

## Extracellular Vesicle Crosstalk Between the Myocardium and Immune System Upon Infarction

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In the current issue of this Journal, Loyer et al<sup>1</sup> present an insightful introduction to the potential crosstalk between cardiac extracellular vesicles (EVs) and the immune response post-myocardial infarction (MI). This timely article addresses the growing need within the field to further understand the functionality of EVs, particularly those released during MI, complementing the growing literature to date associating EVs with cardiovascular disease.<sup>2</sup>

### Article, see p 100

It is now firmly understood that a biphasic immune response is one of the primary drivers of cardiac repair and remodeling post-MI, and importantly, this two-stage response is critical for proper healing. Neutrophils, quickly followed by monocytes, are the first immune responders to the site of infarction. The short-lived neutrophil has classically been seen to detrimentally influence cardiac remodeling, in which armory carried by the neutrophil to kill invading microbes actually leads to further damage during sterile injury through the generation of excess reactive oxygen species and release of proinflammatory cytokines. However, recent studies by Horckmans et al<sup>3</sup> revealed through neutrophil depletion experiments that the neutrophil may be capable of orchestrating a polarization of macrophages toward a reparative phenotype, revealing a beneficial role of the neutrophil during MI.

Twenty-four hours post-MI, the dominating immune cells within the heart are monocytes and macrophages, the cell types that are the focus of the publication by Loyer et al in this issue.<sup>1</sup> Neutrophil (CD45<sup>+</sup>/CD11b<sup>+</sup>/Ly6G<sup>High</sup>/Ly6C<sup>+</sup>) numbers are transient and begin to diminish 24 hours post-infarct in comparison to the ever-increasing number of monocytes (CD45<sup>+</sup>/CD11b<sup>+</sup>/Ly6G<sup>-</sup>/Ly6C<sup>+</sup>) in the first week post-infarct. Ly6C<sup>High</sup> monocytes with a proinflammatory phenotype initially control the immune response, followed by a temporal shift toward Ly6C<sup>Low</sup> monocytes, favoring repair, a topic expertly reviewed elsewhere.<sup>4</sup> The phenotypic and cell type shift within the myocardium post-infarct relies heavily on cellular crosstalk, which can be conducted through the release and uptake of EVs. The relevance of EV exchange of biological material to macrophages has been previously shown in the

context of putative “endothelial progenitor” cultures from peripheral blood mononuclear cells.<sup>5</sup> EVs from contaminating platelets carrying “endothelial” markers, particularly platelet endothelial cell adhesion molecule, also known as cluster of differentiation 31 (CD31) and von Willebrand factor, were entrapped by macrophages, temporarily influencing their “endothelial marker” positivity, leading to a gross misinterpretation of their “endothelial progenitor” potential.<sup>5,6</sup>

Loyer et al demonstrated in a murine model of coronary artery ligation that large and small EV numbers, of sizes 252±18 and 118±4 nm, respectively, are transiently increased in the myocardium and that on infarct, these EVs are primarily derived from cardiomyocytes (caveolin-3<sup>+</sup> and troponin T<sup>+</sup>) and the endothelium (CD31<sup>+</sup> and CD41<sup>-</sup>). Cardiomyocyte-specific expression of  $\alpha$ -actin-conjugated GFP (green fluorescent protein) was then utilized to show cardiomyocyte EVs being taken up by neutrophils and monocytes, which was argued to partly explain the transient nature of EV number post-infarct. However, conclusive evidence for full EV uptake rather than fluorescence being transferred by EV attachment to the outer plasma membrane of cells is missing. Lastly, large EVs but not small EVs from ischemic hearts were shown to cause an increase in proinflammatory IL-6 (interleukin-6) and chemokines CCL2 and CCL7 (C-C motif chemokine ligand 2 and 7, respectively; Figure). Because GFP fluorescence is being preferentially associated with monocytes, the potential role that these EVs could play on neutrophil functionality was not further explored. The study then concluded with the finding that EVs of similar size to those seen in mice post-infarction could be obtained from human heart tissue.

There are many avenues of future research arising from the work presented by Loyer et al. The mechanism behind the ability of large EVs, but not small EVs, to influence the levels of proinflammatory proteins within monocytes needs to be answered. The change in monocyte signaling by EVs could be because of cell-surface receptor activation or through the delivery of cargo, and further research to elucidate this would be of considerable interest. Higher resolution imaging should be used to confirm vesicle uptake. Alternatively, the binding of EVs to the plasma membrane of immune cells could be causing the fluorescence observed, with the importance of EV glycosylation and its biological role now becoming an emerging focus.<sup>7</sup>

One particular question arose with regard to the relevance of human myocardial EVs analyzed in this study, which were from patients receiving surgical intervention to treat aortic stenosis through valve replacement, a key difference, of course, being ischemia of the myocardium, it is merely shown that EVs of similar sizes to those seen in mice post-infarct can be isolated from human heart tissue. It would be of interest to determine whether the EVs isolated from human hearts have the same ability to influence the inflammatory profile

