Adult Bone Marrow–Derived Stem Cells for Organ Regeneration and Repair

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Stem cells have been recognized as a potential tool for the development of innovative therapeutic strategies. There are in general two types of stem cells, embryonic and adult stem cells. While embryonic stem cell therapy has been riddled with problems of allogeneic rejection and ethical concerns, adult stem cells have long been used in the treatment of hematological malignancies. With the recognition of additional, potentially therapeutic characteristics, bone marrow–derived stem cells have become a tool in regenerative medicine. The bone marrow is an ideal source of stem cells because it is easily accessible and harbors two types of stem cells. Hematopoietic stem cells give rise to all blood cell types and have been shown to exhibit plasticity, while multipotent marrow stromal cells are the source of osteocytes, chondrocytes, and fat cells and have been shown to support and generate a large number of different cell types. This review describes the general characteristics of these stem cell populations and their current and potential future applications in regenerative medicine. *Developmental Dynamics 236:3321–3331, 2007.* © 2007 Wiley-Liss, Inc.

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INTRODUCTION

Stem cells have always been fascinating for cell biologists due to their undifferentiated state that can give rise to a highly specialized cell type or organism and their seemingly endless self-renewal potential. Only recently have these cells become of wide public interest, triggered by a number of landmark observations in the late 1990s, namely the discovery of the extensive plasticity of adult stem cells and the successful in vitro culture of human embryonic stem cells (Thomson et al., 1998; Raff, 2003). The therapeutic use of embryonic stem cells (ESCs) is still debated in the public due to ethical concerns, but their application in human therapy is also controversial because of immunological incompatibilities and concerns about uncontrolled development of malignancies or teratomas from administered cells (Hentze et al., 2007). In contrast, adult stem cells are free of such ethical concerns, and they can be used in the autologous setting, thereby avoiding rejection. Furthermore, allogeneic stem cells have already been extensively used in human bone marrow transplantation for the treatment of otherwise deadly diseases (Thomas, 1999). Recognition of these advantages of adult stem cells have translated into the conduct of a large number of clinical trials with bone marrow–derived cells (BMDCs) for organ repair and regeneration, mainly in the treatment of cardiac diseases. Together, the promising results with stem cell therapy have led to the development of a new discipline in medicine, regenerative medicine (van Laake et al., 2006). The scope of this review is to give an overview of the biology of BMDCs and to discuss completed and ongoing clinical trials in which organ repair is enhanced with these cells.

ADULT STEM CELLS

A stem cell is defined as an undifferentiated cell that is capable of asymmetrical cell division, i.e., gives rise to one differentiated cell type while re-

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maining in the tissue of origin as a stem cell, thereby maintaining the renewal capacity of the tissue. Differentiated cells are produced in general through different stages beginning with progenitor cells or transit amplifying cells. Their proliferative activity is much higher than that of stem cells. The stem cell retains its state either through asymmetric cell division, giving rise to one stem cell and one progenitor cell, or by reversal of a progenitor cell back to a stem cell after symmetric division (Raff, 2003; Morrison and Kimble, 2006). While bone marrow, intestine, and lung have well-known stem cell populations, other organs previously thought to be "post-mitotic" and unable to regenerate have also been shown to contain stem cells. The brain (Gage, 2000) and heart (Beltrami et al., 2003) are now recognized to contain defined stem cell populations, while this is possibly true for the kidney (Oliver et al., 2004).

Adult stem cells are vital for continuously renewing tissues such as the bone marrow and intestine, and play an important role for recovery from injury in tissues like the liver (Fausto, 2004). Many diseases, including cancer, have been recognized as being the result of stem cell "defect," a fact that has major implications for their treatment (Reya et al., 2001).

Bone marrow is an ideal tissue for studying stem cells because of its accessibility and because doses and proliferative responses of bone marrow–derived stem cells can be readily investigated. Furthermore, there are a number of well-defined mouse models and cell surface markers that allow effective study of hematopoiesis in healthy and injured mice. Because of these characteristics and the experience of bone marrow transplantation in the treatment of hematological cancers, bone marrow–derived stem cells have also become a major tool in regenerative medicine. The bone marrow harbors two distinct stem cell populations: hematopoietic stem cells (HSC) and multipotent marrow stromal cells (MSC).

