonary veins. Near the transition zone of the lateral mesoderm and head mesenchyme (large arrow), the pulmonary artery (pa) is formed. Bar, 50 µm. AS, aortic sac; cc, coelomic cavity; F, foregut; Lp, liver primordium; PCV, posterior cardinal vein.

Stage HH19 to stage HH27. The lumen of the first and second pairs of pharyngeal arch arteries becomes narrower, which is accompanied by an increase of lumenzized connections with the ventral pharyngeal veins and the anterior cardinal veins (Figures 2C, 4C, and 4D). At stage HH20, the first pair is remodeled into a capillary plexus (Figure 4D); this occurs in the second one at stage HH23 (Figure 6A).

The third pair of pharyngeal arch arteries maintains its wide lumen during these stages. The connections with the developing ventral pharyngeal veins have disappeared at stage HH21 (Figure 6B). However, later on, the endothelial plexus around the third pair extends considerably and merges with the lumenzized MPES (Figures 6B and 6C). As a result, the vascular plexus cranial to the laryngotracheal groove, at the level of the second arches, is connected to the left and right ventral pharyngeal veins as well as to the atrial segment. As will be described below, this last connection can be considered as the cranial tributary of the central pulmonary veins.

The narrow lumen of the fourth and sixth pairs of pharyngeal arch arteries widens to the size of the third from stage HH23 onward (Figures 4D and 6C). The number of connections consisting of endothelial cells and strands of precursors between the arteries, the ventral pharyngeal veins, and the common cardinal veins decreases gradually (Figures 4C and 4D). In some cases, vascular connections located between the fourth and sixth pairs persist shortly, giving the impression of an intermediate fifth pharyngeal arch artery (Figure 4D). The last endothelial connections to disappear are seen at stage HH27 in the medial part of the sixth pair and the cranial part of the pulmonary arteries.

**The Pulmonary Vasculature**

**Stage HH11 to stage HH13.** Between the pharyngeal arches and the midgut, characteristic cylindric splanchnic mesothelium indicates the area of the developing
lungs (Figure 2A). The exact cranial and caudal demarcation is, however, not distinct during these stages. To describe the development of the vascular system in the lungs, this area is divided arbitrarily into three parts with respect to the developing endodermal evaginations of the lung buds, that is, into a dorsal, a ventral, and a caudal aspect.

In the caudal aspect, many endothelial precursors are present, situated between the dorsal aorta, the sinus venosus (Figure 3C), the omphalomesenteric veins, and
the vitelline vessels. At stage HH13, precursor strands run from the lung area to the sinus venosus and vitelline vessels but not to the dorsal aorta.

The dorsal aspect of the lung at stages HH11 and HH12 does not contain endothelial precursors. At stage HH13, two longitudinal, irregular strands are seen (Figure 3D) close to the transition zone of the lateral mesoderm and the head mesenchyme. These precursors cover an area 40 μm wide and will give rise to the pulmonary arteries.

The ventral aspect of the developing lungs is lined by the endocardium of the atrial segment. At stage HH13, the MPES, still connected to the endocardium of the atrium by way of the dorsal mesocardium, lumenizes near the venous pole (Figure 3D), forming the cranial tributary of the central pulmonary veins. Furthermore, at this site there is an increase of endothelial precursors around the MPES.

Stage HH14 to stage HH18. Until stage HH18, the strands of precursors in the dorsal aspect of the lungs (pulmonary arteries) are interrupted at many places (see Figure 2B). Except for their junction with the sixth pair of pharyngeal arch arteries, parts of these strands (Figure 5C) start to lumenize at stage HH17.

The splanchic plexus in the caudal aspect of the lung goes through a number of alterations. At stage HH15, when the endodermal hepatic diverticulum starts to develop, the endothelial precursors contact the dorsal aorta, so the plexus extends from the dorsal aorta via the liver primordium and midgut to the vitelline vessels. The strands lumenize in the areas of the liver and midgut at stage HH16 and consist of many endothelium-lined vessels at stages HH17 and HH18 (Figures 4B and 5D). At the ventral aspect of the developing lungs, no significant alterations are seen in these stages.

