

Cellular Therapy in 2010: Focus on Autoimmune and Cardiac Diseases

Leor Perl MD¹, Adaya Weissler MD¹, Yoseph A. Mekori MD^{1,2,3} and Adam Mor MD^{1,2}

¹Department of Medicine B and ²Laboratory of Allergy and Clinical Immunology, Meir Medical Center, Kfar Saba, and ³Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

ABSTRACT: Stem cell therapy has developed extensively in recent years, leading to several new clinical fields. The use of mesenchymal stromal cells sparks special interest, as it reveals the importance of the paracrine and immunomodulatory effects of these supporting cells, in disease and in cure. This review discusses our current understanding of the basic clinical principles of stem cell therapy and demonstrates the broad range of this treatment modality by examining two relatively new therapeutic niches – autoimmune and cardiac diseases.

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Cellular therapy refers to the use of live cells to replace or repair a damaged organ system. This approach was first used more than 50 years ago when hematopoietic stem cells from the bone marrow of healthy donors (allogeneic transplant) were used to replace the hematopoietic system of a recipient with leukemia after it was ablated with chemotherapy [1]. This method was later extended to include autologous (self-donated) stem cells for various malignant conditions, allowing the use of more intense chemotherapy.

Today, cellular therapy is a mainstay in the treatment of oncologic and hematologic diseases. However, in this article we describe two new therapeutic areas: autoimmune and cardiac. We will concentrate on a new and exciting class of cells in the field of cellular therapy: mesenchymal stromal cells. These cells are known to have special tissue regeneration abilities. In contrast to other transplanted cells, recent literature supports the notion that MSC infusion induces both local and distant cytokine expression, thus explaining their beneficial effects [2]. We will discuss the possible mechanisms by which MSCs induce these effects, focusing on cardiac and autoimmune diseases.

STEM CELL CLASSIFICATION

Stem cells can be classified by their ability to divide and to produce differentiated cells. Totipotent cells have a total

differentiation capacity. They can divide and produce all the different types of cells in an organism, including extra-embryonic tissues. Examples of such cells are the zygotes, those cells formed during sexual reproduction. Totipotent cells can specialize into pluripotent cells that can give rise to most, but not all, of the tissues necessary for fetal development. Pluripotent cells have the potential to differentiate into the three germ layers. These cells undergo further specialization into multipotent cells that give rise to cells that have a particular function. HSCs are multipotent cells.

Stem cells can also be classified into adult stem cells and embryonic stem cells. A typical ASC is one which, on division, results in one daughter cell that can further differentiate and replenish a whole compartment, while the other cell remains fully self-renewing. The best example of this type is the HSC. ASCs are restricted in their cross-tissue differentiation potential. Nevertheless, recent research has demonstrated that pluripotent stem cells can be directly generated from adult fibroblast cultures [3]. ESCs originate earlier in the human development sequence and have different characteristics. In the embryo, once the blastocyst has formed with an inner cell mass and an outer membrane, the ESCs form the inner cell mass. These cells have tremendous plasticity in terms of *in vitro* tissue differentiation and are an unambiguous example of pluripotent cells [Figure 1]. They are able to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. However, the use of embryonic tissue in research and clinical fields has given rise to complex ethical and cultural questions, which are not applicable in adult-derived stem cell therapy [Table 1].

MSC = mesenchymal stromal cell
HSC = hematopoietic stem cell
ASC = adult stem cell
ESC = embryonic stem cell

Figure 1. Differentiation potential of stem cell lineages

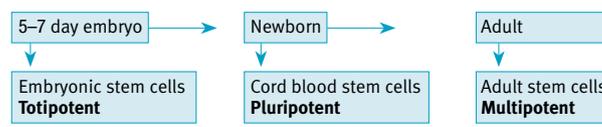


Table 1. Classification of stem cells

Cell type	Embryonic stem cells	Adult stem cells	
		Hematopoietic stem cells	Mesenchymal stem cells
Source and harvesting methods	Mouse grown on a layer of gelatin. Requires the presence of leukemia inhibitory factor Human-grown on a feeder layer of mouse embryonic fibroblasts. Requires the presence of basic fibroblast growth factor	Bone marrow or blood, following pretreatment with granulocyte colony-stimulating factor. Other sources: umbilical cord blood, placenta, fetal liver, and fetal spleen	Bone marrow monocytes are plated directly into cell culture plates or flasks
Implications	Research	Clinical use in leukemia and other cancers through bone marrow transplants	Experimental
Advantages	Unlimited expansion and pluripotency	Clinically proven	Void allorecognition
Concerns	Possible teratoma development. Embryonic stem cells may rapidly differentiate	Limited differentiation potential	Limited differentiation potential
Ethical concerns	The use of embryonic tissue	None	None