HEMATOPOIETIC STEM CELLS

The high regenerative potential of blood cells even after severe losses and the continuous renewal and turnover of lymphocytes are powerful indicators of the regenerative potential of hematopoietic stem cells (HSCs). The German pathologist Julius Cohnheim in 1867 was one of the first to realize that the bone marrow gives rise to circulating cells, including fibroblasts engaged in inflammatory wound healing processes. However, the clonogenicity of blood cell lineages and the concept of stem cell theory were only proven in 1961 (Becker et al., 1963; Siminovitch et al., 1963). HSCs are undifferentiated cells capable of self-renewal and stepwise differentiation into fully specialized cells of the blood, e.g., erythrocytes, thrombocytes, and leukocytes. Although much insight has been gained into the identity of these rare bone marrow cells, their full identity is still debated and there is not one single marker to truly identify this cell (Orlic and Bodine, 1994). Several laboratories have identified cell populations that are highly enriched in HSCs, which requires the demonstration of specific positive markers and the absence of differentiation or lineage markers. Currently, most commonly used are c-kit and sca-1-positivity and lineage negativity or SLAM family markers (Spangrude et al., 1988; Kiel et al., 2005). A definite proof for hematopoietic stem cell activity of a single cell, however, can only be obtained by successful treatment of a lethally irradiated mouse with such a cell. HSCs are the best understood stem cells in the body, mainly due to their easy accessibility, the availability of mouse models including a wide array of surface marker antibodies, and their wide use in clinical applications. However, in the late 1990s, several laboratories discovered surprising and previously unknown properties of HSCs that questioned long-held dogmas regarding the irreversibility of differentiation and lineage commitment (Raff, 2003). It was shown that BMDCs not only commit to their natural lineage, but are also able to differentiate into muscle and liver cells (Ferrari et al., 1998; Gussoni et al., 1999; Petersen et al., 1999). Since these experiments utilized mainly whole bone marrow populations, the cell responsible for these unexpected results could not be identified until it was shown that highly purified HSCs differentiate into parenchymal cells of most tissues after transplantation (Krause et al., 2001). These results are not unequivocally accepted and might be due to a mechanism different from transdifferentiation or plasticity. Alternative explanations for their proposed plasticity included fusion (Terada et al., 2002; Ying et al., 2002), methodological problems (Raff, 2003), restriction to a model system/disease state, and presence of embryonic stem cell–like cells in the injected cell population. Plasticity is not a frequent phenomenon and does not usually occur under steady-state conditions (Wagers et al., 2002; Wagers and Weissman, 2004), however, it can be very powerful in certain model systems, and even if fusion is the primary mechanism, exploitation of stem cell plasticity might yet prove to be a useful therapeutic tool for otherwise incurable diseases.

The concept of HSC plasticity was the main reason for the enthusiasm in the scientific community regarding future treatment strategies in regenerative medicine. It was based on the assumption that transplantation of stem cells capable of parenchymal differentiation are able to replace dead cells in damaged tissues, thereby repairing a critically injured organ. Although this repair process might be true in certain model systems, even if accomplished by cell fusion (Masson et al., 2004), it is a very rare and slow process, thereby rendering it probably not effective in injuries with rapid pathophysiological kinetics such as acute myocardial injury (Balsam et al., 2004). However, besides differentiation, there are other potential mechanisms of action of HSCs in injured tissues. Among them are secretion of various chemokines and cytokines, thereby stimulating regeneration by inhibiting apoptosis, suppressing immune reactions and increasing angiogenesis, enhancement of proliferation of tissue endogenous stem/progenitor cells, and rescue by mitochondrial transfer or cell fusion (Prockop and Olson, 2006; Spees et al., 2006).

TABLE 1. The Major Currently Published Studies Utilizing Stem Cell Mobilization for Tissue Regeneration[a]

Organ and disease	Protocol	Patient number study type	Results	Reference
Heart, STEMI	10 □g/kg G-CSF or Placebo	114, randomized, double blind	No change in infarct size LVEF or restenosis rate	(Zohlnhofer et al., 2006)
Heart, STEMI	10 □g/kg G-CSF or Placebo	78, prospective, randomized, double blind	No change in systolic wall thickness, LVEF	(Ripa et al., 2006)
Heart, STEMI (FIRSTLINE-AMI)	10 □g/kg G-CSF	50, randomized after reperfusion therapy	Better diastolic wall thickness, significant increase in LVEF	(Ince et al., 2005)
Heart, STEMI	5 □g/kg G-CSF (4 days)	20, randomized	Perfusion defect size same, LVEF increase not significant	(Valgimigli et al., 2005)
Heart, STEMI	G-CSF 10 □g/kg/Tag (5 Tage)	44, randomized, double blind, placebo controlled	No significant increase in LVEF after 3 months	(Engelmann et al., 2006)
Heart, coronary artery disease	40 □g GM-CSF intracoronary, 10 □g/kg sc	21, randomized, double blind, placebo	Increase in coronary collateral flow	(Seiler et al., 2001)
Peripheral arterial disease	10 □g/kg GM-CSF every 2 days/2 weeks	40, randomized, placebo	No difference in walking distance until the start of symptoms	(van Royen et al., 2005)
Brain, acute cerebral stroke	15 □g/kg (5 Tage)	10, randomized, blinded	Improvement of the NIHSS at 6 months after treatment	(Shyu et al., 2006)