Stage HH19 to stage HH27. In the dorsal aspect, the pulmonary arteries contain for the greater part a lumen. The proximal parts consist of endothelial precursors up to stage HH21 (see Figure 2C). From this stage onward (about 35 somites), the proximal parts of the pulmonary arteries start to lumenize and to lose their connections with the veins. They still have irregular connections with the sixth pharyngeal arch arteries at stage HH25 (Figure 7A). The more posterior parts of both pulmonary arteries contain a small but circular endothelium-lined lumen, whereas locally mesenchymal condensations already indicate the developing media.

The endothelial precursors ventral to the lung buds lumenize and form the caudal tributaries of the central pulmonary veins at stage HH19. At this stage, the lumen of the pulmonary veins is wider than those of the pulmonary arteries (Figure 5D).

The lumenized connections of the caudal part of the pulmonary vasculature with both the dorsal aorta and the omphalomesenteric veins disappear gradually. The vascular connections with the dorsal aortas disappear at stage HH20 (Figure 4D), whereas the last endothelial connections between the pulmonary and midgut vasculature (Figure 6E) are seen at stage HH27. As a result, each lung bud is dorsally accompanied by a pulmonary artery and ventrally by a vein and by a vascular plexus around its tip.

The Bronchial Vasculature (Stage HH21 to Stage HH28)

As described in the previous section, the connections between the pharyngeal arterial system and both the ventral pharyngeal veins and the cardinal veins disappear from stage HH21 onward (Figure 2D). At the same time, there is a marked increase in the number of endothelial cells and mainly precursors in the mesenchyme, forming an extensive plexus in the area between the sixth pair of pharyngeal arch arteries, the pulmonary arteries, the dorsal aortas, the ventral pharyngeal veins, and the common cardinal veins (Figures 6C and 7A). This plexus again forms lumenized connections with the ventral pharyngeal and cardinal veins until stage HH25. With the growth of this plexus, it becomes very difficult in sectioned material to discriminate between the various vascular systems.

From stage HH26 onward, the dorsal aorta, surrounded by many precursors (Figure 7B), and the dorsal intersegmental arteries become connected again to the splanchic plexus (Figure 7C). As a result, the definitive vascular system of the bronchi, trachea, and esophagus is formed (the pleuro-hilar vessels), which is supplied by the dorsal aorta and the dorsal intersegmental arteries and drained by both the cardinal veins and the central pulmonary veins. Because the area of the lung hilus undergoes many spatial changes in older stages, the bronchial arteries cannot be differentiated into intrapulmonary and extrapulmonary parts at stage HH28. In the proximal parts of the bronchi, the bronchial arteries of the vascular plexus lose most of their contacts with the pulmonary arteries (Figure 7D), whereas more distally they remain inseparable from the pulmonary arteries and veins.

Discussion

Origin of the Splanchic Plexus

A splanchic plexus has been described in several animal species such as chick, cat, and rat, making it
acceptable that it is not a species-specific entity. According to Buell,\textsuperscript{14} the splanchnic plexus arises (HH15) around the foregut by proliferation of angioblastic cells from the dorsal aortas, ventral aortas, cardinal veins, and mainly from the dorsal wall of the atrial segment. Chang\textsuperscript{32} described that angioblastic cells migrate from the endocardial tube as early as at stage HH9 to contribute to the formation of the splanchnic plexus ventrally from the foregut. In these studies, the angioblastic cells were defined morphologically.

In contrast to earlier investigations, we were able to study both the lumenized and nonlumenized parts of the splanchnic vasculature using the combination of the aMBo monoclonal antibody\textsuperscript{36} against endothelial cells and precursors as well as India ink injections. In this way,\textsuperscript{27} we investigated the formation of the vascular system in quail embryos between stages HH5 and HH13. It showed that the initial vascular network, positioned at the interface of splanchnic mesoderm and endoderm, of a 4-somite quail embryo (HH8) is remodeled during the growth of the embryo to give rise to the endocardium of the heart tube, pharyngeal arch arteries, dorsal aortas, and vitelline veins. Therefore, it was concluded that not all endothelial cells in the splanchnic mesoderm around the foregut arise by vasculogenesis but that they also can originate from the initial vascular plexus, a phenomenon also observed in the mouse embryo.\textsuperscript{31} This can explain the observation\textsuperscript{32,33} that even the youngest lung buds used in quail–chick transplantation experiments contain endothelial precursors that have differentiated in situ. These endothelial precursors assemble to form endothelium-lined blood vessels.\textsuperscript{34–36}

