Platelets Take the Lead in Lymphatic Separation

Honsoul Kim, Gou Young Koh

The circulatory system of mammals consists of 2 components, blood vessels and lymphatic vessels, which are counterparts that act in parallel but develop in series. Unlike blood vessels that are a system built up to form a closed circuit carrying circulating blood, lymphatic vessels differ in that it is an open system with unique architecture starting at its periphery as a blunt end specialized to drain excessive interstitial fluid. Lymphatic vessel function is not merely confined to the drainage of excessive fluid, but it also serve as a major route for transportation of immune cells, absorbed lipids, cancer cells and cell debris from local intercellular space into its corresponding draining lymph node and subsequently into the systemic circulation. Blood vessels and lymphatic vessels are strictly separated from each other hence are not directly connected throughout the body, with only a few exceptions. The most typical and universal location of lymphatic vessels are never seen in lymphatic vessels. Aristotle recognized this colorless fluid. "During the late Middle Ages and throughout the Renaissance, scientists must have had numerous questions on the lymphatic vessels. What would be the role of vessels containing colorless fluid? What do they actually contain? How are they formed and different from blood vessels? In fact, some remaining documents from those days witness the efforts of scientists seeking answers to these questions. Approximately a century ago, Sabin, Huntington, and McClure had questioned about the origin of lymphatic vessels in the thoracic duct empties the lymph into the subclavian vein at the lower neck region. Additional lymphaticovenous communications occur in the renal, hepatic and adrenal veins, in the lymph nodes and in other peripheral locations. Under normal conditions, flow through these blood vessel and lymphatic vessel connections is allowed only in an one-way direction by a fine and delicate end-opening valve from the lymphatic "thoracic duct" to the left subclavian vein. Therefore, blood components such as red blood cells and platelets are never seen in lymphatic vessels. Aristotle recognized this and described lymphatic vessels as "vessels containing a colorless fluid."

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The lymphatic vessels start to develop at around embryonic development day 9.5 to 10.5 in mice and weeks 6 to 7 in humans, at a time point when the blood vessels are already functioning. Focal clusters of endothelial cells in the cardinal vein are found to be committed to the lymphatic phenotype. Budding from this nascent lymphatic structure consequently leads to a dynamic and rapid expansion of lymphatic vessels to grow in a centrifugal pattern and the network is further developed under the regulation of several key growth factors and corresponding receptors. However, an unsolved question behind this whole process of the early development of lymphatic system is how the lymphatic vessels are sealed off from the cardinal vein after the nascent lymphatic vessels are established to accomplish lymphatic separation, and what are the key events that orchestrate the separation process. If the sealing is incomplete, connections between the already functioning blood vessels and the newly developing lymphatic vessels would remain open, resulting in a type of lymphaticovenous fistula, and blood components such as red blood cells will freely enter the lymphatic vessels. The existence of these "blood-filled lymphatic vessels," which reflect embryonic hemorrhage, can be used as a hallmark of incomplete sealing between blood vessels and lymphatic vessels. For example, Abtahian et al found such blood-filled lymphatic vessels in Syk and Slp76 knockout mice and described their observations on the occurrence of the nonseparation phenotype. Syk and Slp76 are expressed selectively in hematopoietic cells, suggesting that these cells contribute to the separation of the 2 vascular systems.

In this issue of Circulation Research, Carramolino et al advance our understanding of the biological processes during the lymphatic separation from blood vessel during embryonic development. In this study, the authors unravel a novel role of platelets in the lymphatic separation and prove that if platelets are absent, the lymphatic separation will not take place during development (Figure). Genetically manipulated mice deprived of platelets were adopted, designed by either removing the specific gene responsible for megakaryocyte/platelets biogenesis (Meis1−/− mice, deficient in the transcription factor Meis1) or by constructing a lineage ablational strategy that would show a consequence of megakaryocyte/platelets depletion without affecting other blood cell lineages (PF4-Cre/Rosa26R-LacZ/pf4-Cre/ROSA26R-LacZ/Df hybrid mice conditionally expresses diphtheria toxin specifically to megakaryocyte lineage on Cre recombination). Both models showed blood-filled primary lymphatic sacs and super-

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Platelet aggregation was blocked, either genetically (podoplanin antibody and/or drug such as acetyl salicylic acid, then the formation of platelet plug, which is the key factor that induces the platelets to aggregate with lymphatic vessels, clearly indicating the occurrence of nonseparation. This observation was further fortified by successfully reproducing consistent phenotypes in wild type pregnant females deprived of platelets by administering anti-thrombocyte antibody. These findings are definitive to claim a novel morphological role of platelets in the lymphatic separation (Figure). However, underlying mechanisms about how platelets exert this role remain as an open question.