[a]STEMI, ST elevation myocardial infarction; G-CSF, granulocyte colony stimulating factor; LVEF, left ventricular ejection fraction; sc, subcutaneously.

MULTIPOTENT MESENCHYMAL STROMAL CELLS

The second stem cell population in the bone marrow was discovered and characterized by the groundbreaking work of Friedenstein, who placed whole bone marrow into tissue culture flasks, removed the non-adherent cell population after some time, and characterized adherent colony-forming fibroblast-like cells (Friedenstein et al., 1970, 1974a,b). These rapidly growing cells could be stimulated, by changing growth conditions or transplantation into animals, to differentiate into osteoblasts, chondrocytes, and adipocytes. This work has been corroborated by many groups, and these cells have been referred to by many different names, e.g., colony-forming unit fibroblasts (CFU-F), mesenchymal stem cells, or marrow stromal cells. Due to their multipotency, the current consensus term is "multipotent mesenchymal stromal cells" (Dominici et al., 2006). These cells are defined by their plastic adherence, surface marker expression of CD73, CD90, CD105, absence of CD34, CD45, HLA-DR, and differentiation into adipocytes, osteocytes, and chondrocytes under specific culture conditions. While their capability to give rise to mesenchymal tissues is inherent, many groups have described unexpected differentiation into neural cells (Cho et al., 2005), cardiomyocytes (Pittenger and Martin, 2004), and pneumocytes (Rojas et al., 2005). Although some of these results were obtained in vitro and thereby subject to methodological criticism (Y. Chen et al., 2006), MSCs have been shown to contribute to all organs after systemic infusion, albeit to a varying degree (Devine et al., 2003; Anjos-Afonso et al., 2004). MSCs are not only found in the bone marrow but in nearly every organ (da Silva Meirelles et al., 2006), but little is known about their normal function and capacities. MSCs are easy to expand in vitro and can be genetically altered by viral vectors, which makes them an ideal and safe long-term vehicle for cellular gene therapy (Caplan, 2000).

MSCs are ideal vehicles for cell therapeutic approaches because they are easy to generate, maintain, and expand in culture and because they can be potentially applied, due to their immune-privileged properties, in an allogeneic setting. Furthermore, besides giving rise to a number of cell types, which makes them ideal for tissue engineering to generate mesenchymal structures like bone and cartilage, their unique immunomodulatory

TABLE 2. Clinical Studies Evaluating BMDCs for Organ Regeneration[a]

Organ and disease	Patient number, clinical design, injection route	Cell characteristics	Results	Reference
	a: Smaller and uncontrolled studies utilizing BMDCs			
Transmural MI after PTCA	18, compared to representative control group	Autologous mononuclear bone marrow cells	Functional and metabolic regeneration	(Strauer et al., 2005)
MI	35	CD133 selected cells	Coronary complications, LV performance improvement	(Bartunek et al., 2005)
Chronic myocardial ischemia MI	26, randomized double blind; placebo controlled	Circulating progenitor cells	Improvement of vascular function	(Erbs et al., 2005)
	27 randomized controlled Phase II study (MAGIC)	Infusion of G-CSF mobilized PBMC (n □ 10), Mobilisation with G-CSF (n □ 10)	Improved ventriculat function, increased angiogenesis, higher restenosis rate	(Kang et al. 2004)
MI	20 patients, 13 controls	Autologous unfractioned bone marrow cells, 78 □ 41 □ 10^6	Improved regional and global LV performance	(Fernandez-Aviles et al., 2004)
MI	6 patients	CD133 selected-cells, 1.5 □ 10^6	Global LV improvement increased perfusion	(Stamm et al., 2003)
Severe ischemic heart disease	8 patients	Autologous MNC	Improvement of symptoms and perfusion	(Tse et al., 2003)
Ischemic cardiomyopathy	14 patients 9 controls	Autologous MNC	Improved treadmill performance	(Perin et al., 2004)
Ischemic cardiomyopathy	27, "no-option" patients	Unfractioned autologous BM	No side effects	(Fuchs et al., 2006)
MI	59 (TOPCARE-AMI)	Circulating progenitor cells (CPC), autologous MNC	Positive effects on LV remodeling	(Assmus et al., 2002; Schachinger et al., 2004)
Peripheral vascular disease	47; 25 with unilateral, 22 with bilateral ischemia of the legs	BM MNC, PB MNC as control	Improvement of pain and oxygen tension	(Tatcishi-Yuyama et al., 2002)

