

What's New in Cardiac Cell Therapy? Allogeneic Bone Marrow Stromal Cells as "Universal Donor Cells"

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ABSTRACT Cardiac cell therapies offer distinct and exciting advantages over current treatments to prevent postinfarction heart failure because they can reverse ventricular remodeling and improve function, but only if the implanted stem cells contribute biological functions and achieve prolonged engraftment within the hostile environment of the damaged heart. Unfortunately, function is diminished in autologous stem cells isolated from older patients and those with comorbidities, and so clinical trials testing the implantation of healthy, allogeneic bone marrow–derived stromal cells (MSCs) isolated from young donors are currently underway. MSCs are unique because, in addition to exerting paracrine effects that restore blood flow and recruit endogenous stem cells to the infarct, they exhibit immune-modulating properties in culture that—if retained after allogeneic implantation—imply the cells may escape immune recognition within the heart. At present, the scope of MSC immune modulation after implantation is unclear. doi: 10.1111/j.1540-8191.2009.00984.x *(J Card Surg 2010;25:359-366)*

Heart failure can develop after a myocardial infarction (MI) when cardiomyocyte loss causes the scar to thin and dilate.[1] Conventional medical and surgical treatments have curbed mortality from coronary artery disease over the last several decades, but none of the standard therapies for heart failure contributes new, contractile tissue to replace the myocardial scar. The heart's own capacity for self-renewal is also limited, and so the search is on for innovative strategies that will promote cardiac regeneration. Of these, cell therapy has perhaps the greatest potential to actually restore cardiac function, and discoveries about the regenerative properties of stem and progenitor cells have propelled research in this field.

To date, the safety and prolonged functional benefits of implanted cells have been clearly demonstrated in preclinical experiments. In the initial clinical trials, autologous cell therapy given after an acute MI modestly improved left ventricular ejection fraction and reduced infarct size, increasing exercise capacity or reducing the 5-year risk of recurrent MI (vs. control treatment).[2-4] But even though a recent report found that cell therapy was at least as effective as existing drug therapies for preventing heart failure after an MI,[5] declining stem cell function in aging patients and the effort required to prepare unique donor cells for individual recipients preclude widespread clinical application at this time.[6]

At this point, researchers are seeking a "universal donor" cell that is both highly regenerative and tolerated by allogeneic recipients. The ultimate goal is a ready, "off-the-shelf" supply of progenitor cells to treat those at high risk of developing heart failure, for example, older patients undergoing coronary bypass surgery who have limited stem cell function. A growing body of research has focused on multipotent bone marrow mesenchymal stromal cells (MSCs) as ideal candidates, owing to their unique regenerative and immune modulating properties. In this review, we consider the utility of allogeneic MSCs as universal donor cells for cardiac regeneration. First, we briefly review the cellular alternatives available. Then, we discuss the fundamental characteristics of MSCs, update the current status of their use in clinical trials, and examine *in vitro* and *in vivo* evidence that might determine whether MSCs avoid immune rejection from the heart.

CELL THERAPY FOR CARDIAC REPAIR: WHICH CELLS ARE BEST?

Cell therapy—with cells delivered to the heart via cytokine mobilization, infusion through the coronary arteries, or direct myocardial injection—has been

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under investigation since the mid-1990s.[7-9] Certainly, the wide variety of cell types that have been considered (summarized in Fig. 1) reflects the huge experimental effort directed toward developing and translating this therapy to improve functional outcomes for patients who experience an extensive MI.

