Del-etion of Microvesicles From the Circulation

Pierre-Emmanuel Rautou, MD, PhD; Nigel Mackman, PhD

Microvesicles (also called microparticles) have become a hot topic recently and transport proteins, mRNA, and microRNA. They have been proposed to play roles in numerous processes, including coagulation, inflammation, immune response, cell activation, and cancer. Microvesicles are small (0.1–1 μm) membrane vesicles that are released from activated and apoptotic cells. They contain proteins from their parental cell and are characterized by surface exposure of negatively charged phospholipids, such as phosphatidylserine. Platelets are the primary source of microvesicles in the circulation of healthy individuals, although other cells also release microvesicles. Increased levels of microvesicles are observed in a variety of cardiovascular diseases, including unstable angina, atherosclerosis, and inflammatory vascular diseases. Some microvesicle populations are considered as surrogate biomarkers of vascular disorders and of thrombotic risk.

The steady-state level of microvesicles in the circulation reflects a balance between microvesicle generation and clearance. Numerous studies have analyzed the mechanisms of microvesicle formation. Stimulation of cells leads to elevated levels of intracellular Ca²⁺, which results in increased phosphatidylserine on the cell surface, membrane blebbing, and subsequent shedding of microvesicles. The importance of phosphatidylserine exposure on platelets and possibly microvesicles in hemostasis is demonstrated by Scott syndrome, a rare bleeding disorder associated with a defect in phosphatidylserine externalization and in microvesicle generation. Recently, it was reported that Scott syndrome patients have a mutation in a bidirectional, nonselective Ca²⁺-dependent channel called TMEM16F.

In this issue of Circulation, Dasgupta and colleagues describe a new pathway of microvesicle clearance from the circulation that involves binding of phosphatidylserine-positive microvesicles to endothelial cells (Figure). Previously, Dasgupta and colleagues reported that the major pathway for the removal of microvesicles from the circulation was via binding to splenic macrophages (Figure). This pathway requires the presence of a glycoprotein called lactadherin (also known as milk fat globule-epidermal growth factor 8), which is secreted by activated macrophages and immature dendritic cells. Lactadherin is a bifunctional protein that contains both discoidin I–like domains, which bind to phosphatidylserine on the surface of apoptotic cells and microvesicles, and a tripeptide Arg-Gly-Asp (RGD) motif, which binds to cellular integrins, such as αvβ3. In vitro studies demonstrated that lactadherin enhances platelet microvesicle binding and phagocytosis by macrophages. Lactadherin is not detected free in plasma of healthy individuals but has been found on the surface of platelet microvesicles. Interestingly, soluble lactadherin does not bind to αvβ3-positive cells (Dr G. Gilbert, unpublished data, 2008), which suggests that binding of lactadherin to phosphatidylserine-positive microvesicles changes its conformation to allow binding to αvβ3. Taken together, these results indicate that lactadherin bridges the binding of phosphatidylserine-positive microvesicles to splenic macrophages and facilitates their removal from the circulation. Under normal conditions, this appears to be a major pathway for microvesicle clearance from the circulation. Indeed, lactadherin-deficient mice have higher basal levels of circulating microvesicles than control mice, which results in a hypercoagulable state. In addition, a deficiency of lactadherin in bone marrow cells results in increased levels of circulating microvesicles in a mouse model of atherosclerosis.