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properties make them promising candidates for the treatment for a large number of inflammatory and immune system–mediated diseases (X. Chen et al., 2006).

ORGAN REGENERATION AND REPAIR

There are remarkable examples of tissue regeneration in the animal kingdom and they are valuable tools in the study of organ regeneration (Holstein et al., 2003; Poss et al., 2003). For unknown reasons, humans have lost this dramatic ability to regenerate tissues. Nevertheless, some organs still exhibit remarkable autoregenerative capacity after severe injury. Perhaps best recognized is the regrowth of liver parenchyma, a fact that must have been known in ancient times, giving rise to the Prometheus myth (Chen and Chen, 1994).

The regeneration of a limb in lower vertebrates, e.g., an amputated amphibian limb, involves several stages, starting with wound healing, continuing through demolition, phagocytosis, and dedifferentiation to a blastema formation, which represents the base for morphogenesis and regrowth of the regenerated limb (Carlson, 2005).

Blastema formation is essential for the whole organ regeneration process, a phenomenon that does not occur in humans. However, injury to an organ leads to a process of cellular dedifferentiation with the expression of embryonic transcription factors that induce a developmental program. This is exemplified by the ischemically injured kidney, where highly specialized polarized epithelial cells dedifferentiate and express Pax-2, an important embryonic transcription factor (Imgrund et al., 1999). On the other hand, the continued expression or misexpression of embryonic Pax-2 is also a sign of renal disease and might lead to a relentless loss of renal functions (Dressler and Woolf, 1999). The reason for these differences in programs leading to organ regeneration is unknown and there is speculation that humans have lost this capacity during evolution (Tanaka, 2003).

Although regeneration is closely linked to stem cells, they are not necessarily a prerequisite for regenera-

TABLE 2. (Continued)

Organ and disease	Patient number, clinical design, injection route	Cell characteristics	Results	Reference
	b: Large randomized trials utilizing BMDCs			
Acute MI	33 cell infusion, 34 placebo, randomized, double blind placebo controlled	Autologous mononuclear BM cells after Ficoll-separation, 3 □ 10^8	No difference in global LV function	(Janssens et al., 2006)
MI (STEMI)	47 cell infusion, 50 no infusion (ASTAMI)	Autologous mononuclear BM cells after Ficoll-separation, 7 □ 10^7 Zellen	No difference compared to control group	(Lunde et al., 2005)
MI	60 patients, randomized (BOOST)	Autologous unfractioned BM cells, 2.5 □ 10^9	After 6 months: LVEF 6% higher in treated compared to controls: No difference after 18 months	(Wollert et al., 2004)
MI	101 cell infusions, 98 Placebo infusions, double blind, placebo, multicenter (REPAIR-AMI)	MNC fraction after BM-Aspiration and Ficoll separation, 2.4 □ 10^8	LVEF improvement compared to placebo (5.5% vs. 3%) after 4 months. Endpoints after 1 year significantly improved	(Schachinger et al., 2006b)
Chronic left ventricular dysfunction	24 circulating progenitor cells (CPC), 28 BM cells, 23 no infusion, randomized, crossover design (TOPCARE-CHD)	MNC fraction after BM-aspiration and Ficoll separation, 7 □ 10^7, or peripheral blood cells	Highest increase of LVEF (2.9%) in BM group compared to CPC or control	(Assmus et al., 2006)

[a]LVEF, left ventricular ejection fraction; BMDCs, bone marrow derived cells; MNCs, mononuclear cells; PB, peripheral blood.

