The origin of the endothelial cells: an evo-devo approach for the invertebrate/vertebrate transition of the circulatory system

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SUMMARY Circulatory systems of vertebrate and invertebrate metazoans are very different. Large vessels of invertebrates are constituted of spaces and lacunae located between the basement membranes of endodermal and mesodermal epithelia, and they lack an endothelial lining. Myoepithelial differentiation of the coelomic cells covering hemal spaces is a frequent event, and myoepithelial cells often form microvessels in some large invertebrates. There is no phylogenetic theory about the origin of the endothelial cells in vertebrates. We herein propose that endothelial cells originated from a type of specialized blood cells, called amoebocytes, that adhere to the vascular basement membrane. The transition between amoebocytes and endothelium involved the acquisition of an epithelial phenotype. We suggest that immunological cooperation was the earliest function of these protoendothelial cells. Furthermore, their ability to transiently recover the migratory, invasive phenotype of amoebocytes (i.e., the angiogenic phenotype) allowed for vascular growth from the original visceral areas to the well-developed somatic areas of vertebrates (especially the tail, head, and neural tube). We also hypothesize that pericytes and smooth muscle cells derived from myoepithelial cells detached from the coelomic lining. As the origin of blood cells in invertebrates is probably coelomic, our hypothesis relates the origin of all the elements of the circulatory system with the coelomic wall. We have collected from the literature a number of comparative and developmental data supporting our hypothesis, for example the localization of the vascular endothelial growth factor receptor-2 ortholog in hemocytes of Drosophila or the fact that circulating progenitors can differentiate into endothelial cells even in adult vertebrates.

INTRODUCTION Circulatory systems of vertebrate and invertebrate metazoans are very different. In fact, features of the circulatory system were the earliest criteria used to establish a classification of animals. Aristotle proposed to split the animal world into two groups, “with blood” and “without blood,” that is, vertebrates and invertebrates. Aristotle indeed meant “with red blood” because he thought that invertebrates had no blood at all.

A main difference between invertebrate and vertebrate cardiovascular system is the presence of a true endothelium, that is, an adluminal continuous layer of epithelial cells interconnected by specialized junctional complexes. Large vessels of invertebrates are constituted of spaces and lacunae located between the basement membranes of endodermal and coelomic epithelia, or between two coelomic epithelia. Cells adhered to the luminal surface of these basement membranes are occasionally present and, in some cases, abundant (reviewed in Casley-Smith 1980; Ruppert and Carle 1983).

Myoepithelial differentiation of the coelomic cells covering hemal spaces is frequent, and these myoepithelial cells can either remain in the coelomic lining or delaminate and constitute a differentiated cell layer comprised between the hemal cavity and the coelomic epithelium. Microvessels are frequently lined by the basement membrane of myoepithelial cells whose contractility can regulate the blood flow. Most invertebrate hearts are also constituted by a single layer of myoepithelial cells that are sometimes continuous with the coelomic epithelium. Instead, vertebrates show a closed, endothelium-lined system of vessels endowed with smooth muscle or pericytes, as well as a thick-walled heart.

The term “endothelium” has been either restricted to the continuous cell layer of the vertebrates, as we are assuming here, or applied to all the cells able to adhere to the luminal surface of the vascular basement membrane (Casley-Smith 1980). Anyhow, there is no phylogenetic theory about the origin of vertebrate endothelial cells or a comprehensive approach to the transition between the vertebrate and the invertebrate types of circulatory system.