This article is in perfect harmony with the recent article reported by Uhrin et al, which shares the same perspective regarding the role of platelets for embryonic developmental separation of blood and lymphatic circulation. In the present article, the authors have indicated podoplanin, which is expressed in cardinal vein and Prox-1 and the cardinal veins (Figure). If the platelet aggregation/activation process was blocked, either genetically (podoplanin−/− mice or kindling-3−/− mice) or by a specific blocking antibody (anti-podoplanin) and/or drug such as acetyl salicylic acid, then the formation of platelet plug, which is the initial event of the separation of lymphatic vessel and blood vessel, was prohibited. Consequently, blood-filled lymphatic vessels were persistently or transiently observed, indicating “nonseparation” had occurred. Podoplanin is a small O- and N-glycosylated transmembrane protein and activates C-type lectin receptor (CLEC)-2 in platelets and promotes platelet aggregation (Figure). Notably, activation of Syk via Slp76 is stimulated by activation of CLEC-2 in the platelets, indicating that a cascade of molecular interactions occur for the podoplanin-induced platelet aggregation and the lymphatic separation (Figure). The crucial role of podoplanin in platelet aggregation and the lymphatic separation is also observed in the mice lacking T-synthetase, a glycosyltransferase encoded by the gene Clgal1 that is critical for the biosynthesis of core-1-derived O-glycans, in endothelial and hematopoietic cells. T-synthetase−/− mice develop blood-filled lymphatic vessels resembling podoplanin−/− mice and display dramatic reduction of podoplanin expression.

The crucial role of platelets in vascular remodeling by separating vascular compartments is not an event confined to the developmental stage but is also observed at the postnatal period. Echtler et al have described their observations of platelet adhesion and aggregation at the ductus arteriosus in newborn mice, of which the consequence is a thrombotic occlusion resulting in ultimate reorganization of the vascular structure that depicts the mechanism of ductus arteriosus closure, showing that platelets can effectively contribute to the division of vascular compartments even after birth.

Despite the progress recently achieved, our knowledge accounting for the mechanism of vascular remodeling remains far from satisfactory. Fortunately, the remarkable advance in optics opened a new era in the field of imaging techniques and is nowadays offering new methods to enable scientists to directly visualize “events occurring in the vessel.” A recent reports visualized hematopoietic stem cells arising from the aortic endothelium in zebrafish using combinations of fluorescent reporter transgenes by real-time imaging confocal microscopy. Another study adopting intravital imaging successfully identified the posterior cardinal vein as the ultimate source of lymphatic vessels in the zebrafish during embryonic development. Therefore, we hope that combining these 2 studies together may provide a way to directly visualize and trace the whole process of how platelets contribute to and accomplish the lymphatic separation from blood vessels.

Conceptually, to maintain the integrity of the whole system that is holding dynamically circulating fluid, proper valve function and adequately sealing off the wall of the conduit between the supply source and drainage system would be necessary to construct a pipeline. Otherwise, unwarranted fluid leakage out of the circuit would occur, especially during a period when the system is not fully prepared (during embryonic development). Likewise, mammals, including humans and mice, adopt sealing and valve systems in the vast network of blood vessels and lymphatic vessels, which are controlled under an extremely delicate balance to maintain

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**Non-standard Abbreviations and Acronyms**

<table>
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<th>Acronym</th>
<th>Description</th>
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<tr>
<td>CLEC-2</td>
<td>C-type lectin receptor 2</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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<td>VEGFR</td>
<td>vascular endothelial growth factor receptor</td>
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proper circulation and regulate fluid drainage during the early stages of life. Lymphatic separation can be considered as a tool to ensure this complex balance and recent studies, including this issue, shed light on the responsible molecules and cell types that control lymphatic separation. However, as mentioned by Uhrin et al, questions remain to some degree in certain events, like the \( \text{NF-E2} \)-deficient mice that do not show the occurrence of nonseparation phenotype in spite of marked platelet reduction. Given that this process of lymphatic separation during the embryonic period is not simple, we believe that responsible molecular and cellular events remain to be further identified.

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**Disclosures**

None.

**References**


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