Finally, stem cells can also be classified based on their anatomic origin. MSCs, unlike HSCs, originate in the stromal component of the bone marrow and are multipotent cells. They lack typical hematopoietic cell surface markers, such as CD34 and CD45, and have the potential to differentiate into several types of tissue cells, such as skeletal muscle, adipose, bone, cartilage and even pancreatic cells [Figure 2] [4]. MSCs were first discovered in the 1960s and were regarded as colony-forming unit-fibroblasts. Later, they were shown to have the technically attractive ability

Mesenchymal stromal cells have immunomodulatory functions, thus making them important candidates for the treatment of autoimmune conditions

to be induced into a specific course of differentiation according to external stimuli. These cells have been tested to treat several conditions, including cardiac and autoimmune diseases. Unlike HSCs, they do not replace damaged organs. It is thought that they home into the damaged tissue, where they exert a local paracrine healing effect. An additional advantage of these cells is that pretreatment with chemotherapy is not required, thanks to the unique immunotolerance capabilities of the cells.

Classically, MSCs are derived from the bone marrow. However, there are other sources for harvesting these cells. This is important in the clinical context of future therapeutic modalities, considering the invasive nature of bone marrow aspiration. It is also possible to derive MSCs from peripheral blood and the umbilical cord. One promising method is based on harvesting these cells from the adipose tissue. A recent article compared MSCs derived from the adipose tissue to marrow-derived MSCs, and showed similar morphologic properties and *in vivo* behavior [5].

CELLULAR THERAPY FOR AUTOIMMUNE DISEASES

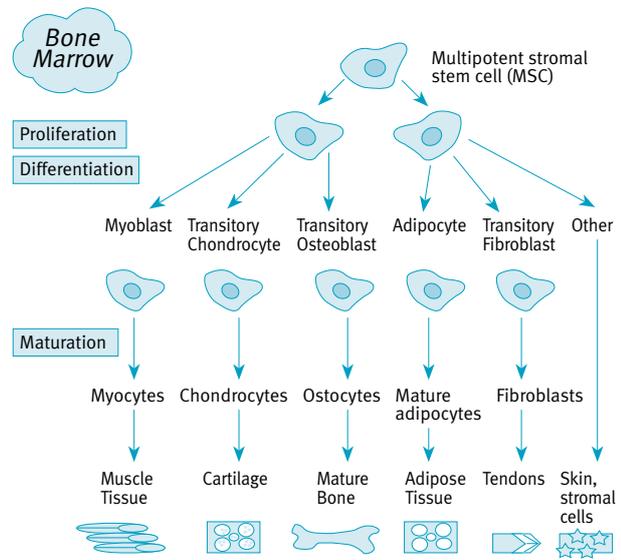
Over the last decade, more than 1000 patients with an autoimmune disease have been treated with different cellular therapies, leading to sustained remission in about 30% [6]. The first patients were treated with autologous HSC transplantation for

life-threatening autoimmune diseases refractory to conventional therapy. The principle of this treatment is termed HSC support. The goal is to allow the use of more intense immunosuppression for patients in whom bone marrow toxicity has restricted dose escalation. The aim is to decrease or even eradicate autoreactive immune cells through conditioning with highly active cytotoxic agents. Initially, it was thought that complete ablation of autoreactive cells would be required for durable remissions, but recent data suggest that a significant

debulking of these cells would allow normal immune regulation mechanisms to gain control of the system [7].

To obtain cells for autologous HSC transplantation, stem cells are mobilized from the bone marrow to the peripheral blood before conditioning, which permits collection. This is

Figure 2. Differentiation of stem cells



achieved by either administering granulocyte colony-stimulating factor or by cyclophosphamide rebound mobilization effect. The cellular marker used for collection is CD34. The term "conditioning" relates to the administration of an immunosuppressive regimen to create bone marrow insufficiency (leading to pancytopenia). The aim is to eliminate most of the B and T lymphocytes. Consequently, re-transfusing autologous HSCs reduces hematotoxicity, the main limiting step. By and large, allogeneic HSC transplantation is more complicated, as it is associated with graft-versus-host disease, which carries significant morbidity and mortality [8].