From the ontogenetic point of view, there still remain many uncertainties about the origin and differentiation of the endothelial cells throughout embryogenesis. It has been established that endothelial cells arise from mesodermal progenitors, but two different hypotheses have been suggested for their lineage. The classical hypothesis states that the primitive endothelium and the blood cells share a common progenitor cell. Furthermore, their ability to transiently recover the migratory, invasive phenotype of amoebocytes (i.e., the angiogenic phenotype) allowed for vascular growth from the original visceral areas to the well-developed somatic areas of vertebrates (especially the tail, head, and neural tube). We also hypothesize that pericytes and smooth muscle cells derived from myoepithelial cells detached from the coelomic lining. As the origin of blood cells in invertebrates is probably coelomic, our hypothesis relates the origin of all the elements of the circulatory system with the coelomic wall. We have collected from the literature a number of comparative and developmental data supporting our hypothesis, for example the localization of the vascular endothelial growth factor receptor-2 ortholog in hemocytes of Drosophila or the fact that circulating progenitors can differentiate into endothelial cells even in adult vertebrates.
called the “hemangioblast” (Sabin 1920), which originates from the mesoderm, mainly in the areas closer to the endoderm and also in the aorta–gonad–mesonephros region. Direct evidence on the existence of hemangioblasts has been reported (Choi et al. 1998; Nishikawa et al. 1998).

Recently, this view has been partially challenged by the identification of a common vascular progenitor, isolated from stem cells, that is able to differentiate into endothelial and smooth muscle cells (Yamashita et al. 2000; Watabe et al. 2003). On the other hand, it has been proposed that only the primitive (embryonic) hematopoietic lineage is derived from hemangioblasts, whereas definitive hematopoietic stem cells would arise from a “hemogenic” endothelium located in the large vessels of the embryo (Nishikawa et al. 2000). The issue has been made even more complex by the discovery of circulating progenitors of endothelial cells that can be mobilized from the bone marrow by vascular endothelial growth factor (VEGF) and incorporated in the endothelial lining in areas of active vascular growth (Asahara et al. 1997, 1999). All these hypotheses are apparently difficult to reconcile.

The aim of this article is to use an evo-devo approach in order to elaborate an ontogenetic and phylogenetic hypothesis about the origin of the endothelium. We think that endothelial cells originated from a type of specialized blood cells, called amoebocytes, that normally adhere and circulate over the vascular basement membrane. The transition between amoebocytes and endothelium would be accounted by the acquisition of an epithelial phenotype. As these amoebocytes are considered true “endothelial cells” by Casley-Smith (1980), and quoted as a possible origin of the endothelium by Kuprijanov (1990), we acknowledge both authors as forerunners of our proposal.

This hypothesis is supported by recent results on the origin and differentiation of the endothelial cells in the embryo, and also from data from comparative anatomy and embryology. We think that this hypothesis, which stresses the coelomic origin of the elements of the vertebrate circulatory system, is able to synthesize most of the current knowledge about the ontogenetic development of the cardiovascular system, including the hemangioblast, the common vascular progenitor and the hemogenic endothelium hypotheses. We also believe that our model can account for many of the hitherto unexplained singularities of the endothelial cells, particularly their potential to acquire an angiogenic, highly invasive phenotype.

CIRCULATORY SYSTEMS IN INVERTEBRATES: ORGANIZATION AND DEVELOPMENT

Transport of oxygen, nutrients, and metabolic waste in invertebrates is performed by two systems of cavities: the coelom and the hemal system. Coelomic cavities are lined by a mesothelium (mesodermal epithelium), whereas hemal cavities are lined by the basement membranes of different types of epithelia, that is, a more or less thick layer of extracellular matrix. Hemal cavities can be found between the endodermal epithelium and the mesothelium, between two mesothelia (for example in the mesenteries), or surrounded by a single layer of mesothelial or myoepithelial cells (Fig. 1, A and B). As we have stated above, this latter cell type is considered a muscular differentiation of the mesothelial cells, and constitute the vascular wall of microvessels in some large invertebrates, such as the cephalopods. Visceral muscle is also derived from coelomic epithelial cells, at least in some cases, such as in sea urchin embryos (Burke and Alvarez 1988). Even the adult mesothelium can regenerate visceral and somatic muscle in echinoderms (Dolmatov and Ginanova 2001; García-Arrarás and Greenberg 2001). As myoepithelial cells are contractile, their presence renders some vessels capable of beating. It occurs, for example, in many vessels of the cephalochordate Branchiostoma (Ruppert 1997).