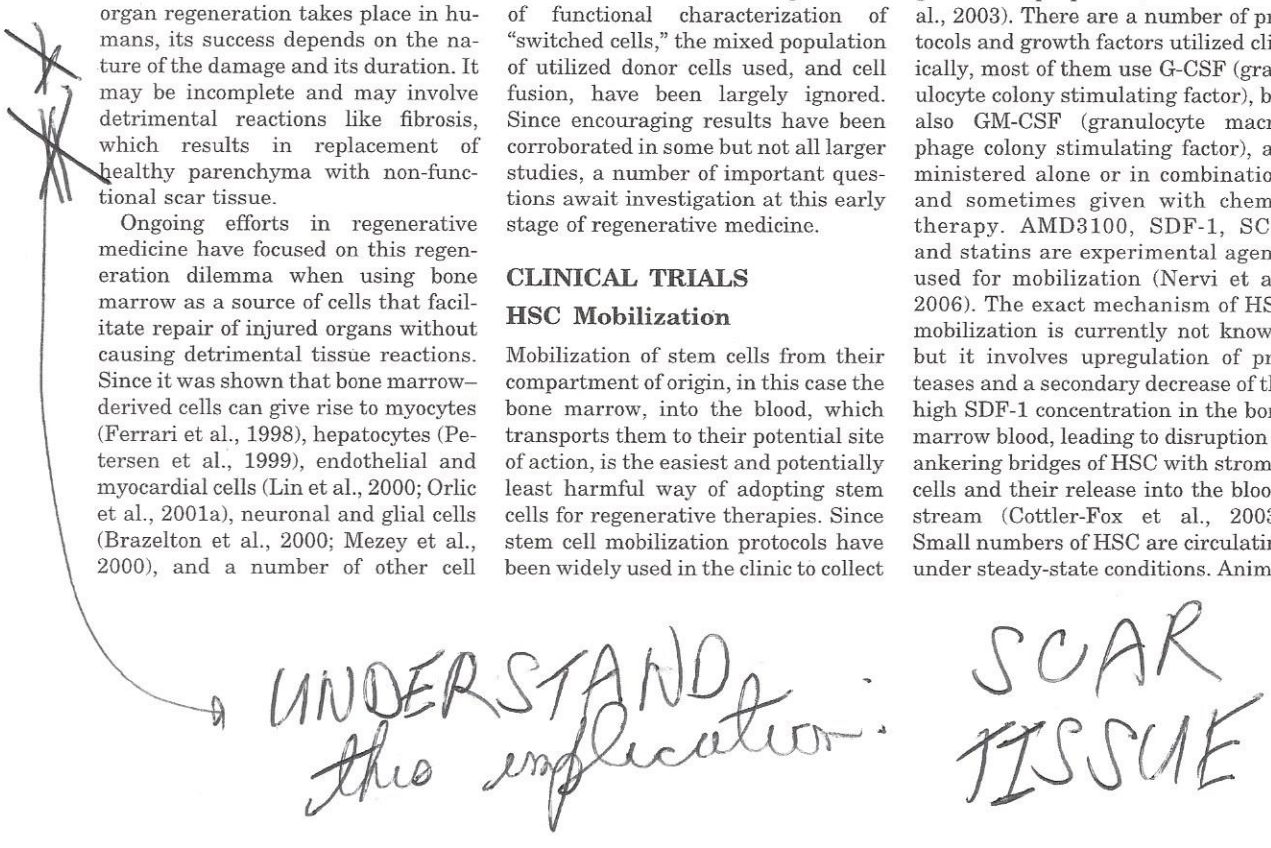
tion, a fact that is illustrated by the liver, where parenchymal cells mainly contribute to organ regeneration and only in severe injury is a stem cell–like cell, the oval cell, recruited. While organ regeneration takes place in humans, its success depends on the nature of the damage and its duration. It may be incomplete and may involve detrimental reactions like fibrosis, which results in replacement of healthy parenchyma with non-functional scar tissue.

Ongoing efforts in regenerative medicine have focused on this regeneration dilemma when using bone marrow as a source of cells that facilitate repair of injured organs without causing detrimental tissue reactions. Since it was shown that bone marrow–derived cells can give rise to myocytes (Ferrari et al., 1998), hepatocytes (Petersen et al., 1999), endothelial and myocardial cells (Lin et al., 2000; Orlic et al., 2001a), neuronal and glial cells (Brazelton et al., 2000; Mezey et al., 2000), and a number of other cell types (Krause et al., 2001), bone marrow–derived cells arte currently the preferred cell type used in regenerative medicine. A number of problems with these studies, including the lack of functional characterization of "switched cells," the mixed population of utilized donor cells used, and cell fusion, have been largely ignored. Since encouraging results have been corroborated in some but not all larger studies, a number of important questions await investigation at this early stage of regenerative medicine.