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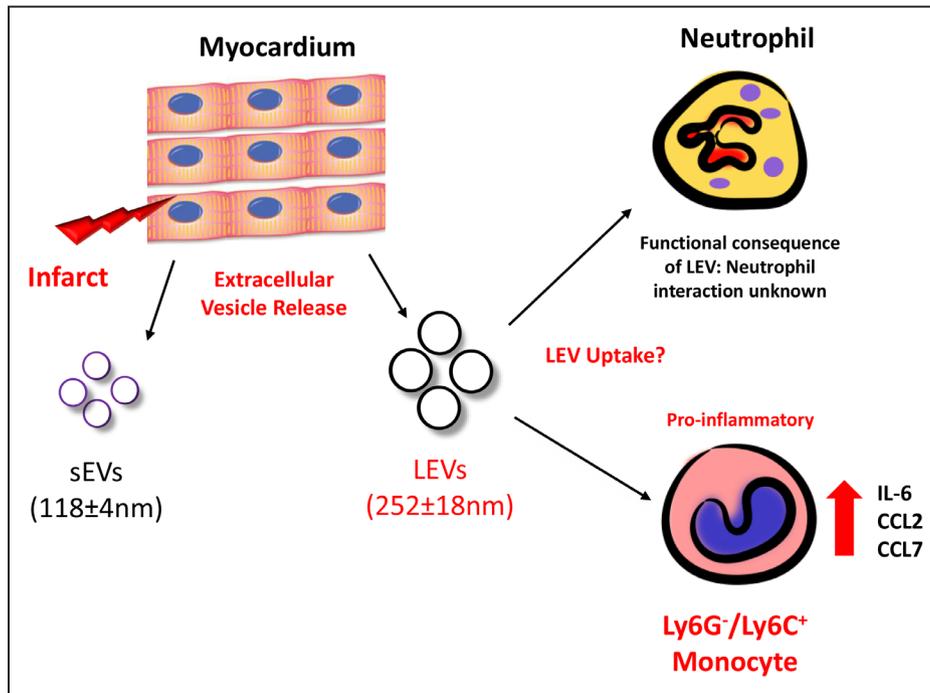
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**Figure.** Extracellular vesicles (EVs) released from cardiomyocytes post-infarct have a proinflammatory effect on monocytes.

Myocardial infarction led to the release of small EVs (sEV) and large EVs (LEV), primarily from cardiomyocytes and the endothelium. While the contents of these EVs remain to be explored, the EVs were shown to bind to neutrophils and monocytes that transiently infiltrate the tissue during sterile inflammation. LEVs, but not sEVs, are then taken up by monocytes and lead to an increase in the proinflammatory cytokines IL-6 (interleukin-6), CCL2 (C-C motif chemokine ligand 2) and CCL7 (C-C motif chemokine ligand 7).

of immune cells. The compositional differences between these human EVs and the EVs observed post-infarct in mice, whether protein or RNA screens, would also be an interesting comparison. Mass spectrometry-based proteomic analyses of differing subpopulations of EVs offer an unbiased and comprehensive approach, now becoming favored in the field of vesicle study.<sup>8,9</sup> A comprehensive understanding of the core signaling protagonists at play could allow the future harboring of particular EV populations for a therapeutic goal.

The phenotypic understanding, of not only the EVs released by the myocardium post-infarct but also the invading immune subpopulations by methods described above, would benefit this area of study immensely. Infiltration of monocytes into the damaged myocardium will also lead to cell differentiation into macrophages, marked by F4/80 expression in mice. However, future studies will need to investigate whether F4/80<sup>+</sup> inflammatory cells can uptake the myocardium-derived EVs. Ly6C levels upon the membrane of monocytes is now a commonly used readout of the inflammatory phenotype of this cell population, and indeed, Loyer et al showed that large EVs from the infarcted myocardium can influence proinflammatory cytokine levels in Ly6C<sup>+</sup> monocytes, but they did not investigate differing subpopulations of monocytes.<sup>10</sup> Human monocyte subpopulations are classically defined through differences in CD14 and CD16 levels, generating 3 distinct phenotypes. The commonalities and differences between human and mouse monocyte populations need to be further explored. How human monocytes are influenced by the EVs isolated from human hearts would be an interesting avenue of research.

EVs hold an enticing potential to be utilized as therapeutic delivery systems in the treatment of cardiovascular disease.<sup>11</sup> EVs have many intrinsic properties that suit their role as therapeutic delivery systems, such as their stability in circulation, natural targeting to certain cellular populations, and the protection of cargo from degradation pathways. Currently, EV use as vectors has been heavily biased toward microRNA delivery. Although most microRNAs are ubiquitously expressed, miR-126 is abundant in endothelium-derived<sup>12</sup> and platelet-derived microvesicles,<sup>13</sup> whereas miR-122 is secreted in EV of the liver.<sup>14</sup> It could be envisaged that the targeting of EVs to the endothelium could be utilized in the regulation of the immune response in a MI setting.<sup>15</sup> However, it is debatable whether the microRNA cargo within EV would be sufficient to mediate lasting biological effects in the recipient cell.

To conclude, Loyer et al published a set of exciting results that for the first time revealed EV release on MI. EV from cardiomyocytes are then taken up by infiltrating monocytes and regulate inflammation. Further investigations regarding this interaction after MI are warranted and will advance the field of EV research.

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### Disclosures

None.

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