Candidate cell types

Based on the fact that cell loss is a main feature of the pathology associated with an MI, the initial rationale for cell therapy was that *muscle cells*; specifically, *cardiomyocytes*, *skeletal myoblasts*, or *smooth muscle cells*, would contribute to functional recovery by replacing lost cardiomyocytes. Instead, preclinical work found that the functional effects resulted from paracrine influences of the engrafted muscle cells (e.g., increasing elasticity in the infarct, promoting angiogenesis, and reducing matrix disruption).[10] With a view to regenerating the damaged myocardium, attention next shifted to *tissue progenitor cells*, including two types of cells from the bone marrow—hematopoietic *endothelial progenitor cells* and multipotent MSCs—that can promote angiogenesis or provide paracrine support to maintain surviving cardiomyocytes.[11,12] *Cardiac stem cells* are endogenous cardiac cells that might produce cardiomyocytes,[13] but their origin and value for cardiac repair are unclear at this time. Most recently, researchers have sought *pluripotent stem cells* from which to create cardiomyocytes for myocardial regeneration. *Embryonic stem cells* can produce any adult cell and potentially regenerate the myocardium,[14] and highly potent *induced pluripotent stem cells* (iPS cells) are genetically reprogrammed adult somatic cells[15,16] that may provide an autologous source of pluripotent cells for cardiac repair. However, iPS cells are currently derived using viral delivery systems,[17] and both types may be predisposed to tumor formation *in vivo*.[18] Although cardiac, embryonic, and iPS cells are not yet ready for clinical use, candidate cells of all types have been repeatedly shown to engraft in the damaged myocardium, and in many cases, to produce measurable functional improvements.

Allogeneic versus autologous donor cells

Generally, implanted cells derived from an *allogeneic* source (another donor; e.g., embryonic stem cells) rather than an *autologous* source (the patient themselves; e.g., iPS cells), will be vulnerable to rejection by a hostile recipient immune system. Therapies using promising pluripotent candidates are still in the very early stages of development, and so other, better-characterized autologous cell types (e.g., bone marrow cells) are of more immediate utility. But adult bone marrow cells often have limited multipotency or are too few within the target tissue,[17] and their translation to clinical use has not been straightforward. Studies performed in young animals demonstrated that autologous bone marrow cells implanted after an MI contributed significantly to functional repair[8,19,20] (reviewed by Dimmeler et al.[21]), but early clinical trials (reviewed by Menasche[22]) found that the effects of autologous cells were comparatively subdued (though still clinically significant) in aged patients—likely due to an age-related decline in the regenerative capacity of the autologous cells.[23,24] Indeed, stem cell number and function are known to deteriorate with normal aging (reviewed by Rando[25]), effectively reducing their engraftment rate and paracrine effects. Since sufficient usable progenitor cells are unlikely to be isolated from aged patients with degenerative or comorbid conditions, cell therapies using more robust progenitor cells from an allogeneic source—specifically, young, healthy donors—may ultimately be preferable for clinical purposes. Numerous reports documenting the unique immune properties of MSCs[26-30] predict that these cells may avoid the difficulties associated with immune rejection *in vivo*. If so, then MSCs isolated from healthy donors could be the highly sought after universal donor cells for allogeneic cell therapy.

WHY ARE MSCS GOOD CANDIDATES FOR CARDIAC REPAIR?

MSCs, believed by most investigators to be multipotent (see review by Prockop[31]), are a rare population of nonhematopoietic cells that exist in several postnatal tissues. They are most abundant in the bone marrow where, accounting for 0.001% to 0.01% of nucleated cells, they are approximately 10-fold fewer than the hematopoietic cells.[32] They are also found in the gut, lung, liver, adipose, and dental pulp.[33-35] MSCs typically occupy a perivascular niche, supporting and contributing to the maintenance of connective and skeletal tissues.[36] Although normally quiescent, adult MSCs can divide rapidly in culture to produce osteogenic, chondrogenic, adipogenic, endothelial, and myogenic cells.[29,37]

MSCs are good candidate cells for cell therapy because they are multipotent[29] and are easily isolated, expanded, and genetically modified *in vitro*.[38,39] However, a nonuniformity of culture and characterization techniques and the lack of a definitive MSC-specific phenotyping surface marker have complicated the reproducible isolation of identical populations for study. Cells expanded in culture may also accumulate molecular alterations, though these can be avoided by testing expanded MSC products for normal karyotype.[40] MSCs have been intensively studied in basic and preclinical studies. In animals, bone marrow– or adipose-derived MSCs have been shown to improve perfusion, attenuate myocardial scar, and restore cardiac function after an MI whether derived from an autologous or an allogeneic source.[41,42] These outcomes do not appear to be achieved by direct regeneration of lost cells, but rather through paracrine facilitation of endogenous repair processes[43] (summarized in Fig. 2). Acting via cell–cell interaction or the release of soluble factors that activate resident or remote stem cells, implanted MSCs boost angiogenesis, stimulate endogenous cell homing,[39,44] and help to stabilize the extracellular matrix.[38] A few reports caution that MSCs could differentiate into bone-forming osteoblasts within infarcted

