Editorial Commentary

The dual role of mesenchymal stem cell-derived exosomes modulates the phagocytic activity of macrophages and promotes cardiac recovery after heart attack

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The World Health Organization considers ischemic heart disease (IHD) as the leading cause of death worldwide, accounting for 16% of total deaths worldwide (https://www.who.int/news-room/fact-sheets/detail/the-top-10-causesof-death). Since 2000, the largest increase in deaths has been for this disease, rising by more than 2 to 8.9 million deaths in 2019 (1). The most prevalent clinical manifestations and complications of IHD are acute myocardial infarction (AMI), cardiac arrhythmias, recurrent myocardial infarction (MI), heart failure (HF) and cardiac arrest (2). Current knowledge about the risk of recurrent MI and approved therapies do not acceptably reduce the threat to patients surviving a second AMI (3). Cardiac remodelling following an ischemic event leads to adverse phenomena resulting in progressive HF (4). In particular, the inflammatory response triggered by the release of danger factors from necrotic cardiomyocytes drives the migration of inflammatory cells into the compromised tissue (4,5). The resolution of the inflammatory response is achieved through the replacement of the necrotized myocardium with granulation tissue represented by collagen scars (6). Although capable of maintaining heart function, collagen scars do not preclude HF afterwards massive MI. Since the extent of MI and the severity of remodelling depend on the inflammatory response of immune cells, several studies have shown the key role of innate immune cells in the post-MI response (7,8). In AMI patients, a series of mediators administers the inflammatory response through dynamically modulating the pro-inflammatory and anti-inflammatory reparative phases (9,10). During the last two decades ground-breaking research has identified small endosomal derived membrane microvesicles, known as exosomes (11,12). They are secreted by immune cells as the main interactors in the regulation of reparatory responses following prolonged ischemia (13). Many researchers in the field have fuelled their minds to develop new therapies aimed at modulating the immune response after AMI (14,15). In an interesting review written by Patil and colleagues (16), the central role that exosomes play in heart disease and therapy is pointed out. They underline an active post-MI role of exosomes derived from immune cells. In a recent issue of *Circulation Research*, Patil and colleagues also showed that awareness and improvement of the phagocytic function of immune cells is an important gain in understanding the pathophysiological mechanisms of repair (17). Specifically, they set up a pilot study aimed to verify whether and how "mesenchymal stem cell-derived exosomes (MSC-Exo) can act as opsonin for apoptotic cells and/or trigger 'eat me' phagocytic signaling in resident/recruited phagocytes after myocardial ischemic injury" (17).

Patil and colleagues used mouse MSCs and verified MSC-Exo-mediated opsonization of apoptotic cardiomyocytes. The authors then determined the mechanism by which MSC-Exo modulates the phagocytic activity of macrophages (*Figure 1*). The hypothesis proposed by the authors focuses on milk fat globule-EGF factor 8 (MFGE8), one of the main opsonins capable of binding apoptotic cells. The authors demonstrated an enrichment of MSC-Exo in MFGE8 protein and mRNA and verified the role of this factor in apoptotic cell clearance. They

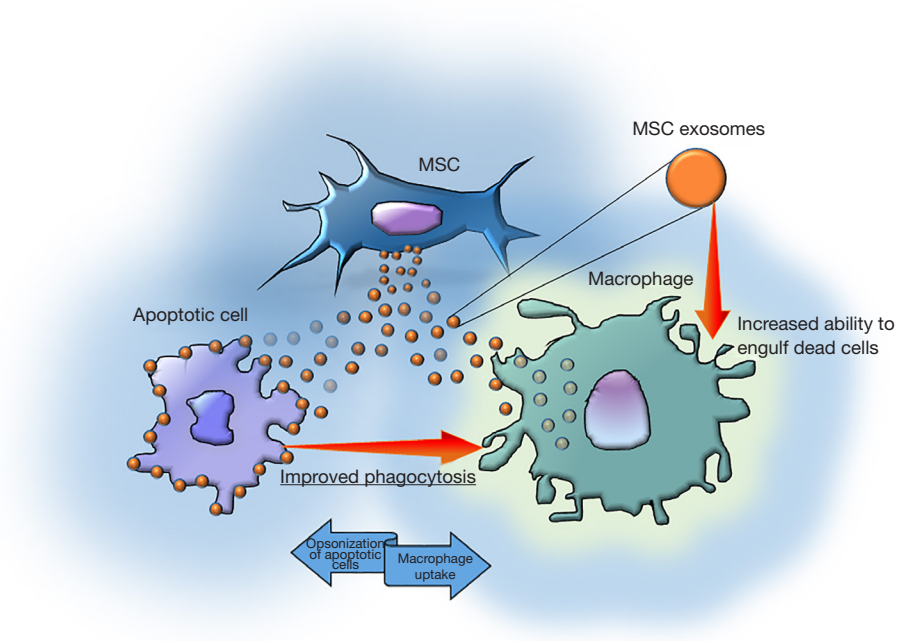

Figure 1 Schematic representation of the involvement of MSC-Exo in the modulation of the phagocytic activity of macrophages. MSC, mesenchymal stem cell; MSC-Exo, mesenchymal stem cell-derived exosomes.

have shown both increased opsonization of dead cells and binding of MFGE8-presenting MSC-Exo to phagocytes which in turn induces activation of phagocytic signalling which increases their ability to remove dead cells.

