Clinical and experimental observations on the inflammatory response following a myocardial infarction

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Induction of a monocyte/macrophage phenotype switch by mesenchymal stem cells might contribute to improved infarct healing post acute myocardial infarction

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Review

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Abstract

Inadequate healing following acute myocardial infarction (MI) can lead to the development of heart failure. The ischemic myocardium triggers an inflammatory response that clears cell debris and initiates the onset of scar tissue formation. The duration and intensity of this inflammatory response have been linked to the cardiac functioning post-MI. In order to diminish scar tissue formation and stimulate regeneration of cardiac tissue, mesenchymal stem cell (MSC) have been applied post-MI and showed beneficial effects on cardiac function. However, other than the expected regeneration of cardiac tissue, modulation of the inflammatory response post-MI, especially related to an effect on monocytes/macrophages, was recently found to be an important aspect of MSC therapy. In healing post-MI, monocytes and macrophages are key players that can either stimulate or repress inflammation using different phenotypes. Increased levels of the pro-inflammatory phenotype have clinically been associated with poor cardiac functional outcome post-MI. MSC have been suggested to switch the monocytes/macrophages phenotype into a more anti-inflammatory state and might therefore beneficially influence the duration and intensity of the inflammatory response and subsequent cardiac function post-MI. To gain more insight into this effect of MSC, this review provides an overview of the most relevant studies regarding this modulatory effect of MSC on monocytes/macrophages including its mechanisms to improve cardiac functioning post-MI.
1 Introduction

During acute myocardial infarction (MI), occlusion of a coronary artery generates an ischemic environment which culminates in apoptotic and necrotic cell death of cardiomyocytes. These cells then need to be removed by recruited neutrophils and monocytes/macrophages. Subsequently, granulation tissue is formed, eventually resulting in scar tissue formation in the infarcted area of the heart. This loss of viable cardiomyocytes following MI can ultimately result in heart failure, since the endogenous regeneration potential of cardiomyocytes is limited. Even though progenitor cells and stem cells can be actively mobilized to take part in the cardiac repair after MI, this endogenous restoration of the jeopardized heart is not sufficient to prevent subsequent heart failure. In order to restore the lost cardiomyocytes, mesenchymal stem cell (MSC) therapy post-MI has recently been studied.

MSC are considered as a potential cell type for tissue regeneration because of their potential to differentiate into cardiomyocytes, their paracrine effect, such as the ability to secrete beneficial cytokines, and their immediate availability from a patient’s bone marrow or adipose tissue. Although clear evidence for the in vivo differentiation of the transplanted MSC in the heart into a cardiomyocyte has been demonstrated in animal MI studies, this capacity remains questionable in humans. Albeit, the possible effects of MSC on the cardiac function in animal studies were especially related to its inhibition of the inflammatory response, its reduction of apoptosis and the stimulation of neovascularisation other than regeneration of cardiomyocytes. In addition, patient studies showed that injection of MSC post-MI contributed to increased angiogenesis, reduced thickening of the myocardial wall and improved cardiac function, although MSC engraftment and retention within the infarcted heart appeared to be limited (less than 5%). This implies that the observed improvements are predominantly related to paracrine effects of MSC instead of cardiomyocyte regeneration. Following MI, an inflammatory response is induced that activates the innate immune system. This initial pro-inflammatory environment is then followed by a milder inflammatory response in order to repair the infarcted myocardium. Monocytes and their descendant macrophages are key players in this healing response and have the ability to both stimulate and repress inflammation using different phenotypes. Both phases of inflammation are needed for the infarcted area to heal properly. Exaggeration of either one of these phases can negatively influence myocardial healing, facilitating heart failure development.

It has been demonstrated that injection of MSC into the ischemic heart can convert the highly inflammatory environment following MI into a less inflammatory state by influencing the monocytes and macrophage phenotype. For this it is important to understand the mechanism(s) of this MSC immunomodulatory effect post-MI more accurately. In this review, an overview of the effects of MSC therapy post-MI on the inflammatory response is provided, in particular related to monocytes/macrophages.
CHAPTER 3

2 Mesenchymal stem cell therapy following MI

2.1 Bone marrow and adipose tissue derived MSC

MSC can be isolated for therapeutic use from different tissues such as the bone marrow or the adipose tissue. The bone marrow contains several populations of stem cells, including haematopoietic stem cells, endothelial progenitor cells and MSC. Therapeutic use of BMSC post-MI has been studied extensively in pre-clinical and clinical trials and has proven to be safe for clinical purposes. In 2002, Zuk et al. showed that adipose tissue forms another potential source of MSC, currently known as adipose derived stem cells (ASC). Similar to BMSC, ASC can be isolated and used autologously. However, isolation of adipose tissue provides up to a hundred times more MSC per gram tissue in comparison with bone marrow, making these cells an interesting alternative for therapeutic use.