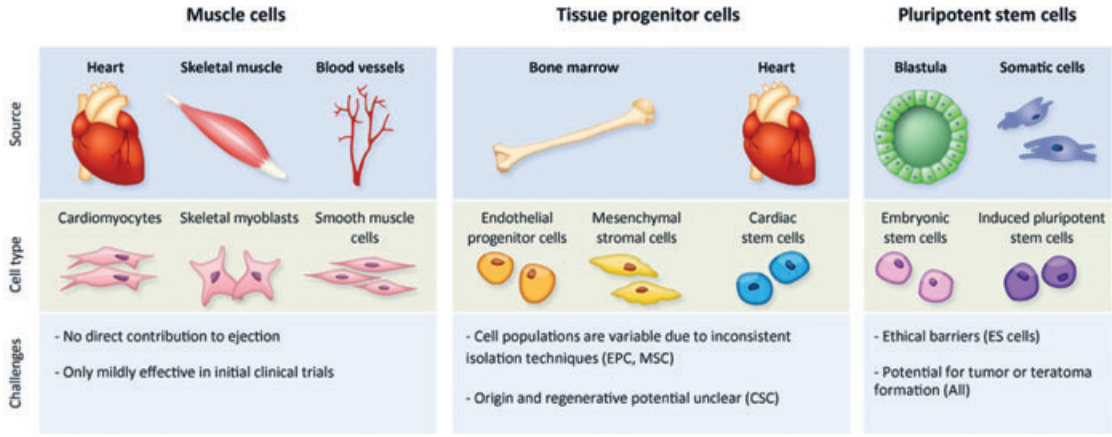


Figure 1. *Candidate cell types for cardiac cell therapy. Cells from three main categories (muscle cells, tissue progenitor cells, and pluripotent stem cells) have been evaluated for cardiac cell therapy. Each category comprises various cell types derived from multiple tissues, and each is associated with a different set of practical challenges. EPC = endothelial progenitor cells; MSC = mesenchymal stromal cells; CSC = cardiac stem cells; ES cells = embryonic stem cells.*