To date, about 900 patients have received autologous HSC transplantations and about 50 an allogeneic HSC transplantation [6]. These cases include autologous HSC transplantation in 250 multiple sclerosis patients, 170 systemic sclerosis, 80 systemic lupus erythematosus, and 100 rheumatoid arthritis patients. Most often, peripheral blood stem cells were used as the source. *In vitro* CD34-positive selection was performed in only one-third of the patients. Two studies showed that about half of all SLE patients achieved remission post-transplantation [9,10]. Around one-third of multiple sclerosis and systemic sclerosis patients went into remission. So far, there is no indication that toxicity occurs in patients with autoimmune disease. Experience with allogeneic HSC transplantation for autoimmune diseases is limited.

As mentioned earlier, MSCs are capable of differentiating *in vitro* and *in vivo* into different lineages, including adipose, bone, cartilage, muscle, and myelosupportive stroma [Figure 2]. MSCs can be collected from bone marrow, skeletal muscle, adipose tissue, synovial membranes, and other connective tissues, as well as from cord blood. *In vitro* MSCs have enormous auto-proliferative potential. Nevertheless, these cells exhibit antiproliferative and anti-inflammatory properties *in vitro* and *in vivo*, making them appealing candidates for regulating autoimmune processes. It is likely that the therapeutic potential is obtained by local paracrine production of humeral factors. A great advantage to this system is that MSCs may be infused without any preconditioning. The mechanisms underlying the immunosuppressive effect of MSCs remain to be fully clarified. *Ex vivo* expanded allogeneic MSCs have been infused in several phase I studies [6]. No adverse events have been observed and no ectopic tissue formation has been noted. After infusion, MSCs remain in circulation for no more than one hour. Most cases were patients with severe GvHD. There are several ongoing phase I clinical trials for autoimmune diseases, including multiple sclerosis, type I diabetes mellitus, systemic sclerosis and SLE [6].

SLE = systemic lupus erythematosus
GvHD = graft-vs-host disease

STEM CELLS AND CARDIAC REGENERATIVE THERAPY

The morbidity and mortality associated with myocardial diseases are mostly secondary to permanent loss of myocardial tissue, thus making cell therapy a potentially crucial treatment modality in the field of cardiac disease. Myocardial tissue had been considered incapable of regeneration. However, in 2001, Orlic et al. [11] showed that injections of bone marrow cells into infarcted mice hearts resulted in improved cardiac function. Two years later, Beltrami et al. [12] demonstrated that cardiac progenitor cells are capable of differentiation into cardiomyocytes, endothelial, and smooth muscle cells. This group also showed that injection of these cells into damaged hearts improved cardiac function through regeneration of the myocardium itself.

Until now, research has focused on the types of cells that are capable of either regenerating into viable cardiac tissue or of supporting cells that promote the process of regeneration. Supporting cells release cytokines that are involved in the cross-talk between different cells and their surroundings. Several types of cells are potential progenitors of regenerated cardiac tissue or may have the capability of supporting the regeneration process. These include mainly ESCs and MSCs, but other cell types such as resident cardiac progenitor cells, skeletal myoblasts, and endothelial progenitor cells are included as well.

In the last decade, the idea of MSC-based cardiac regeneration became part of mainstream cardiac research. Several studies have supported the possibility that MSCs have the potential to promote cardiac regeneration. MSCs are multipotent cells with limited capacity for differentiation and proliferation, and after several division cycles they show evidence of senescence [13]. Strikingly, it was suggested that these cells have the potential to become mature, beating cardiac myocytes [14,15]. When MSCs are exposed to the DNA demethylating agent (5-azacytidine), they express specific cardiac genes, adopt myotube morphology, produce intercalated disks, and have other functions associated with myocytes. Another *in vitro* method to facilitate MSC differentiation into myocytes involves the use of different growth factors [16]. MSCs have several potential advantages. They have excellent homing capabilities, necessary for implanting into injured heart tissue, and the benefit of being "immunotolerant," i.e., inducing a low host-immune response, therefore possibly surviving better in an allogeneic transplant setting. Another important advantage is their potential ability to promote angiogenesis, a process that is crucial for ischemic tissue.