The other main component of the invertebrate circulatory systems are blood cells. The diversity of structure and function among invertebrate hemocytes is wide. However, invertebrate blood cells participate in functions similar to their counterparts in vertebrates, that is, blood coagulation, immunological defense, and oxygen transport (for a description of the echinoderm blood cells’ functions, see Cavey and Markel 1994). Among the immunological functions, phagocytosis, aggregation, encapsulation, and graft rejection have been reported for example in holothurians (reviewed in Smiley 1994). It is important to remark that there are free, circulating cells called coelomocytes in the coelomic spaces of invertebrates as well as into the connective tissue. In many cases coelomocytes and hemocytes are indistinguishable, and they receive their names according to the cavity where they are found, that is, coelomic and hemal cavities. In other cases, the term “hemocyte” is used to design the cells carrying pigments, irrespective of whether they are located in the coelomic or hemal cavities (Smiley 1994).

The embryonic origin of coelomocytes and hemocytes in invertebrates is not well known. However, most descriptive and experimental approaches point to the coelomic epithelium as the most probable progenitor tissue of these circulating cell types, as described in several groups of echinoderms (Holland et al. 1965; Smiley 1986; reviewed in Chia and Xing 1996). In urochordates, hemocyte progenitors (hemoblasts) form nodules composed of proliferating cells surrounded by differentiating hemocytes (Burighel and Cloney 1997). Interestingly, differentiation of hemoblasts into smooth muscle cells has been reported (Freeman 1964). Bossche and Jangoux (1976) have shown that delamination of mesothelial cells to form phagocytic cells occurs even in the adult starfish when these echinoderms are injected with carbon particles in the coelom.

The morphological and functional similarities between hemocytes, coelomocytes, and coelomic epithelium of inver-
tebrates are striking. For example, some hemichordate species bear free hemoglobin in the circulatory fluid. This molecule is synthesized and released by cells from the coelomic epithelium (Benito and Pardos 1997). A similar process occurs in polychaete annelids (Friedman and Weiss 1980). Hemoglobin is also found in hemocytes of other invertebrates (annelids, echinurids, mollusks, phoronids, nemerteans, and echinoderms, reviewed in Glomski and Tamburlin 1990) and also in coelomocytes of ophiurids (Christensen et al. 2003). Thus, synthesis of hemoglobin establishes another relationship between coelomic epithelium, coelomocytes, and hemocytes.

On the other hand, coelomocytes and hemocytes are sometimes ciliated, as are frequently the mesothelial cells. This is the case of the echinoids (Cavey and Märkel 1994) and cephalochordates (Ruppert 1997). In other cases, coelomocytes are not ciliated, but they show residual centrioles probably derived from a ciliated ancestral condition (hemichordates, Benito and Pardos 1997; asteroids, Chia and Koss