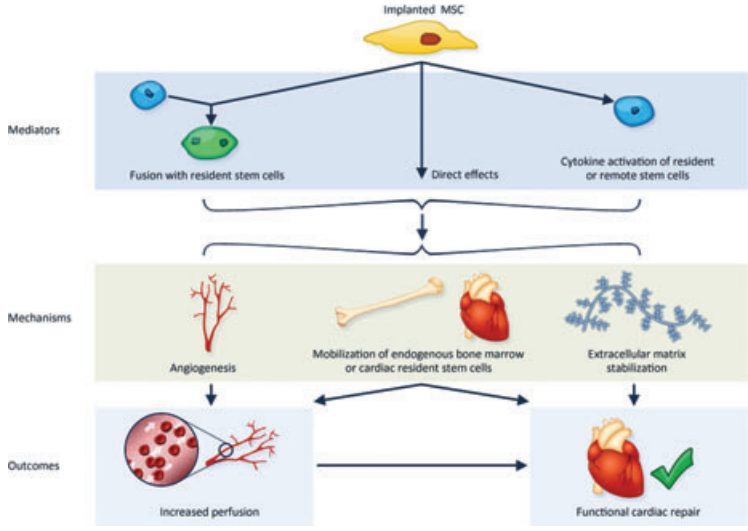
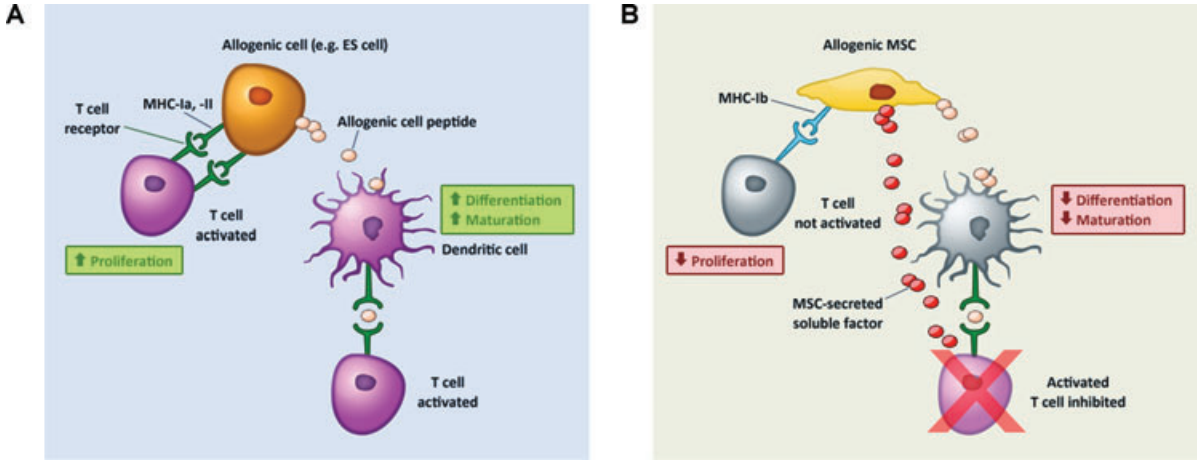


Figure 2. *Mechanisms by which implanted MSCs may achieve cardiac repair. MSCs appear to facilitate endogenous reparative processes that include angiogenesis, endogenous cell recruitment, and extracellular matrix stabilization. These paracrine effects are achieved through cell-cell interaction and/or the release of soluble factors that activate resident or remote stem cells. Implanted MSCs may also fuse with endogenous cells in small numbers, but direct, in vivo transdifferentiation of MSCs into cardiomyocytes occurs very infrequently. The net outcomes of MSC implantation are increased perfusion, support and protection of cardiomyocytes, and ultimately, improved function.*



mouse hearts, suggesting that their developmental fate may not be restricted by the surrounding tissue after implantation.[45] Fortunately, extensive numbers of unwanted cells have not been documented in most large animal studies or clinical trials. MSCs are most safely delivered by direct myocardial injection because they are large enough (approximately 10 to 20 μm in diameter) to restrict distal blood flow (no-reflow) if they are infused through the coronary arteries.[46,47]

Universal donor cells for allogeneic cell therapy?

Due in part to their ease of expansion—billions can be generated from a modest percutaneous bone marrow aspirate[32]—MSCs have the exciting potential for commercial preparation as an off-the-shelf therapy. They also exhibit unique immune characteristics in culture, including low cell surface expression of major histocompatibility complex antigens (involved in eliciting an alloimmune response) and the ability to modulate immune reactions.[48-50] However, while MSC immune modulating properties are well defined *in vitro*, conflicting reports from relatively few preclinical studies of allogeneic MSCs as cardiac therapeutics highlight the need for caution when applying the *in vitro* findings to an *in vivo* scenario. The concern is that the implanted cells may initially engraft in the infarcted heart, but eventually be rejected. Obviously, this possibility has implications for the utility of allogeneic MSCs as universal donor cells. As researchers attempt to understand exactly how MSCs behave *in vivo*, clinical trials are already progressing.

TOWARD CLINICAL APPLICATION: WILL ALLOGENEIC MSCS BE EFFECTIVE?