2.2 MSC therapy and cardiac function

The functional therapeutic effect post-MI of BMSC and ASC appear to be similar, regarding their functional characteristics. The immunophenotype between BMSC and ASC is namely shown to be >90% identical. Furthermore, they are both able to differentiate into cardiomyocytes and endothelial cells in vitro and can home to injured or inflammatory tissue in vivo. Finally they both induce paracrine effects, such as the secretion of growth factors and cytokines.

Notwithstanding, differences in the therapeutic capacity post-MI BMSC and ASC have been described in pre-clinical studies. Rasmussen et al. showed that intramyocardial injection of ASC seven days post-MI improved left ventricular ejection fraction (LVEF) during a 4-week follow up and diminished scar wall thinning as compared to the saline group. In contrast, BMSC did not show any significant improvement in these parameters. This was also observed by Paul et al. when injecting human ASC and BMSC intramyocardially in rats 10 minutes post-MI. The group that received ASC showed, better than the BMSC group, a remarkable recovery of ventricular performance 6 weeks post-MI, as measured using echocardiography. However, the results of these two studies are in contrast with results of Karpov et al. In their rat study, intramyocardial injection of BMSC seven days post-MI resulted in decreased left ventricle (LV) scar area whereas injected ASC did not show this response. Moreover, injection of the BMSC, in contrast to ASC, resulted in better preservation of the LV contraction ability post-MI as studied in a Langendorff perfusion model two weeks post-MI.

In the last decades, several clinical trials have been initiated to investigate the effect of MSC therapy on cardiac function post-MI. The REPAIR-MI trial, a large randomized trial in 2006 that included 204 patients (NCT00279175), reported that intracoronary BMSC therapy significantly improved LV function. However, subsequent trials did not show an effect of BMSC therapy post-MI on cardiac function. The first clinical trial that used ASC as cellular therapy following MI (the APOLLO trial, NCT00442806) was performed recently, but also this stem cell trial showed only moderate beneficial effects on infarct size, perfusion and cardiac function.
One of the major drawbacks of stem cell therapy is that only a low number of the administered stem cells applied to the heart home to the infarcted area. When administered, most of the stem cells die within the first week after transplantation.\textsuperscript{36, 37} It is hypothesized that the inflammatory environment of the infarcted myocardium negatively modulates the survival of MSC and thereby their function.

3 The inflammatory response post-MI and its interaction with MSC

3.1 Phases of healing post-MI

Following MI, the cardiac healing response can be divided into three subsequent, partly overlapping phases; (1) the inflammatory phase, (2) the proliferative phase and (3) the maturation phase, all characterized by a typical infiltrate of inflammatory cells.\textsuperscript{38, 39} This healing response has recently been reviewed extensively by Frangiogiannis\textsuperscript{38} and is only described here briefly to highlight the roles of monocytes/macrophages during this response. During the first few hours post-MI, the innate immune system is immediately triggered in response to ischemic injury. Cells of the myeloid lineage, including neutrophilic granulocytes and monocytes, are then quickly recruited to the infarcted area. In this phase, irreversibly damaged cardiomyocytes are eliminated and extracellular matrix degradation is promoted.\textsuperscript{40} Monocytes are a heterogeneous pool of cells that display different receptor repertoires allowing them to be mobilized selectively. Once infiltrated into the myocardial tissue, monocytes can differentiate amongst others into macrophages where environmental stimuli can influence their phenotype. During the inflammatory phase, the classical monocyte subset is recruited from the bloodstream into the myocardium through MCP-1/CCR2 interaction.\textsuperscript{39, 41} After infiltration into this pro-inflammatory environment, the monocytes can differentiate into M1 macrophages.\textsuperscript{40} This then contributes to the production of nitric oxide (NO) and pro-inflammatory cytokines such as interleukin (IL)-6, tumour necrosis factor (TNF)-α, interferon (IFN)-γ and IL-1β.\textsuperscript{39} After approximately three days, when cell debris is cleared, the non-classical monocytes are recruited into the myocardium through fractalkine/CX3CR1 during the proliferative phase and can differentiate into M2 macrophages. This subset exhibits a pro-resolution profile and produces cytokines and growth factors e.g. IL-10, transforming growth factor (TGF)-beta and vascular endothelial growth factor (VEGF) to repress inflammation and coordinate formation of granulation tissue.\textsuperscript{40, 42} This proliferation phase is mainly associated with ventricular remodelling, such as matrix deposition and generation of a microvascular network.\textsuperscript{7, 43, 44} Finally, during the maturation phase seven days post-MI, vascular cells and fibroblasts undergo apoptosis resulting in a collagen-based scar maturates.