Fig. 1. Relationships between the circulatory system and the coelomic epithelium. (A) Hemal space (H) between the endodermal epithelium of the gut (EN) and a layer of myoepithelial cells (ME) in an oligochaete annelid. Note the presence of amoebocytes (arrows) adhered to the basement membranes of both epithelia. (B) Hemal space (H) in the hemichordate Balanoglossus, lined by the basement membranes of the EN and the coelomic epithelium (CE). CO, coelom. (C) Heart of the urochordate Clavelina in transverse section. The heart is constituted of a coelomic myoepithelium (ME) forming a tube around the hemal space (H). Another coelomic epithelium forms the pericardium (PE). PC, pericardial cavity. (D) Chick embryo, stage HH18, semithin section. Cells from the CE show signs of migration (arrow) in the proximity of the areas of the aorta (AO) where the clusters of hematopoietic stem cells (HSC) appear. (E) Chick embryo, stage HH17, immunostained with an anti-cytokeratin antibody. Cytokeratin is present in the cells of the CE and, transiently, in the mesenchymal cells that delaminate from it. Note the signs of migration in coelomic cells (arrow), the abundance of cytokeratin-positive cells in the mesentery and the presence of immunoreactive cells in the aortic wall (arrowhead). (F) Quail embryo, stage HH21, immunostained with an anti-WT1 antibody. Wilms’ tumor-associated transcription factor (WT1) is expressed in coelomic cells and upregulated in the proximity of the aorta (arrows). Note the presence of WT1-positive cells in the lateral and ventral areas of the aorta (arrowheads). CV, cardinal vein. (G) Quail embryo, stage HH17, double immunostaining of the AO with an anti-cytokeratin antibody (green) and with the anti-endothelial/hematopoietic antigen QH1 (red). Cytokeratin-positive cells are apparently migrating from the CE to the aortic wall, where expression of QH1 reveals the differentiation of endothelial and hematopoietic progenitor cells. (H) Chick embryo, stage HH 19, semithin section of the liver. The CE of the liver also shows signs of migration (arrow) toward the areas where mesenchymal cells are forming the hepatic sinusoids (S). HE, hepatocytes. (I) Quail embryo, stage HH20, double immunostaining of the developing liver with an anti-cytokeratin antibody (green) and the anti-endothelial/ hematopoietic antigen QH1 (red). Coelomic cells are delaminating from the CE and incorporating into the sinusoids (S) (arrowhead). Note the double-stained cell (arrow).
Mesothelial cells and coelomocytes sometimes share phagocytic properties, as in ophiuroids (Byrne 1994) and cephalochordates (Ruppert 1997).

A type of hemocyte, sometimes called “amoebocyte,” has been shown to be able to adhere to the basement membrane lining the hemal cavities (Fig. 1A). This has been reported in many invertebrate groups including annelids, mollusks, echinoderms, and cephalochordates (Fernández et al. 1992; Smiley 1994; Ruppert 1997). In some cases, the adherent amoebocytes are so abundant that they acquire the appearance of an endothelium. This is the case of some vessels in holothurians and cephalopods (Smiley 1994; Budelmann et al. 1997). For this reason, some authors have claimed that these animal groups actually bear an endothelium-lined vascular system (Ruppert and Carle 1983). However, we have herein assumed that a true endothelium must be characterized by the presence of intercellular junctions, and the adherent amoebocytes are free cells that migrate throughout the inner surface of the vessels.

Nemerteans constitute the only invertebrate group in which a closed, endothelium-lined vascular system had been described. Surprisingly, this group was considered as acelo-mate, and thus the coincidence of an advanced type of hemal system with the lack of coelomic cavities raised strong difficulties from the phylogenetical point of view. However, detailed embryological studies have demonstrated that the presumed vessels formed from solid cords of mesodermal cells through a process identical to the schizocoelic formation of the coelom in protostomes (Turbèville 1991). Thus, the circulatory system of nemerteans is currently considered a specialized coelomic system, and these worms have been included among the coelomate protostomes. This situation is not exceptional. It was known that two circulatory systems coexist in the arrhynchobdellid hirudineans (Fernández et al. 1992). One of them is a typical hemal system with walls constituted of basement membranes and the other one, much more developed, is constituted of a network of mesothelium-lined coelomic channels that spread throughout the body as a capillary network.

In summary, two main kinds of cell types are involved in the invertebrate circulatory systems, contractile myoepithelial cells and blood cells, and both cell types are probably derived from the coelomic epithelium during the development. This must be kept in mind when considering the transition between the invertebrate and vertebrate types of circulatory systems.