In the youngest embryos studied, three parts of the splanchnic plexus actually belong to the initial vascular network of the 4-somite embryo\textsuperscript{31}: 1) the cranial part that gives rise to the pharyngeal arch arteries, 2) the MPES, which is derived from the proendocardium during the formation of the endocardial heart tube and the dorsal mesocardium, and 3) the caudal part located in the lung area between the dorsal aortas, sinus venosus, and vitelline vessels. Mitosis of precursors and remodeling of vessels (e.g., the first pharyngeal arch) may contribute to the expansion of the plexus. The splanchnic plexus merges fluently with the endothelial cells and precursors in the lateral mesoderm and the head mesenchyme. The latter two are developmentally lagging behind because in these areas more precursors are seen, whereas the ventral part of the splanchnic plexus contains more endothelial cells lining a lumen.

**Development of the Pulmonary Artery and Vein**

Many investigators studied the development of the pulmonary arteries and the pulmonary veins but were only able to describe the development of the lumened part of the vascular system because of the techniques available. Congdon,\textsuperscript{22} for instance, described that the pulmonary artery develops as a splanchnic sprout from the aortic sac, whereas Huntingdon\textsuperscript{18} described a number of sprouts from the dorsal aortas. According to other investigators,\textsuperscript{14,19,23,37} the pulmonary vein develops by a proliferation of the endocardium of the sinus venosus into an originally avascular splanchnic mesoderm. Brown\textsuperscript{38} and Squier\textsuperscript{39} came close to our observations in stating that the pulmonary artery and vein develop from a network of capillaries around the foregut. The connection of this network (or so-called splanchnic plexus) to the aortic sac and the sinus venosus has been described as giving rise to the pulmonary artery\textsuperscript{17,38} and pulmonary vein,\textsuperscript{38} respectively.

Our results, using the immunohistochemical approach, show that the pulmonary artery and vein develop from this splanchnic plexus by connecting to preexisting vessels. Outgrowth of endothelial sprouts from existing vessels (the dorsal aortas, the cardinal veins, and the atrial segment) into areas lacking endothelial precursors was not observed. Precursors were always present surrounding the large lumenized arteries and veins before new vessels arose.

The varying descriptions on the assumed outgrowth of the pulmonary arteries from the aortic sac,\textsuperscript{19,22} the dorsal aortas,\textsuperscript{18} or from the sixth pair of pharyngeal arch arteries\textsuperscript{39,40} can be understood by (species-specific) variations in lumination of the splanchnic plexus. The same holds for the view that the central pulmonary veins sprout from the atrial wall, whereas our studies show that this endothelium of the pulmonary veins has been connected to the atrial endocardium right from the beginning.

**Pulmonary Versus Bronchial Vasculature**

There is limited literature available concentrating specifically on the development of the bronchial system. The description of Boyden\textsuperscript{24,25} refers to one normal and one abnormal human embryo in which the pulmonary arteries are established in the seventh week of gestation. The bronchial arteries develop as lumenized sprouts from the descending aorta to the bronchi in the ninth to 12th week of gestation. According to Boyden, the time lag between the formation of both indicates the development of two separate systems. As our serial sections show, it is virtually impossible to study the development of the very complex bronchial system without a specific endothelial staining.

Our data show that the bronchial vessels arise from the same splanchnic endothelial plexus as the pulmonary vessels. From stage HH21 onward, the vascular plexus between the pulmonary arteries and the systemic veins is remodeled to give rise to the bronchial vessels at stage HH26. The number of connections of both the pulmonary arteries and systemic veins with bronchial vessels related to the esophagus, trachea, and proximal parts of the bronchi decreases. This part of the bronchial system, defined as pleuro-hilar vessels,\textsuperscript{2} will be drained by the azygos, hemiazygos, or one of the intercostal veins.\textsuperscript{2,5} Our work is supported by the work of Shaner,\textsuperscript{31} who stated that the (extrapulmonary) bronchial veins are remnants of connections of the pulmonary plexus with the systemic veins.