Accumulating evidence suggests that an exaggerated inflammatory phase post-MI directly negatively affects the prognosis of MI patients.\textsuperscript{45, 46} Using cardiac magnetic resonance imaging van der Laan \textit{et al.}\textsuperscript{46} showed that a high level of circulating classical monocytes is associated with impaired functional outcome of MI patients (Figure 1). This has also been described by Tsujioka \textit{et al.}\textsuperscript{45} who showed that a peak in the level of classical monocyte in the blood early
post-MI was negatively associated with myocardial salvage.

It has been suggested that cytokines secreted by administered MSC, convert the microenvironment of the infarct post-MI into a milder inflammatory state. More specifically, MSC might even improve infarct repair via a switch of the monocytes and macrophages into an anti-inflammatory phenotype and thereby beneficially influence short- and long-term functional outcome. On the other hand, the inflammatory response might also negatively affect stem cell therapy post-MI. Thus, it is important to gain insight into the mutual interaction between the inflammatory response post-MI, including the monocytes/macrophages and the applied MSC.

3.2 Effects of the inflammatory response on MSC

As mentioned earlier, it has been hypothesized that a highly inflammatory environment negatively influences the survival and functioning of applied MSC, although clear evidence for this hypothesis is lacking. Albeit, pre-clinical studies demonstrated that post-MI, the therapeutic effect of MSC strongly depends on the timing of MSC injection.

In animal studies, the majority administered MSC immediately post-MI. We recently have performed a study that compared the effect of intravenous ASC injection at different time points post-MI in rats. We then found that ASC administration at seven days post-MI resulted in a significant infarct size reduction whereas administration at day one post-MI did not have this effect. In addition, a recent meta-analysis of stem cell therapy in MI models of

![Figure 1. The association between circulating levels of classical monocytes and recovery of regional myocardial function.](image)

The change in wall thickening in dysfunctional segments from baseline to 4 months is depicted for tertiles of classical monocytes, (I, II and III where tertile III represents the highest level of monocytes). Data are presented as mean ± SD. P values for the change between baseline and follow-up within each tertile were calculated with paired Student t test. For the change in wall thickening from baseline to 4-month follow-up, P values were determined by the Spearman correlation test. Base denotes baseline; FU, follow-up. (Reprinted from American Heart Journal 163, van der Laan et al., A proinflammatory monocyte response is associated with myocardial injury and impaired functional outcome in patients with ST-segment elevation myocardial infarction: Monocytes and myocardial infarction, 57-65, 2012, with permission from Elsevier)
large animal showed that the LVEF is mostly preserved when stem cells are injected at one week post-MI. These studies thus indicate that improvement of the therapeutic effect using MSC is especially achieved when MSC are applied during the end of the proliferative phase, thus when the infarct environment has switched towards a more reparative state. This implies that inflammatory cells can affect MSC functioning. One in vitro study analyzed the effect of M1 and M2 macrophages on BMSC. They found that M1 macrophages and their secreted cytokines inhibited growth of BMSC whereas presence of M2 macrophages stimulated BMSC growth. Thereby they concluded that to improve MSC retention, MSC should be administered during the proliferation phase post-MI thus when M2 macrophages are abundantly present.

To address the effect of the inflammatory response on administered BMSC, Holladay et al. transfected BMSC with the anti-inflammatory cytokine IL-10 and injected these intramyocardial immediately following MI induction in rats. Indeed, the retention of the transfected BMSC in the heart was found to be fivefold higher as compared to control BMSC at four weeks post-MI. This coincided with improved cardiac function as determined using LVEF and was associated with a decrease in M1 macrophage accumulation in the heart at that time point. This implies that repression of the pro-inflammatory environment protected the BMSC retention in the heart. The improved LVEF post-MI in part can be by the increased MSC retention. Otherwise, M2 macrophage levels were also found to be increased in the heart of IL-10 transfected BMSC, which could imply that the improved therapeutic effects of BMSC on cardiac function post-MI is linked to the MSC modulation of the macrophage phenotypes.