**THE INVERTEBRATE–VERTEBRATE TRANSITION OF THE CIRCULATORY SYSTEM. A HYPOTHESIS**

We think that the transition between the invertebrate and vertebrate circulatory systems could have occurred in the following way (Fig. 2):

- Pericytes and smooth muscle cells derived from myoepithelial cells detached from the coelomic lining. This provides a rationale for the origin of muscular progenitors of the aortic media and coronary arteries from the coelomic epithelium, as we will discuss below.
- Adherent amoebocytes developed an epithelial phenotype through the acquisition of specialized intercellular junctions and the ability to secrete basement membrane proteins. We think that this is a key event in order to explain the origin of a new cell type in vertebrates.
- These protoendothelial cells also acquired the ability to transiently shift between a quiescent, epithelial phenotype and a proliferative, migratory, and invasive phenotype, when stimulated by specific signals. This is the cellular basis of angiogenesis, and this ability allowed the growth of the vascular tree from the original visceral areas (close to the coelom and digestive tract) to somatic areas (especially tail, head, neural tube, and, later, limbs).
- Early endothelial and smooth muscle cells also developed a system of molecular cross-talking. In this way, endothelial cells produce signals to recruit and stabilize smooth muscle through platelet-derived growth factor and transforming growth factor-β signals, and smooth muscle controls the endothelial state through angiopoietins/Tie receptors (Folkman and d’Amore 1996).
- The basement membrane of the primitive tubular myoepithelial heart (Fig. 1C) was also colonized, as the remaining vessels, by endothelial cells that formed the endocardium. Proliferation of the myoepithelium and its differentiation in a special type of striated muscle accounts
for the major differences between vertebrate and invertebrate hearts.

DEVELOPMENTAL SUPPORT TO THE HYPOTHESIS

We are suggesting that endothelial cells appeared through re-epithelialization of adhering blood cells, whereas perivascular cells (smooth muscle, pericytes) would derive from coelomic-derived myoepithelial cells. If we assume that both vertebrate cell types are derivatives of the coelomic epithelium (and we have shown that most reports agree with this idea), then all the cell lineages that compose the vertebrate circulatory system would ultimately derive from embryonic coelomic cells. We think that only in this way the hemangioblast hypothesis could be reconciled with the vascular progenitor hypothesis, since the main vascular cells types, that is, blood cells, endothelium and perivascular cells, share a common phylogenetic and ontogenetic ancestor.

On the other hand, it has been well established that coronary perivascular cells arise from progenitors originated in the epicardium, that is, the coelomic epithelium of the heart (Vrancken Peeters et al. 1999). We have proposed elsewhere that cells delaminated from the coelomic epithelium migrate to the ventral part of the dorsal aorta and contribute, at least, to aortic smooth muscle (Pérez-Pomares et al. 1999) (Fig. 1, D–G). Thus, the differentiation of embryonic cells derived from the coelomic wall into smooth muscle has been well documented and could be a general event.

Regarding the endothelium, an origin from coelomic progenitors has also been proposed for the heart and liver (Le Douarin 1975; Moore et al. 1998; Pérez-Pomares et al. 2003). It is important to remark that the origin of the earliest embryonic endothelial cells, those from the endocardium, also derive from the same coelomic epithelium that will give rise to the myocardium (Sugi and Markwald 1996). Thus, the endocardium and the myocardium would be derivatives from a coelomic epithelium, according to our proposal. We can also quote, in this regard, the existence of cell lines with the ability to differentiate into both endothelial and myocardial lineages (Eisenberg and Bader 1995; Eisenberg and Markwald 1997).

We think that the differentiation of the earliest endothelial cells in the endoderm/mesoderm interphase also recapitulates the origin of the vertebrate circulatory system from an ancestral hemal system that, in invertebrates, is always related to the digestive tube and viscerae. This also emphasizes the importance of angiogenesis, a process which enabled the expansion of the circulatory system from its visceral origin to the somatic areas that are greatly developed in vertebrates (head, neural tube, tail, somitic muscle, and limbs).