Around the distal parts of the bronchi, the pulmonary and bronchial vessels remain inseparable during the described stages of development. Many strands of precursors connect both systems. This explains the feasibility of an extensive anastomosing system between the intrapulmonary bronchial and pulmonary system in the normal lung.\textsuperscript{4–7} It can also be seen as an explanation for the common venous drainage of the pulmonary and bronchial arteries through the pulmonary veins described by Marchand and coworkers\textsuperscript{2} and Schraunfgel.\textsuperscript{8}
FIGURE 5. Panel A: Transverse section through the cranial part of the lung area of an HH17 embryo (28 somites). The developing sixth pharyngeal arch artery (VI) is connected in adjacent sections (dots) to the dorsal aorta (Dao), pulmonary artery (pa), and the common cardinal vein (CCV). Around the foregut, precursors (arrows) and the midpharyngeal endothelial strand (mpes) are visible. Panel B: Transverse section of HH17 embryos (28 somites) shows the fourth (IV) and sixth (VI) pharyngeal arch arteries in the splanchnic plexus. The plexus is connected to the ventral pharyngeal veins (VPV). Panel C: Transverse section just cranial through the area of the lung buds of an HH18 embryo (29 somites). At this stage, the proximal part of the pulmonary artery still consists of precursors. Some precursors are present in the ventral aspect of the lungs (arrows). An artifact in the endocardium of the atrium (A) is indicated with an asterisk. Panel D: Transverse section through the lung buds of an HH17 embryo (28 somites). In this area, the splanchnic plexus consists of many endothelium-lined vessels. They are connected to the dorsal aorta in adjacent sections (dots). Bars, 50 μm. AS, aortic sac; A, atrial segment; cc, coelomic cavity; F, foregut; O, outflow tract of the heart.
Figure 6. Panel A: Transverse section through the second pharyngeal arch of an HH25-staged embryo. The second pharyngeal arch artery is remodeled into a capillary plexus. Venous drainage occurs by the anterior cardinal vein (ACV) and the ventral pharyngeal vein (VPV). Note the hemopoietic stem cells, which can be distinguished from endothelial precursors because they are always round and larger (arrows). Bar, 200 μm. Panel B: Transverse section through an HH21 embryo (35 somites). The connections of the third pair of pharyngeal arch arteries (III) with the ventral pharyngeal veins have disappeared. The midpharyngeal endothelial strand (mpes) is lumenized over its complete length. Bar, 100 μm. Panel C: Frontal section through an HH25 embryo. The ventral pharyngeal veins and the midpharyngeal endothelial strand extend between the large lumenized pharyngeal arch arteries (III, IV, VI). Bar, 200 μm. Panel D: Transverse section through an HH19 embryo (33 somites). The lumina of the central pulmonary veins (pv) are wider than those of the pulmonary arteries (pa). Bar, 100 μm. Panel E: Frontal section through the lung bud of an HH25 embryo. The lung bud is accompanied by a pulmonary artery and pulmonary vein. The dots indicate a connection between the pulmonary and the midgut (mg) vasculature in adjacent sections. Bar, 100 μm. A, atrial segment; B, bronchus; CCV, common cardinal vein; Dao, dorsal aorta; E, endocardium; F, foregut; O, outflow tract of the heart.