The new pathway of microvesicle clearance described by Dasgupta and colleagues involves endothelial cells and a bridging glycoprotein called developmental endothelial locus-1 (Del-1). A previous study reported uptake of microvesicles by endothelial cells in the liver by a phosphatidylserine-dependent mechanism. Del-1 was discovered as an αvβ3 binding protein that regulates angiogenesis during embryogenesis. Del-1 is secreted by endothelial cells and, similar to lactadherin, has discoidin I–like domains and an RGD motif that mediate binding to both phosphatidylserine on microvesicles and αvβ3 on cells. In adult mice, Del-1 mRNA is expressed in the lungs and brain, with no expression in the liver or spleen. Interestingly, Dasgupta and colleagues found Del-1 in the plasma of healthy individuals. Moreover, a significant number of microvesicles from platelets and red blood cells bound Del-1 in flow cytometry experiments, whereas only a small number of microvesicles from monocytes and endothelial cells were positive. Del-1 is likely binding to phosphatidylserine on microvesicles in a similar manner to lactadherin; however, it is unclear why Del-1 appears to bind selectively to different populations of microvesicles. One might have expected a higher percentage of Del-1 binding to microvesicles derived from endothelial cells, because these cells express Del-1. In addition, Del-1 has been shown to bind the leukocyte integrin αLβ2 (also called CD11a). Therefore, one could also have expected higher binding of Del-1 to microvesicles derived from monocytes.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.
From the Department of Medicine, Division of Hematology & Oncology, McAllister Heart Institute, University of North Carolina at Chapel Hill.
Correspondence to Nigel Mackman, PhD, University of North Carolina at Chapel Hill; Chapel Hill, NC. E-mail nrmackman@med.unc.edu (Circulation. 2012;125:1601-1604.)
© 2012 American Heart Association, Inc.
Circulation is available at http://circ.ahajournals.org
DOI: 10.1161/CIRCULATIONAHA.112.094920
Importantly, in contrast to the results observed with lactadherin-deficient mice, Del-1–deficient mice do not have increased basal levels of microvesicles compared with controls.5 The authors hypothesize that this lack of change in basal microvesicle level may be caused by compensation by other pathways.

Dasgupta and colleagues5 found that binding of platelet microvesicles to cultured human umbilical vein endothelial cells and human microvascular endothelial cells was inhibited by blocking phosphatidylserine or αvβ3. Similar results were observed with microvesicles from red blood cells. However, no studies were presented with microvesicles from monocytes or endothelial cells, which have lower levels of Del-1 binding in the circulation. Next, fluorescently labeled human platelet microvesicles were injected into mice, and their uptake was measured in endothelial cells in various tissues. Del-1 deficiency led to a 50% reduction in microvesicle uptake by lung and liver endothelial cells but no change in uptake by splenic endothelial cells.5 These differences may reflect heterogeneous αvβ3 expression in the endothelium of the different tissues. For instance, relatively high levels of αvβ3 are observed in lung microvascular endothelium, with weaker expression in other organs.12 Despite differences in processing of these exogenous microvesicles, as noted above, there was no change in levels of endogenous microvesicles in Del-1–deficient mice. Therefore, these results should be interpreted cautiously.

Endotoxemia leads to increased levels of circulating microvesicles in mice.3 Interestingly, administration of lipopolysaccharide dramatically decreases lactadherin expression in the spleen, which would limit the effectiveness of this pathway to clear the elevated levels of microvesicles.7 Dasgupta and colleagues5 determined whether a deficiency in Del-1 affected levels of microvesicles after challenge with lipopolysaccharide. They found significantly higher levels of microvesicles in the plasma of Del-1–deficient mice compared with controls. This result suggests that the Del-1 clearance pathway may be “turned on” during pathological conditions that are associated with elevated levels of circulating microvesicles. At present, it is not clear how the Del-1

### Table. Studies Assessing Clearance of Microvesicles In Vivo

<table>
<thead>
<tr>
<th>Cell Origin of MVs</th>
<th>Organs Implicated</th>
<th>Main Cells Implicated</th>
<th>Molecules Implicated</th>
<th>Kinetics of MV Clearance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet MVs</td>
<td>Spleen: + +</td>
<td>Macrophages</td>
<td>Lactadherin</td>
<td>Uptake assessed 5 min after injection</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Liver: Not assessed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lungs: Not assessed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidneys: Not assessed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet MVs</td>
<td>+ +</td>
<td>Endothelial cells</td>
<td>Del-1 in lungs and liver but not in spleen</td>
<td>Uptake assessed 5 min after injection</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte MVs</td>
<td>+/−</td>
<td>In the liver: 92% by Kupffer cells</td>
<td>Phosphatidylserine</td>
<td>Within 5 min, 80% of MVs are cleared from the circulation</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cell MVs</td>
<td>+ +</td>
<td>Monocyte/ macrophages plus other undetermined cells</td>
<td>Not assessed</td>
<td>Uptake assessed 5 min after injection</td>
<td>17</td>
</tr>
<tr>
<td>Tumor cell MVs</td>
<td>+ +</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>Not assessed</td>
<td>Detectable in plasma up to 30 min after injection</td>
</tr>
</tbody>
</table>

