Del-ition of Microvesicles from the Circulation

**Running title:** Rautou et al.; Clearance of microvesicles

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**Abbreviations and acronyms:**
Del-1 -- Developmental endothelial locus-1;
MV -- microvesicle;
PS -- phosphatidylserine.
Microvesicles (MVs) (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. MVs 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 (PS). Platelets are the primary source of MVs in the circulation of healthy individuals, although other cells also release MVs. Increased levels of MVs are observed in a variety of cardiovascular diseases, including unstable angina, atherosclerosis and inflammatory vascular diseases. Some MV populations are considered as surrogate biomarkers of vascular disorders and of thrombotic risk.

The steady-state level of MVs in the circulation reflects a balance between MV generation and clearance. Numerous studies have analyzed the mechanisms of MV formation. Stimulation of cells leads to elevated levels of intracellular Ca\(^{2+}\) that results in increased PS on the cell surface, membrane blebbing and subsequent shedding of MVs. The importance of PS exposure on platelets, and possibly, MVs in hemostasis is demonstrated by Scott syndrome, a rare bleeding disorder associated with a defect in PS externalization and in MV generation. Recently, it was reported that Scott syndrome patients have a mutation in a bidirectional, non-selective Ca\(^{2+}\)-dependent channel dubbed “scramblase” called TMEM16F.

In this issue of Circulation, Dasgupta and colleagues describe a new pathway of MV clearance from the circulation that involves binding of PS\(^+\) MVs to endothelial cells (Figure 1). Previously, Dasgupta and colleagues reported that the major pathway for the removal of MVs from the circulation was via binding to splenic macrophages (Figure 1). This pathway requires the presence of a glycoprotein called lactadherin (also known as milk fat globule-epidermal
growth factor), 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 PS on the surface of apoptotic cells and MVs, and a tripeptide Arg-Gly-Asp (RGD) motif, which binds to cellular integrins, such as $\alpha v \beta 3$. In vitro studies demonstrated that lactadherin enhances platelet MV binding and phagocytosis by macrophages. Lactadherin is not detected free in plasma of healthy individuals but has been found on the surface of platelet MVs. Interestingly, soluble lactadherin does not bind to $\alpha v \beta 3$-positive cells (Dr. G. Gilbert, unpublished data), suggesting that binding of lactadherin to PS$^+$ MVs changes its conformation to allow binding to $\alpha v \beta 3$. Taken together, these results indicate that lactadherin bridges the binding of PS$^+$ MVs to splenic macrophages and facilitates their removal from the circulation. Under normal conditions, this appears to be a major pathway for MV clearance from the circulation. Indeed, lactadherin deficient mice have higher basal levels of circulating MVs than control mice resulting in a hypercoagulable state. In addition, a deficiency of lactadherin in bone marrow cells results in increased levels of circulating MVs in a mouse model of atherosclerosis.

The new pathway of MV 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 MVs by endothelial cells in the liver by a PS-dependent mechanism. Del-1 was discovered as a $\alpha v \beta 3$ binding protein that regulates angiogenesis during embryogenesis. Del-1 is secreted by endothelial cells and, similarly to lactadherin, has discoidin I-like domains and an RGD motif that mediate binding to both PS on MVs and $\alpha v \beta 3$ 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 MVs from platelets and red blood cells
bound Del-1 in flow cytometry experiments, whereas only a small number of MVs from
monocytes and endothelial cells were positive. Del-1 is likely binding to PS on MVs in a similar
manner to lactadherin. However, it is unclear why Del-1 appears to bind selectivity to different
populations of MVs. One might have expected a higher percentage of Del-1 binding to MVs
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).11 Therefore, one could also have
expected higher binding of Del-1 to MVs derived from monocytes. Importantly, in contrast to the
results observed with lactadherin deficient mice, Del-1 deficient mice do not have increased
basal levels of MVs compared to controls.5 The authors hypothesize that this lack of change in
basal MV level may be due to compensation by other pathways.