Systemic–Pulmonary Connections

During development, the splanchnic plexus is connected to systemic arteries and veins. These connections are transient except for those that give rise to the bronchial arteries and pleuro-hilar veins. In various species, these connections have been described during development with the dorsal aortas,12,17–19,24,25,41 the gut plexus,16,21 and the cardinal veins.20,21 Abnormal congenital venous drainage is usually explained as persisting connections of the pulmonary veins with the gut plexus or cardinal veins.20,21,38 Abnormal arterial supply to the lungs, however, could be less easily explained on the basis of our knowledge of vessel formation in the embryo. It has been suggested that these abnormal vessels either reflect persisting aorto-pulmonary connections during the establishment of the
FiguRe 7. Panel A: Transverse section through an HH25 embryo. The proximal part of the lumenized pulmonary arteries (pa) contains an irregular lumen. Many strands of the vascular plexus, such as the ventral pharyngeal vein (VPV), are connected to the pulmonary artery and to the sixth pharyngeal arch artery (VI). Bar, 200 µm. Panel B: Transverse section through an HH21 embryo (35 somites). Many endothelial cells and precursors (arrows) are present surrounding the dorsal aorta (Dao), which they do not contact. Bar, 50 µm. Panel C: Transverse section through an HH28 embryo. The dorsal aorta and the dorsal intersegmental arteries (Dia) are connected to the vascular plexus around the larynx, esophagus, trachea, and bronchi. Bar, 100 µm. Panel D: Transverse section through an HH28 embryo. The pulmonary artery is part of an extended vascular plexus, which gives rise to the bronchial and pleuro-hilar vessels. The plexus is supplied by the dorsal aorta and drained by the common cardinal vein (CCV), ventral pharyngeal vein, and the central pulmonary veins (pv). Bar, 100 µm. A, atrial segment; F, foregut; Oe, esophagus; Tr, trachea.

It can be deduced from the present data that, in case of obstruction of the pulmonary outflow tract, systemic–pulmonary collateral arteries may arise during two embryonic periods. First, the caudal part of the pulmonary vasculature is connected to the dorsal aorta from stage HH15 to stage HH20. These connections are comparable with the transient aortopulmonary connections, as have been described in rat, cat, and chick embryos. During maldevelopment of the pulmonary part of the outflow tract, these transient connections may persist as systemic–pulmonary collateral arteries.

Second, the vascular plexus that gives rise to the bronchial arteries and pleuro-hilar vessels is connected to the dorsal aorta from stage HH26 onward. As described in the previous section, the pulmonary arteries are connected through this plexus to the dorsal aorta.
and intersegmental arteries. It is feasible that during maldevelopment, this plexus may be used for the formation of systemic–pulmonary collateral arteries as well. The place of origin, course, and branching pattern of the extrapulmonary part of the collateral arteries may vary because of the exact timing and degree of maldevelopment that occurs. Also, the intrapulmonary connections can be expected to be influenced in their extent and intensity by the developing malformation.

Last of all, it is possible that at a much later stage during development and even postnatally on the basis of a developing pulmonary obstruction, the already-existent bronchial arteries are recruited as an alternative supply route and thus develop into systemic–pulmonary connections.

Clinical Implications

There are several clinically relevant questions that need differentiation between the various types of aortopulmonary connections. Proposed classifications differentiate between major aortopulmonary collateral arteries and bronchial arteries. These are in part based on already-mentioned developmental concepts that appeared to be supported by histological evidence. This search for differentiation continues, as is evident from recent publications on the arterial supply to the lungs in tetralogy of Fallot.

Our studies on normal development indicate (with regard to the background of collateral artery supply) that there are potentially two periods. This finding, however, will not lead to an easy differentiation in the postnatal period between the various collateral arteries. This is supported by a study that is in progress by us in which the normal/abnormal histology of the vessel wall is used as a possible differentiating factor. This study is based on earlier published material of Liao et al confirming the great variability that can be seen in the collateral vessels. These can show a muscular, musculoelastico-elastic vessel wall that might be compromised by intimal thickening, in some cases mimicking the wall structure of the ductus arteriosus. Our preliminary data show that this approach does not allow a differentiation between, for example, collaterals that are derived from a bronchial artery or from another arterial system during development. In fact, this is in line with our data on normal development of the vascular system in the lung area as explained in the present study. It might be relevant in this respect to mention that we can support those studies that indicate that it is highly unlikely to find a ductus arteriosus and a collateral artery supply to the same lung. A clear embryological explanation is still lacking.

In conclusion, we feel that there is a need for further research into the development of abnormal connections between the systemic and pulmonary system, both extrapulmonary and intrapulmonary, preferably in an experimental setting.

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