The authors claim the involvement of MSC-Exo in cardiac repair and their therapeutic potential in the improvement of heart function (18,19). They demonstrated *in vitro* the link between exosomes and apoptotic and necrotic H9c2, showing their ability to behave as opsonins towards apoptotic cells. They also showed the same ability towards cardiac fibroblasts. In both cases the bond is achieved through the interaction between MFGE8 and the phosphatidylserine (PS) exposed on the outer layer of the plasma membrane. It has also been verified that the phagocytic index significantly improved when H9c2 were exposed to the opsonization by MSC-Exo compared to the same non-exposed cells. In addition, an *in vivo* evaluation was performed to verify whether in these conditions the biological significance of the exosome-mediated opsonization had the same result on phagocytosis; therefore, by injecting apoptotic cardio-myoblasts into the mouse peritoneum, they discovered similar effects to the *in vitro* results. Nevertheless, in the complex study by Patil and colleagues, the most interesting finding may be considered the dual role of the MSC-Exo. Indeed, following the uptake in macrophages MSC-Exo are also able to activate intracellular signalling stimulating the efferocytosis, namely the biological process by which apoptotic cells are cleared by phagocytic cells (20).

Interestingly, and in accordance with the literature data (21,22), the authors highlighted an increase in the expression of integrin αVβ3 (MFGE8 receptor), an up regulation of its downstream RhoA and RAC1/2/3 and the inhibition of PTEN expression which regulates RAC expression levels. The biological significance of these molecular events ensures a transformation of the cytoskeleton, a rearrangement of actin, and facilitates the phagocytic activity of apoptotic cells (23).

Therefore, using a specific siRNA they produced MFGE8-knockdown (KD) MSC-Exo to evaluate the phagocytic activity of macrophages. Interestingly, they found that RAW 264.7 cells (monocyte/macrophage-like cells) used as a model, lost phagocytosis-related signalling. Intriguingly, the result described above was totally switched to a downregulation of both integrin αVβ3 and Rho-A and Rac 1/2/3 with an upregulation of PTEN. Furthermore, phagocytosis analysis revealed a reduced uptake of dead cells by macrophages pre-treated with MFGE8-KD MSC-Exo.

In light of the above, it can be said that the authors have

clearly demonstrated that the biological effects of MSC-Exo on macrophage activity are largely dependent on the presence/absence of MFGE8.

The central goal formulated by the authors was further confirmed by the intramyocardial administration of MSC-Exo. Treatment with MSC-Exo immediately after left anterior descending (LAD) ligation and a second dose of MSC-Exo administered intravenously by injection into the tail vein 24 hours after surgery was performed. From these experiments, the *in situ* engulfment of dying heart cells, the infiltration of inflammatory cells, and the impact on remodelling and repair of tissues dependent on these phenomena were assessed.

The authors analyzed the results of this experimental set, 7 days after the surgical manoeuvres on heart sections. They assessed cTnT (cardiomyocyte marker) positivity within $CD68^+$ macrophage cytoplasm and macrophage phagocytosis of apoptotic cells in myocardial tissue staining sections with the TUNEL assay kit. Observing an increase in the number of cytoplasmic cTnT-positive $CD68^+$ cells and a significant increase in the number of TUNEL-positive $CD68^+$ cells in the cytoplasm compared to mice that received PBS, post-MI.

Furthermore, *in vivo* intramyocardial administration of MSC-Exo revealed another interesting result regarding the resolution of inflammation, also in this case the administration of MSC-Exo significantly increased the clearance of dying cells, a result of great importance because it is associated with the downregulation of mRNA expression of myocardial pro-inflammatory cytokines such as iNOS, IL6 and CCL4, and upregulation of anti-inflammatory cytokine mRNA such as CCL24 and IL10. To corroborate these results, the authors also performed the evaluation of the neo-vascularization ascertained with staining for $CD31^+$ cells and the evaluation of the functionality of the left ventricle by means of echocardiography. The results agree with all others supporting the evidence that MSC-Exo contributes to preserve heart function after myocardial ischemic injury, activating phagocytic macrophage signaling which promotes apoptotic cell clearance and reduces scar formation and fibrosis in the left ventricle. The latest analysis performed by the authors to verify whether myocardial repair and resolution of inflammation were mediated by MFGE8 was conducted by evaluating the effect on survival and remodelling of the left ventricle. This revealed that administration of MFGE8-deficient MSC-Exo failed to protect mice from MI complications.

As described by several authors the resident macrophages of the heart represent the tip of the balance in insult response, orchestrating the immune response and a series of events concerning cardiac repair, leading to a more or less compromised cardiac performance (24,25).

From the study by Patil and colleagues it is clear that the knowledge of exosomes is fundamental to improve pathological conditions that today lead in most cases to negative outcomes. This knowledge is key to developing new therapies that could have benefits for infarct patients.

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Footnote

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