Dasgupta and colleagues 5 found that binding of platelet MVs to cultured human
umbilical vein endothelial cells and human microvascular endothelial cells was inhibited by
blocking PS or αvβ3. Similar results were observed with MVs from red blood cells. However, no
studies were presented with MVs from monocytes or endothelial cells that have lower levels of
Del-1 binding in the circulation. Next, fluorescently-labeled human platelet MVs were injected
into mice and uptake measured in endothelial cells in various tissues. Del-1 deficiency led to a
50% reduction in MV uptake by lung and liver endothelial cells but no change in uptake by
splenic endothelial cells.5 These differences may reflect differences in 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 MVs, as noted above there was no difference in levels of
endogenous MVs in Del-1 deficient mice. Therefore, these results should be interpreted
cautiously.
Endotoxemia leads to increased levels of circulating MVs in mice. 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 MVs. Dasgupta and colleagues determined if a deficiency in Del-1 affected levels of MVs after challenge with lipopolysaccharide. They found significantly higher levels of MVs in the plasma of Del-1 deficient mice compared to 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 MVs. At present, it is not clear how the Del-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 TNF-α.

Other molecules beside lactadherin and Del-1 bind to PS+ MVs. For instance, the bridging molecule called growth-arrest-specific 6 (also known as GAS6) can bind to PS on cells and the phagocyte receptor tyrosine kinase MER. However, we did not find a difference in the number of PS+ MVs 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). β2-glycoprotein I is another PS binding protein that binds platelet MVs and promotes their phagocytosis by macrophages in a PS-dependent manner. Ligand-receptor interactions may also contribute to MV clearance. Binding of monocyte-derived MVs to activated platelets was shown to be dependent on both PS and P-selectin glycoprotein ligand-1. A similar dual interaction may occur between monocyte MVs and activated endothelium (Figure 1). Therefore, we propose a general mechanism for MV uptake by macrophages and endothelial cells that involves both binding via PS bridging molecules, such as lactadherin and Del-1, to αvβ3 and other ligand-receptor interactions. The relative contribution

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of each of these different pathways to MV clearance from the circulation remains to be
determined.

Interestingly, different organs have been implicated in MV clearance depending on the
cell origin of the MVs (Table 1). This may be due to differences in protein and lipid
composition of MVs from different cell types. Interestingly, splenectomy is associated with
increased levels of circulating red blood cell and leukocyte MVs in patients. 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 MVs. In rodent studies, MVs are cleared very rapidly (Table 1). In
contrast, platelet MVs were cleared more slowly in patients receiving platelet transfusions.
Importantly, efficient clearance of circulating MVs is needed in pathological conditions. One
study found that a 30 minutes exposure to second-hand smoke was associated with an increase in
circulating endothelial cell-derived MVs for up to 24 hours that may reflect ongoing endothelial
cell activation and/or a defect in clearance.

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

In conclusion, this study not only highlights a new mechanism for the clearance of
circulating MVs but also provides a new perspective for in vitro studies showing that different
MV can regulate endothelial cell functions. Since 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 MVs may be targeted to endothelial cells in various organs by specific interactions similar to the delivery of letters using zipcodes. Further studies are needed to better understand how MVs modulate endothelial cell biology in vivo.

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Conflict of Interest Disclosures: None

References:


## Table 1. 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>Kinetic of MV clearance</th>
<th>References</th>
</tr>
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<tbody>
<tr>
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<td>Spleen</td>
<td>Liver</td>
<td>Lungs</td>
<td>Kidneys</td>
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<td></td>
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<td>Macrophages</td>
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<tr>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>Endothelial cells</td>
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<td>Erythrocyte MVs</td>
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<td>+++</td>
<td>+/−</td>
<td>+/−</td>
<td>In the liver: 92% by Kupffer cells</td>
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<tr>
<td>Endothelial cell MVs</td>
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<td>++</td>
<td>++</td>
<td>-</td>
<td>Monocyte/macrophages + other undetermined cells</td>
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<tr>
<td>Tumor cell MVs</td>
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<td>Not assessed</td>
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**Figure Legend:**

**Figure 1.** Pathways for clearance of microvesicles from the circulation. In normal conditions, the lactadherin pathway mediates clearance of MVs. Macrophages in the spleen secrete lactadherin and this facilitates capture of circulating MVs. In endotoxemia, the Del-1 pathway mediates clearance of MVs. We speculate that this pathway is turned on by the increased αvβ3 expression on the surface of endothelial cells. Activated endothelial cells also express adhesion molecules, such as P-selectin, which can capture leukocyte MVs containing P-selectin glycoprotein ligand-1 (PSGL-1). LMV, leukocyte microvesicle; PMV, platelet microvesicle.
Macrophage

Phosphatidylserine
Del-1
Lactadherin

Blood

Activated endothelial cell

PMV

PMV

PSGL-1

Blood

Lungs and Liver

Spleen

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