3.3 MSC modulation of the monocyte and macrophage phenotype post-MI

Dayan et al. studied in a mouse MI model the effect of BMSC on monocyte/macrophage accumulation within the infarcted heart and cardiac function, as measured by echocardiography. They found that intravenous infusion of BMSC at day 2 post-MI increased the incidence of M2 macrophage accumulation into the infarcted myocardium, and improved cardiac function at 2 and 4 weeks post-MI. Moreover, they found increased circulating non-classical monocytes in the blood and an unaltered expression of chemokines (CX3CL1 and CCL2), vascular adhesion molecules (VCAM, ICAM and E-selectin) and monocyte/macrophage endothelial adhesion receptors (CD62L) in the infarcted heart 24 hours after infusion of the BMSC. They suggested that this indicates a BMSC-mediated phenotypic switch in the circulating monocytes, other than affecting the migration process. Furthermore, they showed that the proportion of M2 macrophages increased in the heart when isolated macrophages were cultured in the presence of BMSC or with BMSC-conditioned medium, which in part was related BMSC-secretion of the anti-inflammatory cytokine IL-10.

Only a few studies analysed the effect and underlying mechanism(s) by which MSC can modulate monocytes/macrophages and focused on isolated BMSC. All these studies report that in vitro the presence of BMSC reduces the amount of the M1 macrophage phenotype amongst the isolated macrophages, while the M2 phenotype is promoted. Kim and Hematti demonstrated that co-culturing with BMSC increased the expression of the typical M2 macrophage marker CD206 on macrophages derived from human peripheral blood as compared to macrophages that were cultured alone. Also, the cytokine profile of the co-
cultured macrophages suggested a M2 profile, with increased IL-10 and decreased IL-12 and TNF-α production. A similar effect of BMSC on macrophages was demonstrated by Maggini et al. They namely showed that BMSC induced a switch of cultured mouse macrophages into an anti-inflammatory phenotype, coinciding with a low production of TNF-α and IL-6 and an increased expression of IL-10.

Next to IL-10, production of indoleamine-pyrole 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE-2) have also been implicated in the modulatory effects of BMSC on monocyte/macrophage phenotype. Expression of the intracellular enzyme IDO is partly controlled by the NF-κB transcription factor and is upregulated in pro-inflammatory environments. The production of PGE-2 is also partly controlled by NF-kB and released through a pro-inflammatory pathway involving cyclooxygenase-2. Both IDO and PGE-2 were shown to be produced by BMSC. They were also demonstrated to be involved in stimulation of IL-10 secretion by macrophages, while inhibition of both IDO and PGE-2 reduced the immunomodulatory effect of MSC on monocytes/macrophages. These results thus suggest that excretion of PGE-2/IDO by MSC contributes to their ability to switch macrophages into an M2 phenotype as schematically demonstrated in Figure 2.

Figure 2. Schematic overview of possible monocyte and macrophage modulation by MSC.
The pro-inflammatory components PGE-2 and IDO could stimulate MSC to switch the monocytes/macrophages into an anti-inflammatory phenotype post-MI. This then can repress the inflammatory environment with increased IL-10 secretion and decreased IL-6 and TNF-α secretion. M1: M1 macrophage; M2: M2 macrophage; PGE-2: prostaglandin-E2; IDO: indoleamine-pyrole 2,3-dioxygenase.
4 Conclusions

In conclusion, animal studies show that MSC isolated from either the bone marrow or the adipose tissue, have the potential to improve cardiac function post-MI. However, the exact mechanism of action still remains to be elucidated. It is suggested that not the differentiation capacity but the immunomodulatory function of MSC is the essential mechanism of action. The highly inflammatory environment post-MI is hypothesized to negatively influence the survival rate and functioning of the MSC. Therefore, MSC should theoretically be applied during the proliferative phase post-MI to improve MSC functioning in the infarcted myocardium. However, in vitro and a few in vivo studies show that BMSC can also modulate monocytes and macrophages into an anti-inflammatory phenotype. In addition, clinical studies demonstrated that an exaggerated pro-inflammatory response with increased blood levels of classical monocytes following MI negatively influences the cardiac outcome. This led to the hypothesis that BMSC improve infarct repair through its modulation capacity on monocytes/macrophages and thereby beneficially switch the highly inflammatory environment post-MI into a more reparative state. Theoretically, it can then be stated that MSC should be applied during the inflammatory phase to stimulate repression of inflammation and induce scar tissue formation in the infarcted area of the heart through switching the monocyte/macrophage phenotype. However, since the inflammatory response also involves a wide scale of other important cellular infiltrates that can influence the MSC viability, interaction of the pro-inflammatory environment on the MSC remains to be further determined in order to understand and improve MSC therapy and functioning post-MI.

References


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