The role of MSCs in clinical cardiac regenerative therapy is uncertain. While it remains unclear whether or not these cells can integrate into the electromechanical system of myo-

Mesenchymal stromal cells improve ventricular function after a myocardial infarction

cytes *in vivo* as do other cardiac progenitor cells, it is likely that they exert their beneficial effect through a paracrine cytoprotective influence. This most likely contributes to the processes of myocardial remodeling, reduction of infarct size, scar formation, vascular repair, angiogenesis, recruitment of other regenerative factors, and ultimately homing of stem cells, thus possibly facilitating myocyte regeneration.

How do MSCs reach the cardiac tissue and, consequently, induce these beneficial effects? It has been shown that stem cell mobilization to the heart occurs naturally. Steingen and co-authors [17] demonstrated that the process of transmigration of MSCs across the endothelial barrier and their subsequent invasion into their target tissue depends on the interaction of adhesion molecules such as VCAM-1 (vascular cell adhesion molecule-1) and VLA-4 (very late antigen-4) with endothelial cells. It was later shown that platelets are a key player in the proliferation, recruitment and integration of MSC into endothelial and cardiac tissue, a process mediated mostly by platelet-derived basic fibroblast growth factor [18]. The pathway involving SDF-1 (stromal cell-derived factor-1) was shown to account for the crucial process of stem cell homing to the injured myocardial tissue. Askari and collaborators [19] demonstrated in a rat model of ischemic cardiomyopathy that SDF-1 was up-regulated immediately after an infarction (and down-regulated 7 days later) and correlated with stem cell homing into the cardiac tissue. Therefore, it is possible to employ MSCs through direct transplantation or by chemically amplifying a naturally occurring response to cardiac injury.

Several mediators have been postulated as potential factors associated with the MSC paracrine effects on cardiac remodeling, cytoprotection, and angiogenesis after intramyocardial (direct injection of cells into infarcted myocardium), intracardiac (injection of cells into cardiac chambers), intravenous, or intracoronary cell transplantation. These include a number of cytokines and growth factors, such as HIF-1 α (hypoxia inducible factor-1 alpha), hepatocyte growth factor-1, mesenchymal SCF (stem cell factor), VEGF (vascular endothelial growth factor), insulin-like growth factor-1, tumor necrosis factor-alpha and others. Notably, hypoxia activates and stabilizes the transcription factor HIF-1 α , which is known to regulate genes related to pro-angiogenic and cytoprotective processes. MSCs have been shown to activate this pathway in a low oxygen environment, and to secrete VEGF, a pro-angiogenic factor, thus preventing apoptosis induced by oxidative stress and ultimately preserving native myocytes after myocardial infarction [20]. Another cardioprotective factor, HGF-1, which has potential angiogenic, anti-apoptotic, anti-fibrotic and anti-inflammatory benefits, was recently examined in a swine model. In this study, MSCs were transplanted

into hearts with and without the co-administration of HGF-1, stimulating angiogenesis and cardiomyocyte regeneration, and improving cardiac function [21].

A promising biological process involves the tyrosine kinase receptor *c-kit* and its ligand, SCF. This signaling pathway is crucial for cellular maintenance, habitual function, and activation. Moreover, most precursor cells that have been shown to improve cardiac function after direct myocardial injection express *c-kit*. A study recently published by Fazel et al. [22] has shown that while cardiac transplantation of MSCs does not induce cardiomyogenesis, it does mobilize progenitor cells, induces angiogenesis, and improves cardiac repair after a myocardial infarction. It was shown that a myocardial infarction up-regulates the expression of SCF and that transplantation of MSCs increases this expression.

A prominent feature of the beneficial effects of MSCs on the infarcted heart is the ability to influence the myocardial matrix. For example, in an animal model of doxorubicin-induced cardiomyopathy, it was shown that MSCs mediate extracellular matrix repair and reduce scar formation. This is accomplished by their effect on factors such as the ECM degrading enzymes matrix metalloproteinases and their tissue inhibitors. Induction of heart failure resulted in a unique profile of enzyme expression. While MMP1, 2 and 9 were over-expressed, the levels of TIMP-3 decreased. Injection of MSCs into the left ventricle resulted in higher MMP expression, and also in higher TIMP levels, resulting in changes

in the collagen content [23]. In a mouse model, transplantation of MSCs over-expressing TIMP-3 reduced the pathologic MMP induction and subsequent remodeling of the heart follow-

ing an infarction, favorably affecting matrix modulation and enabling functional recovery of the cardiac chambers [24].