MSCs are already in clinical use, principally for the treatment of autoimmune diseases. For cardiac applications, clinical trials assessing the safety and efficacy of whole bone marrow or autologous MSCs have been well ahead of those using allogeneic MSCs. Two randomized, placebo-controlled, multicenter trials are now underway in the United States testing allogeneic MSC therapy to prevent congestive heart failure.[36,51]

The Provacel trial (sponsored by Osiris Therapeutics, Inc. Columbia, MD, USA) is assessing the safety of allogeneic MSCs infused intravenously in patients after an MI. Positive six-month results were reported by Zambrano and colleagues at the 2007 American Heart Association Scientific Sessions meeting.[52] The authors reported that ejection fraction was significantly increased compared to baseline at three and six months after MSC therapy in patients with infarcts resulting from occlusion of the left anterior descending (LAD) coronary artery, but not in those with non-LAD infarcts or placebo patients.

The Revascor trial (sponsored by Angioblast Systems, Inc., New York, NY, USA) is evaluating the safety and feasibility of the lowest of three doses of allogeneic MSCs delivered by single injection into the damaged myocardium of patients with congestive heart failure. The specialized cells were injected through a left ventricular catheter after endocardial mapping. The positive three months results of this trial were reported online [http://www.angioblast.com]. Ejection fraction was significantly increased compared to baseline at three months after MSC therapy. The mean difference in ejection fraction between cell and placebo recipients was also significant at three months.

The initial clinical reports on allogeneic MSC therapy to prevent heart failure are certainly encouraging, but the trials enrolled a limited number of patients who were evaluated shortly after treatment. In addition, these studies will not be able to determine how long the allogeneic MSCs survived because the implanted cells were not labeled. Preclinical studies have neither convincingly documented the late engraftment of allogeneic MSCs implanted into the heart nor determined whether their effects on cardiac function will persist in the long-term—though a clinical trial currently in development will directly compare allogeneic and autologous preparations of MSCs.[53] Better prediction of clinical outcomes will require an understanding of the nature and extent of MSC immune interactions in an allogeneic cardiac environment. To this end, let us now examine what is currently known about the immune properties of MSCs in culture and after implantation.

DO ALLOGENEIC MSCS REALLY RESIST IMMUNE REJECTION FROM THE HEART?

Whether MSCs are able to engraft in an allogeneic environment without activating immune cells and undergoing the usual course of recognition and rejection—that is, whether they are immunoprivileged[54]—depends on both the immune properties of the MSCs themselves, and the interactions between the MSCs and local immune system molecules (reviewed by Nauta and Fibbe[40]). The immune properties and interactions of MSCs have been best characterized *in vitro*.

Evidence from the *in vitro* studies

In culture, MSCs exhibit a predominantly immunosuppressive phenotype, whereby they inhibit

Figure 3. *Mechanisms by which MSCs are immunosuppressive in vitro. The alloimmune interactions of potential donor cells can be observed by culturing them with peripheral blood cells [T cells and antigen presenting cells (dendritic cells)] from a potential recipient. (A) Normally, allogeneic cells (e.g., embryonic stem cells, ES cells) induce T-cell activation and proliferation when cell surface antigens from the major histocompatibility complex (MHC-Ia, MHC-II) are recognized by T-cell receptors, or when peptides from the allogeneic cells are recognized by dendritic cells, which in turn activate the T cells. (B) MSCs lack expression of immunogenic MHC-Ia and MHC-II antigens. Instead, they express MHC-Ib, which prevents T-cell activation and proliferation. Allogeneic MSCs may also avoid T-cell activation by suppressing dendritic cell differentiation and maturation, or directly inhibit activated T cells by secreting immunosuppressive soluble factors.*

alloimmune responses. The main interactions are summarized in Figure 3. For example, the fact that MSCs generally do not elicit T-cell proliferative responses from allogeneic peripheral blood mononuclear cells in a mixed lymphocyte reaction (MLR) suggests that the cells are hypoimmunogenic.[32,55] MSCs likely do not effect immune modulation by suppressing immune responses directly, but rather by suppressing T-cell proliferation.[56] MSC inhibitory effects appear to be transient, but persist during expansion in culture.[30] Furthermore, MSCs arrest T-cell division,[27] inhibit the differentiation and maturation of dendritic (antigen presenting) cells,[28] and decrease the production of inflammatory cytokines.[26]