CLINICAL TRIALS

HSC Mobilization

Mobilization of stem cells from their compartment of origin, in this case the bone marrow, into the blood, which transports them to their potential site of action, is the easiest and potentially least harmful way of adopting stem cells for regenerative therapies. Since stem cell mobilization protocols have been widely used in the clinic to collect stem cells for autologous or allogeneic stem cell transplantation, clinicians have much experience with these regimens when they are adopted for regenerative purposes (Cottler-Fox et al., 2003). There are a number of protocols and growth factors utilized clinically, most of them use G-CSF (granulocyte colony stimulating factor), but also GM-CSF (granulocyte macrophage colony stimulating factor), administered alone or in combination, and sometimes given with chemotherapy. AMD3100, SDF-1, SCF, and statins are experimental agents used for mobilization (Nervi et al., 2006). The exact mechanism of HSC mobilization is currently not known, but it involves upregulation of proteases and a secondary decrease of the high SDF-1 concentration in the bone marrow blood, leading to disruption of ankering bridges of HSC with stromal cells and their release into the bloodstream (Cottler-Fox et al., 2003). Small numbers of HSC are circulating under steady-state conditions. Animal



models have shown that stem cell mobilization is effective in organ protection, e.g., of the heart (Orlic et al., 2001b). However, since most regimens not only increase the circulating stem cell pool, hematopoietic growth factors like G-CSF and GM-CSF also induce a marked peripheral granulocytosis. This can lead to a harmful situation, as we showed in experimental acute renal failure, where the mobilization-associated granulocytosis greatly worsened the outcome (Tögel et al., 2004).

The easy availability of mobilization regimens and the existing clinical experience led to early adoption of this treatment approach for clinical purposes. Small trials, mainly in myocardial infarction patients, showed the feasibility and safety of the mobilization approach and paved the way for larger randomized controlled studies (Table 1). The FIRSTLINE-AMI trial documented a significant increase in left ventricular ejection fraction in patients treated with G-CSF (Ince et al., 2005), patients with coronary artery disease showed increased collateral flow with GM-CSF treatment (Seiler et al., 2001), and there was an improvement of the NIHSS 6 months after treatment of stroke patients. However, these promising results were not corroborated by other investigators and the largest currently published trial showed no change in infarct size, LVEF, or restenosis rate after G-CSF treatment (Zohlnhofer et al., 2007).

Despite these sobering results, the end of the road for stem cell mobilization has not been reached, and a number of unanswered questions remain: which disease can be treated with mobilization? What is the ideal mobilization regimen? What is the best time point to start such treatment?

HSC Injection

The rationale for therapeutic HSC administration is similar to that for stem cell mobilization. Based on experimental animal studies, showing differentiation into myocytes, vascular cells, and many other cell types, different preparations of bone marrow cells containing different doses of HSC were used for clinical trials after myocardial infarction. Most of the studies (Table 2) used autologous mononuclear cell (MNC) preparations; only some selected the cells based on a surface marker, CD133 (Stamm et al., 2007). MNC preparations contain mainly different stages of progenitor cells and true HSCs and MSCs are rare in these autografts. Smaller studies established the safety of this approach and demonstrated functional and metabolic regeneration of myocardial tissue (Strauer et al., 2005), improvement of vascular function (Erbs et al., 2005), global left ventricular performance (Stamm et al., 2003; Fernandez-Aviles et al., 2004), and peripheral vascular disease (Tateishi-Yuyama et al., 2002). Based on these promising results, larger randomized multicenter trials were conducted. Two trials did not show an improvement of cardiac function in patients treated with autologous bone marrow (Lunde et al., 2005; Janssens et al., 2006), and the BOOST trial documented an improvement in LVEF after 6 months but no difference after 18 months. In contrast, the REPAIR-AMI trial showed a significant improvement in the treatment group after 4 months and all endpoints were still significantly better one year after treatment (Schachinger et al., 2006a). Perhaps the most significant finding in this trial was the fact that patients with the worst ejection fraction before treatment benefited the most, and that a longer time span between infarction and treatment resulted in a better outcome. These results provide valuable clues for the design of future of trials. The TOPCARE trial compared bone marrow–derived MNCs with peripheral blood cells and concluded that a treatment effect was exclusively reached in the MNC-treated group (Assmus et al., 2006).