The possibility of an origin of hematopoietic progenitors from coelomic-derived cells is not so well grounded and lacks experimental support. This hypothesis was raised by Olah et al. (1988) for the lateroventral areas of the dorsal aorta from chick embryos, where the earliest intraembryonic hematopoietic cell clusters appear. Accordingly, we have described an active migration of coelomic cells to the aorta coinciding with the appearance of these hematopoietic progenitors (Pérez-Pomares et al. 1999). On the other hand, it is important to remark that primary hematopoietic foci always appear between the endoderm and coelomic epithelium, the mesenteries and the aorta–gonad–mesonephros region, areas rich in coelomic-derived cells as suggested by the presence of mesenchymal cells expressing mesothelial markers such as cytokeratin or the transcription factor WT1 (Fig. 1, E–I).

COMPARATIVE SUPPORT TO THE HYPOTHESIS

Despite the lack of endothelial cells in invertebrates, it is surprising that the endothelial-specific signaling system VEGF/VEGF receptor (VEGFR) can be found in some of these animals, playing roles that support our hypothesis. A VEGFR-like molecule has been localized in the hemocytes of Drosophila (Heino et al. 2001), according to our proposal of a hemocytic origin of the endothelium. Furthermore, VEGF signals seem to regulate hemocytic migration in this species, according to the role played by VEGF as the main angiogenic triggering signal.

On the other hand, the botryoid tissue of the leeches (blood-sucking annelids) has been shown to respond to vertebrate VEGF (Eguileor et al. 2001; Tettamanti et al. 2003). This tissue is probably derived from the coelomic epithelium (Fernández et al. 1992) and it is thought to play a defensive role through phagocytosis. Interestingly, when leeches are injured or when they are injected with vertebrate VEGF, botryoid tissue reorganizes itself in hollow channels lined by endothelial-like cells, thus allowing defensive hemocytes to reach the place of an eventual infection. This is a surprising example of constitution of a transient, endothelial-like tissue in invertebrates from coelomic-derived cells associated with a defensive response and emphasizes the primary role of the endothelium in immunological cooperation.

THE ENDOTHELIUM AS A LAYER OF RE-EPITHELIALIZED BLOOD CELLS

If we regard the endothelium as a layer of re-epithelialized blood cells, we can explain some of their singularities. For example, endothelial cells in mammals form an epithelium with a mesenchymal type of intermediate filaments, because they are composed of vimentin. Most vertebrate epithelial cells contain cytokeratin-constituted intermediate filaments. Indeed, leukocytes and hematopoietic cells also show intermediate
filaments composed of vimentin. However, from the phylogenetic point of view, the presence of cytokeratin in the endothelium of anamniotes such as rainbow trout and *Xenopus* might be relevant (Jahn et al. 1987; Markl and Franke 1988).

On the other hand, the molecules involved in the endothelial intercellular junctions are highly specific. This is what we should expect from an evolutionary novelty as the proposed development of these junctions from originally free cells. Vascular endothelial (VE)-cadherin is only found in the endothelium, whereas PECAM is shared by the endothelium and some types of blood cells. The molecules involved in the cross-talking between endothelium and vascular smooth muscle are also specific, and we can consider them as evolutionary novelties of vertebrates. This is the case of the tyrosine kinase receptors Tie-1 and Tie-2 and their ligands, the angiopoietins.

It was well known that endothelial cells share a number of molecules with blood cells or their progenitors, a fact usually related to the common origin of these cell types from the hemangioblast. This is the case of CD34, PECAM, and VEGFR-2. However, it is less known that mesothelial cells also share a number of molecular resemblances with endothelial cells, including the receptor for acetylated low-density lipoprotein (Van Hinsbergh et al. 1990; Eid et al. 1992), vascular cell adhesion molecule, and CD44 (Gardner et al. 1995; Muller and Yoshida 1995; Topley et al. 1996). Like endothelium, mesothelial cells produce prostaglandin I₂ (Satoh and Prescott 1987), angiotsin-converting enzyme (Chung-Welch et al. 1989), and plasminogen activator inhibitors (Van Hinsbergh et al. 1990). It is not surprising that cultured mesothelial cells have sometimes been mistaken for microvascular endothelial cells, from which they can be distinguished by their angiogenic response (reviewed in Van Hinsbergh et al. 1990).