Largely responsible for the immunosuppressive properties of MSCs *in vitro* is a unique expression pattern of cell surface antigens from the major histocompatibility complex (MHC, or ''human leukocyte antigen system'' in humans). The MHC is a large genomic region or gene family found in most vertebrates that plays an important role in the immune system and autoimmunity. Proteins encoded by the MHC are expressed on the cell surface. MSCs lack surface expression of MHC class Ia and MHC class II molecules.[57,58] When present, these antigens are normally recognized by cytotoxic T cells (Fig. 3) and the cells carrying them are subsequently eliminated by cytolysis. MSCs also lack expression of costimulatory molecules (including CD40, CD40 ligand, CD80, and CD86) that are required for T-cell induction,[59] but they do express MHC class Ib molecules[60]—immunosuppressive molecules that inhibit CD4+ T-cell responses and natural killer cell–mediated cytolysis.[61,62]

MSC immune modulation is mediated by direct cell contact with immune cells, and maintained by the release of soluble factors that can inhibit activated T cells;[36] these include transforming growth factor β1, hepatocyte growth factor, interleukin-10, and prostaglandin E-2[50,54] (Fig. 3). MSCs also secrete factors that prevent the release of harmful cytokines from natural killer cells[26] and suppress the proliferation or terminal differentiation of B lymphocytes[63] in culture.

Despite the apparent hypoimmunogenicity of MSCs in their naïve state, the cells actually have a "dual" immune character, whereby they become immunogenic—able to stimulate alloimmune responses—in specific culture conditions (e.g., hypoxic or inflammatory).[64] For instance, compared to naïve MSCs, those cocultured with proinflammatory cytokines exhibit increased surface expression of immunogenic MHC class I and class II antigens.[29,55,59] The human form of MHC class II can also be upregulated in culture by exposing human MSCs to the soluble cytokine interferon-γ.[58] Recent evidence shows that interferon-γ stimulation causes MSCs to function as antigen-presenting cells—immune accessory cells that induce antigen-specific T-cell responses by binding foreign antigens.[65,66]

There is also evidence that differentiation along certain lineages can affect MSC immune properties *in vitro*. Specifically, peripheral blood lymphocyte stimulation (in an MLR culture) and cytotoxicity were increased approximately eightfold and fourfold, respectively, compared to undifferentiated cells for rat MSCs that underwent chondrogenic differentiation. Expression of costimulatory B7 molecules (required to fully activate cytotoxic T cells) was also upregulated in the differentiated cells.[67]

Outcomes from the *in vivo* preclinical studies

Clearly, MSCs' immune characteristics have been well defined *in vitro*. Considerably less obvious is whether the various immune interactions of these cells proceed similarly within the intricate (and clinically relevant) environment of the myocardium. One concern is that culture expansion to produce MSC populations for *in vitro* study could alter the fundamental properties of the cells.[40] As well, conflicting results from preclinical studies performed to date have prevented a clear consensus on the *in vivo* fate of allogeneic MSCs.

The early experiments demonstrated that allogeneic MSCs are immunoprivileged in noncardiac tissues such as uterus and skin, evidenced by long-term engraftment in nonimmunosuppressed recipients.[68,69] Later, the viability, retention (for up to 8 to 12 weeks), and differentiation of allogeneic MSCs implanted into the infarcted myocardium without immunosuppression were reported in several porcine[41,70,71] or murine[72] models. There has also been evidence of MSC immunoprivilege in xenographic transplantation experiments. For example, Chiu and colleagues found that mouse MSCs implanted into adult rats produced stable cardiac chimeras with no rejection for 12 weeks.[73] The mouse cells homed to the infarcted myocardium and differentiated into several cell phenotypes over a period of six weeks after coronary artery ligation, and significantly improved ventricular function.[74] The same group reported similar findings (engraftment for at least eight weeks) when human MSCs were implanted into the rat myocardium.[75]