MSC

Initial attempts at using MSCs in the clinical setting have been made as an adjunct to stem cell transplants, either to improve engraftment in autologous stem cell transplantation or in hematological malignancies treated with HLA-matched BMT from siblings (Table 3) (Koc et al., 2000; Lazarus et al., 2005). The safety of autologous and allogeneic MSC products has been established in these trials and no toxicity was documented. Due to the ability of MSCs to differentiate into osteocytes, they have been used as adjunct therapy to marrow transplantation in osteogenesis imperfecta (Horwitz et al., 1999, 2002). The rationale was to introduce cells with a healthy gene that produce physiologic bone matrix. The fracture rate of treated children improved and there was only one child without long-term engraftment of MSCs, which was attributed to rejection of transplanted cells. Because of the robust immunoregulatory properties of MSCs, these were given to patients with graft versus host disease (GvHD) after allogeneic bone marrow transplantation. Initial treatment of grade IV GvHD with MSC, normally invariably fatal, was successful and the effectiveness was corroborated in a subsequent series of patients (Le Blanc et al., 2004, 2005). While it is difficult to prove a clinical improvement in patients with progressive degenerative diseases like metachromatic leukodystrophy, Hurler syndrome, and amyotrophic lateral sclerosis (ALS) (Koc et al., 2002; Mazzini et al., 2006), MSC-based therapies are currently being tested in heart diseases (S. Chen et al., 2006), morbus Crohn, connective tissue degeneration, and stroke (Bang et al., 2005).

MSCs are ideal for stem cell–based therapy because of their off-the-shelf availability, a feature that cannot be underestimated due to the acute nature of many diseases requiring prompt treatment. Obviously, such immediate availability is not given with autologous cells, since their preparation is time consuming and since the obtained cell product might be damaged by the underlying disease itself. Of note, MSCs are currently the most advanced cell therapy tool because of the availability of three FDA approved products, Prochymal™, Provacel™, and Chondrogen™.

POTENTIAL MECHANISMS OF ACTION

The original rationale for stem cell therapy, based on a large number of pre-clinical studies, was to effect regeneration by integration of administered and differentiated cells into the organ with subsequent functional restitution. However, the finding of cell

TABLE 3. Clinical Studies Using MSC for Organ Regeneration, Tissue Repair, and Engraftment Support of Hematopoietic Stem Cells

Organ or disease	Cell number and application route	Results	Reference
Acute graft versus host disease (GvHD)	2 □ 10^6/kg allogen (third party), intravenous	Complete remission of GvHD after MSC infusion	(Le Blanc et al., 2004)
GvHD	Median 1 □ 10^6/kg (range 0.4–9), intravenous	6 complete remission, 4 improvement	(Le Blanc et al., 2005)
Osteogenesis imperfecta (OI)	Allogen (matched donor), intravenous	Improved fracture rate and growth	(Horwitz et al., 1999)
Hematological diseases treated with HSCT	1, 10, and 50 □ 10^6 total, autologous, intravenous	No side effects	(Lazarus et al., 1995)
Breast cancer	1–2.2 □ 10^6 autologous expanded MSC, intravenous	No toxicity	(Koc et al., 2000)
Osteogenesis imperfecta	1 □ 10^6 and 5 □ 10^6/kg (first/second dose), intravenous	No toxicity, durable engraftment in 5 of 6 patients, acceleration of growth, decreased fracture rate compared to untreated controls	(Horwitz et al., 2002)
Hematological diseases treated with HSC transplantation	1–5 □ 10^6/kg, intravenous	No toxicity	(Lazarus et al., 2005)
Metachromatic leukodystrophy (MLD) and Hurler syndrome	2–10 □ 10^6/kg, HLA identical donor, intravenous	No toxicity, low engraftment, no measurable clinical improvement	(Koc et al., 2002)
Acute MI	8 □ 10^9 cells/ml intracoronary, autologous MSC	LVEF increase in MSC group, improvement of perfusion defect	(Chen et al., 2004)
Amyotrophic lateral sclerosis (ALS)	Autologous MSC, intraspinal	No toxicity	(Mazzini et al., 2006)
Acute MI	Autologous MSC, Intracoronary	Improved contractility	(Katritsis et al., 2007)
Paraplegia	Autologous MSC, differenciated into neurons in culture	No toxicity	(Moviglia et al., 2006)
Acute stroke	Autologous MSC, 1 □ 10^8 cells intravenous	No toxicity	(Bang et al., 2005)

[a]MI, myocardial infarction.