Recent data have also implicated MSCs in cardiac immunomodulation. MSCs affect antigen-presenting cells, directing them towards a suppressor phenotype, ultimately resulting in an attenuated T cell response. Some of the factors mentioned above, such as HGF-1, are known to suppress lymphocyte proliferation and cytotoxic T cell activation. It has recently been reported that MSCs also inhibit natural killer and B cell proliferation and activation, thus becoming immunotolerant donors [25,26], but also possibly favorably affecting myocardial immunomodulation, contributing to an anti-inflammatory counter-response following injury.

Several clinical trials have already examined the possible applications of stem cells in cardiac regeneration therapy.

New techniques, better technologies in stem cell isolation, induction of trans-differentiation, and transplantation may improve clinical results

ECM = extracellular matrix
 MMP = matrix metalloproteinases
 TIMP – tissue inhibitors of metalloproteinases
 HGF-1 = hepatocyte growth factor-1

Most included bone marrow-derived, heterogeneous mononuclear cells. In a randomized, double-blind, placebo-controlled, multicenter trial (the REPAIR-AMI), autologous mononuclear progenitor cells derived from the bone marrow were intracoronarily reinfused early after myocardial infarction and successful reperfusion therapy. The treatment improved global left ventricular function and prevented ventricular end-systolic expansion when assessed 4 months after the event [27]. In yet another trial, intramyocardial injection of autologous bone marrow-derived mononuclear cells in patients with chronic ischemia resistant to medications resulted in an improvement in myocardial perfusion compared to placebo [28].

Regarding experience with isolated MSCs, Hare et al. [29] intravenously infused 60 patients with adult human MSCs following acute myocardial infarctions, in a double-blind, randomized, controlled trial presented at the 2007 American College of Cardiology Annual Scientific Session. The treated patients enjoyed improved global status, longer average time to rehospitalization, and fewer cardiac arrhythmias, such as premature ventricular arrhythmias. In another randomized study, Chen and co-researchers [30] infused mesenchymal stem cells intracoronarily 18 days after a myocardial infarction. There was a significant improvement in ejection fraction in the cell-treated group associated with improved cardiac function, as assessed by echocardiography and nuclear imaging modalities. However, these studies are unique and preliminary and have yet to be repeated in large-scale trials and examined for clinical endpoints. One such study, PROMETHEUS (Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery), will examine the safety and efficacy of intramyocardial administration of autologous MSCs in patients with chronic left ventricular dysfunction secondary to myocardial infarction who are undergoing coronary bypass surgery. Additional trials evaluating the benefit of MSC infusion, either directly into the heart or intravenously, in a variety of cardiac diseases are anticipated. Side effects of MSC cardiac cell therapy are also an issue that is as yet unstudied. Possible adverse effects, reported in several trials of MSC therapy, include the creation of encapsulated intramyocardial ossifications and calcifications [31], the appearance of arrhythmias [32], sarcomas [33], and teratomas [34].

CONCLUSIONS

MSC transplantation has therapeutic potential in both the autoimmune and cardiac fields. Since these cells have immunomodulatory functions, they are important candidates for the treatment of autoimmune conditions. MSCs improve ventricular function after a myocardial infarction, primarily due to paracrine cytokine release that induces angiogenesis,

hypoxia-induced cardioprotection, recruitment of marrow progenitor cells, and a favorable matrix modulation. MSCs have already shown a therapeutic effect in several disorders in humans, including articular cartilage defects [35], osteogenesis imperfecta [36], avascular necrosis [37] and GvHD [38]. Additional clinical trials, and *in vivo* studies in particular, are warranted. New techniques, better technologies in stem cell isolation, induction of trans-differentiation, and transplantation may improve clinical results. The vast range of medical challenges studied throughout the world, now utilizing different aspects of MSC regeneration therapy, will certainly shed light on this fascinating field of cellular therapy.

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Correspondence:

Dr. A. Mor

Laboratory of Allergy and Clinical Immunology, Meir Medical Center, Kfar Saba 44281, Israel

Phone: (972-9) 747-2198

Fax: (972-3) 747-1311

email: Adam.Mor@clalit.org.il

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