MV indicates microvesicle; Del-1, developmental endothelial locus-1.
clearance pathway is upregulated, but it may be by increased expression of αvβ3. Indeed, αvβ3 expression is increased in endothelial cells exposed to inflammatory stimuli, such as tumor necrosis factor-α.13

Other molecules beside lactadherin and Del-1 bind to phosphatidylserine-positive microvesicles. For instance, the bridging molecule called growth-arrest-specific 6 (also known as GAS6) can bind to phosphatidylserine on cells and the phagocyte receptor tyrosine kinase MER.14 However, we did not find a difference in the number of phosphatidylserine-positive microvesicles between growth-arrest-specific 6–deficient mice and controls either at baseline or after lipopolysaccharide challenge (Burnier L, Lee R, Angelillo-Scherrer A, and Mackman N, unpublished data, 2011). β2-Glycoprotein I is another phosphatidylserine binding protein that binds platelet microvesicles and promotes their phagocytosis by macrophages in a phosphatidylserine-dependent manner.15 Ligand-receptor interactions may also contribute to microvesicle clearance. Binding of monocyte-derived microvesicles to activated platelets was shown to be dependent on both phosphatidylserine and P-selectin glycoprotein ligand-1.16 A similar dual interaction may occur between monocyte microvesicles and activated endothelium (Figure). Therefore, we propose a general mechanism for microvesicle uptake by macrophages and endothelial cells that involves both binding via phosphatidylserine bridging molecules, such as lactadherin and Del-1, to αvβ3 and other ligand-receptor interactions. The relative contribution of each of these different pathways to microvesicle clearance from the circulation remains to be determined.

Interestingly, different organs have been implicated in microvesicle clearance depending on the cell origin of the microvesicles (Table).5,6,9,17,18 This may be because of differences in protein and lipid composition of microvesicles from different cell types. Interestingly, splenectomy is associated with increased levels of circulating red blood cell and leukocyte microvesicles in patients.19 This observation is consistent with the lactadherin-macrophage clearance pathway operating mainly in the spleen. Moreover, removal of the spleen in a xenograft tumor model was associated with increased levels of tumor-derived microvesicles.18 In rodent studies, microvesicles are cleared very rapidly (Table). In contrast, platelet microvesicles were cleared more slowly in patients receiving platelet transfusions.20 Importantly, efficient clearance of circulating microvesicles is needed in pathological conditions. One study found that a 30-minute exposure to second-hand smoke was associated with an increase in circulating endothelial cell–derived microvesicles for up to 24 hours, which may reflect ongoing endothelial cell activation and/or a defect in clearance.2

The findings by Dasgupta and colleagues5 have implications beyond the field of microvesicle clearance. These investigators demonstrate that platelet microvesicles can interact with endothelial cells in vivo. This result is highly significant because numerous in vitro and ex vivo studies have shown that various microvesicles can modulate endothelial cell biology by inducing endothelial proliferation, inflammatory phenotype, or dysfunction.2 The in vivo relevance of these studies was so far somewhat uncertain.

In conclusion, the present study not only highlights a new mechanism for the clearance of circulating microvesicles but also sheds new light on in vitro studies showing that different microvesicles can regulate endothelial cell functions. Because the endothelium is positioned at the interface of blood and tissues, it plays a key role in interpreting signals delivered via the circulation. We speculate that different microvesicles may be targeted to endothelial cells in various organs by specific interactions, similar to the delivery of letters using zip codes. Further studies are needed to better understand how microvesicles modulate endothelial cell biology in vivo.

Acknowledgments
We would like to thank our colleagues for critical reading of the manuscript.

Sources of Funding
This work was supported by the Philippe Foundation (to Dr Rautou) and a grant (HL095096; to Dr Mackman) from the National Institutes of Health.

Disclosures
None.

References

Downloaded from http://circ.ahajournals.org/ by guest on April 13, 2017


Key Words: Editorials / endothelial function / endothelium / platelets / clearance / microparticles
Del- et-ion of Microvesicles From the Circulation
Pierre-Emmanuel Rautou and Nigel Mackman

Circulation. 2012;125:1601-1604; originally published online March 2, 2012;
doi: 10.1161/CIRCULATIONAHA.112.094920

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/125/13/1601