fusion (Terada et al., 2002), and the fact that only a small number of transdifferentiated cells (□ 0.1% of all tested cell types) could be detected in the injured organ post transplantation, made it unlikely that cell engraftment is the main therapeutic principle. Fusion can be interpreted as a form of gene delivery into cells with a genetic defect, as illustrated in a model of liver damage caused by a defect in the fumarylacetoacetate hydrolase enzyme. In this model, cell fusion between administered MSC and hepatocytes was the principal therapeutic mechanism, resulting in cure of the disease, animal survival, and replacement of up to half of the total liver cells (Wang et al., 2003). However, cell fusion failed to result in the expression of the desired sarcoglycan gene in a model of myopathy (Lapidos et al., 2004). While mechanisms of action cannot be actively investigated in humans, animal models have provided a large number of other explanations for the observed treatment effects. These are illustrated in Figure 1. BMDSCs secrete a number of cytokines, chemokines, and growth factors, which are potentially disease modifying. Subsequent actions of these factors include stimulation of angiogenesis, suppression of inflammation, inhibition of apoptosis, and enhancement of endogenous repair by stimulation of intrinsic stem and progenitor cell proliferation (Rabb, 2005; Chien, 2006; Tögel et al., 2007). Currently, there is very limited

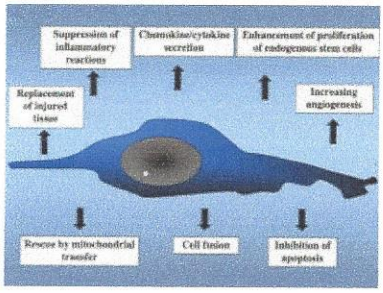


Fig. 1. Mechanisms of action of stem cells in organ regeneration.

knowledge about the in situ secretion patterns of soluble factors at the sites of action, which is an important point to consider, because all expression profiles of factors have been determined in vitro, and because it is well known that cells and their respective gene expression profiles are influenced by local factors.

SIDE EFFECTS AND DANGERS

Major concerns about cell therapy in general are the potential consequences associated with a treatment that results in the long-term or permanent presence of foreign cells in the recipient, i.e., cells that can not be retrieved. Administered cells remain in the body and although many studies have shown only limited or transient engraftment (Tögel et al., 2005), it cannot be excluded that there is long-term engraftment. While pharmacological treatments can be stopped and potential adverse effects are thereby limited, this is obviously not the case for cell therapy. Therefore, this form of treatment demands extraordinary safety precautions. While teratoma formation is a defining criterion for ES cells, and as little as 2 ES cells contaminating a graft of mature cells have been shown to give rise to teratomas (Hentze et al., 2007), adult and BMDCs do not form tumors on a regular basis. However, in vitro and mouse studies have shown that MSCs can undergo transformation or support growth of existing tumors (Djouad et al., 2003; Houghton et al., 2004; Wang et al., 2005; Tolar et al., 2007). So far, there have been no reports of transformation of administered BMDCs into tumors in patients treated with autologous or allogeneic stem cells, but experience is limited and follow-up times are insufficient to allow assessment of true long-term effects.

Another concern, besides tumor formation, is development of fibrosis (Iwano et al., 2002; Russo et al., 2006), since MSCs are fibroblast-like cells and are stimulated by TGF-□, a factor that has been shown to be the major factor in the development of tissue fibrosis. Animal models of BMDC treatment have actually shown that these cell preparations are protective of tissue fibrosis rather than profibrotic (Ortiz et al., 2003; Zhao et al., 2005; Ninichuk et al., 2006), rendering the potential contribution of MSCs to fibrosis insignificant.

Currently, there is also a lack of standardization procedures for stem cell preparations. All studies described in Tables 1–3 used their own stem cell preparations without standardization procedures. It was demonstrated that procedures need to be carefully monitored in order to obtain comparable stem cell preparations (Seeger et al., 2007). As the field of regenerative medicine is still in its beginning, it is of central importance that criteria for standardization of the cell product and therapy are rigorously defined, steps that are obviously vital to the successful conduct of future studies.

FUTURE DIRECTIONS

Bone marrow transplantation has become the main treatment for formerly incurable leukemias and has been successively developed from the experimental animal stage towards as the standard treatment in the clinic. The pioneer of this treatment, E. Donnell Thomas, has been awarded the Nobel prize. As with bone marrow transplantation, stem cell therapy with adult BMDCs for regenerative medicine has leaped from the experimental stage into the clinic and randomized trials have been completed with success. Although stem cell therapy cannot be considered as a new treatment standard yet, it appears very likely that it will develop into an important tool for the innovative treatment of many diseases.

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