Interestingly, while some *in vivo* studies suggest that allogeneic MSCs resist immune rejection from the heart, others have documented the opposite outcome. Specifically, MSCs implanted into allogeneic recipients have been shown to elicit cellular and humoral immune responses[76-78] that either lead to rejection of the implanted allogeneic cells[77] or accelerate the rejection of a donor allograft. For example, MSC injection did not prolong allograft survival in a rat model of heart transplantation, despite the fact that the MSCs inhibited stimulated T-cell proliferation in an MLR culture.[79] MSC priming of skin graft recipients increased the proliferation of CD4+ T cells and also failed to induce allograft tolerance in mice.[76] Similarly, delivering donor MSCs along with allogeneic bone marrow cells induced a memory T-cell response that increased rejection of the donor bone marrow in mice.[78]

When used for cell therapy in the infarcted heart, MSCs are transplantable across allogeneic and even xenographic barriers, and may survive for several months in the myocardial tissue.[72] But even though the implanted MSCs have been associated with transient improvements in global left ventricular

function,[72,80] results from a study in rats suggest that these functional effects do not persist beyond about eight weeks.[72]

As with noncardiac applications, MSCs implanted into the heart are subject to immune reactivity and subsequent rejection in multiple species. In a porcine model, Poncelet et al. documented donor-specific cellular and humoral reactivity (immunoglobulins M and G) with antibody-complement–mediated cytotoxicity in response to allogeneic MSCs delivered by intracardiac injection.[81] In sheep, ejection fraction improved in response to low doses of allogeneic MSCs implanted after an MI, but the cells were not detected at four or eight weeks after injection.[80] Xenographic MSCs were similarly rejected from the infarcted hearts of rats that received human cells.[82]

The variation in preclinical outcomes could, in part, reflect differences in experimental methodologies or in the characteristics of the MSC populations isolated or generated for implantation in the different experiments. But overall, limited engraftment of allogeneic MSCs and transient effects on cardiac function suggest that the *in vitro* immunosuppressive properties of MSCs may not confer lasting immunoprivilege in the infarcted heart.

CONCLUSIONS

Allogeneic cell therapy has the potential to improve functional outcomes and reduce the symptoms of heart failure after an MI; however, the broad application of this treatment will require a simple commercial product of highly regenerative, universal donor cells for the heart. With clinical trials evaluating the safety and feasibility of allogeneic MSCs as cardiac therapeutics already in progress, defining the nature and extent of MSC immunoprivilege is now of primary importance. At this point, the issue remains unresolved.

Preclinical and initial clinical findings document the functional benefits of cell therapy with allogeneic MSCs, and abundant *in vitro* data suggest that the distinctive immune character of MSCs can protect them against rejection from the heart—at least in the short term. Since most assessments of cell engraftment and cardiac function after allogeneic MSC therapy were restricted to the first 8 to 12 weeks after cell implantation, additional *in vivo* studies are needed to clarify whether the functional effects of implanted MSCs persist beyond this time frame. Meanwhile, a growing body of *in vitro* and *in vivo* work cautions that MSC immune character is changeable, raising the possibility that naïvely immunosuppressive MSCs could become immunogenic under the influence of myriad inflammatory cytokines present at the site of a myocardial infarct.[83,84] The effects of local factors on the immune character of implanted MSCs will certainly need to be defined, especially in light of the fact that cytokines such as interferon-γ have been shown to modulate MSC immune properties *in vitro*.[65,66]

If, in fact, allogeneic MSCs are eventually eliminated by the recipient immune system after myocardial implantation, strategies will be required to induce lasting immune tolerance, thereby prolonging the cells' engraftment in the infarcted heart and rendering them suitable for clinical use. Fortunately, compared with the immune reactions that follow allogeneic organ transplantation, those associated with allogeneic cell therapy may be simpler to isolate and modify because individual cells can be treated in culture prior to implantation. As the search for a universal donor cell continues, preclinical and clinical studies are attempting to resolve the extent to which allogeneic MSCs are immunoprivileged within the infarcted heart. The results of these investigations will yield information key to the design and implementation of the next generation of clinical cell therapy trials.

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