We think that a main support for our proposal of a phylogenetic origin of the endothelium from circulating progenitors is that this process still occurs in adult vertebrates (Asahara et al. 1997, 1999). The presence of endothelial progenitors in the bone marrow, which can be mobilized in response to VEGF, circulate in the blood and incorporate into the vessel wall as endothelial cells, might be now regarded in the context of our phylogenetic approach to the origin of the endothelium.

Finally, another strong argument in favor of our proposal is the dynamics of angiogenesis. Endothelial cells constitute a very stable cell type, with an extremely low turnover, when they are in contact with the internal elastic lamina and they receive stabilizing signals from perivascular cells. However, when endothelial cells are stimulated by angiogenic signals (mainly ligands of tyrosine kinase receptors such as VEGFs, FGFs, HGF), they change their phenotype and transform into a proliferative, migratory, and invasive cell type, persisting in this phenotype until the balance between stimulatory and stabilizing signals leads them to reacquire the stable epithelial phenotype. We think that this unique ability of endothelium is related with the conservation of the mechanisms that transformed a free, migratory cell into an epithelial cell. As we have described above, the system VEGF/VEGFR regulates blood cell migration in *Drosophila*, what is in accordance with our proposal.

**ENDOTHELIAL CELLS PROVIDE NEW FUNCTIONS TO THE VERTEBRATE VESSELS**

Circulatory systems of invertebrates perform well and in some cases allow for impressively large body sizes. Giant squids, for example, are among the largest marine animals. Thus, the need for an endothelium related to size and cardiac performance is not obvious. However, we think that the specialization of a free blood cell type that first became adherent and later epithelial, provided vertebrates with at least three advantages related with the higher performance of their circulatory systems. First was the cooperation in defensive tasks between the free cells of the immune system and the endothelium. Cells of the immune system cooperate among themselves to develop an immunitary response. We know that endothelial cells also contribute to this cooperative response by expression of surface molecules that are recognized by circulating cells. In this way, endothelial cells address competent cells to the place of an infection. We have seen how a primitive cooperation of this type can already be observed in the botryoid tissue of leeches, suggesting that this might be the primary function of the endothelium. In addition, endothelial cells would also allow for a fine regulation of blood flow, through the production of nitric oxide. The evolutionary novelty of this mechanism is again suggested by the specificity of the endothelial isoform of the nitric oxide synthase. Finally, as we have stated above, transition between quiescent and migratory phenotypes of endothelial cells allows for controlled invasion of tissues and vascularization of embryonic areas that are originally avascular. This seems to be especially important in vertebrates, because main body parts such as the head, tail, somitic muscle, or limbs are located far away from the visceral areas where the circulatory system develops.

**CONCLUSIONS**

Coelom and epithelial–mesenchymal transitions are main evolutionary advances of metazoa. We have used both features to account for the hitherto unexplained invertebrate/vertebrate transition of the circulatory system. Advances in the performance of the vertebrate circulatory system were essential to increase the size and activity of these animals. We think that these advances developed in response to the need for a mechanism of regulated vascular growth, fine regulation of the blood flow and immunological cooperation. This new view can explain most of the singularities of the endothelium and is compatible with all the current ontogenetic models.
about the origin of these cells, including the hemangioblast, the bipotential vascular progenitor, and the existence of circulating endothelial precursors. At the same time, a number of predictions are implicit in our model, for example:

- Ontogenetic relationships will be established between embryonic coelomic cells and the hemangioblastic lineage.
- Further endothelial markers will be found in blood cells of invertebrates.
- Molecules playing a role in epithelial-mesenchymal transitions will be detected in angiogenic endothelium.
- Molecular regulation of the endothelial differentiation of circulating endothelial progenitors will show similarities to the molecular regulation of the final stages of angiogenesis (morphogenesis and differentiation).

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