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The Immune Status of Mesenchymal Stem Cells and its Relevance for Therapeutic Application

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ABSTRACT

Multipotenciality and anti-inflammatory activity, the two main properties of mesenchymal stem cells (MSCs), underlie their therapeutic prospective. During the past decade numerous studies in animal models and clinical trails explored the potential of MSCs in the treatment of diseases associated with tissue regeneration and inflammatory control. Other qualities of MSCs: ready accessibility in bone marrow and fat tissue, and rapid expansion in culture make the therapeutic use of patients' own cells feasible. The prevailing belief that MSCs are non-immunogenic encouraged the use of unrelated donor cells in immune-competent recipients. The data emerging from studies performed with immune-incompatible cells in animal models for a wide-range of human diseases show, however, conflicting results and cast doubt on the immune privileged status of MSCs. Our analysis of the pre-clinical literature in this review is aimed to gain a better understanding of the therapeutic potential of immune-incompatible MSCs. Emphasis was laid on applications for enhancement of tissue repair in the absence of immune-suppressive therapy.

INTRODUCTION

Research into the therapeutic properties of mesenchymal stem cells (MSCs) has been evolving rapidly during the past decade. The growing interest in these mesoderm-derived stem cells followed the discovery of their multipotent differentiation capacity. Their first use in clinical trials, however, exploited their immune modulatory properties. The initial studies were performed with patient-derived autologous cells. This was practicable due to the presence of MSCs in readily accessible tissues and their extensive expansion potential *ex vivo*.

The therapeutic use, however, of cells from diseased individuals is not always possible. Factors like a patient's age, inheritability of the disorder, disease history and the use of medications by the patient were found to adversely influence the yield as well as the quality of the cells [1-6]. Other drawbacks for the use of autologous cells are the time and costs associated with preparation and quality control for each individual patient

The advantage of cell banks containing large amounts of validated ready-for-use cells as cellular pharmaceuticals is obvious. Moreover, pre-screening of off-the-shelf stem cells for their therapeutic capacity facilitates selection of the most suitable cell sources and donors for each application.

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These factors and the assumption that MSCs are non-immunogenic encouraged the exploitation of allogeneic MSCs for applications in immune-competent patients (reviewed in [7]). In various pre-clinical and clinical studies clear-cut therapeutic effects were achieved with immune-incompatible MSCs. The results from studies of the survival of grafted MSCs in allogeneic and xenogeneic recipients were, however, conflicting; raising the question whether MSCs are genuinely immune privileged.

In an attempt to arrive at a better understanding of the above we have chosen to analyze in this review only studies that contain records of the recipients immune response to the immune-incompatible transplants and a side-by-side comparison of their benefit to that of syngeneic cells.

General properties of MSCs

MSCs are multipotent stem cells present in the stroma of virtually all organs and connective tissues (reviewed in [8]). The precise location and physiological role of these cells are, so far, poorly defined. Recent findings suggest that MSCs represent activated progeny of pericytes that line the abluminal side of blood vessels throughout the body (reviewed in [9]).

MSCs, first isolated from the bone marrow (BM) [10], are defined as plastic-adherent fibroblast-like cells with extensive proliferation capacity in culture and the ability to differentiate *in vitro* to adipocytes, chondrocytes and osteoblasts [11].

MSCs isolated from various tissues share a number of non-hematopoietic cell-surface markers i.e. CD29, CD44, CD73, CD90, CD105 and human major histocompatibility complex (MHC) class I [12].

Furthermore, MSCs have been shown to have pleiotropic immune-modulatory properties *in vitro* including suppression of T cell proliferation in response to alloantigens or mitogens, inhibition of B cell proliferation and antibody production as well as of dendritic cell maturation. Human MSCs are not lysed by freshly isolated allogeneic natural killer (NK) cells but are susceptible to the lytic activity of activated NK cells.

The immune-suppressive activity of MSCs has been demonstrated for a variety of autoimmune conditions including graft-versus-host disease (GvHD) [13] and experimental autoimmune encephalomyelitis (EAE) [14]. The systemic administration of MSCs improved allogeneic hematopoietic stem cell engraftment [15] and prolonged organ allograft survival [16].

The mode by which MSCs mediate their immune-modulatory effects in these models is not clear. The mechanisms suggested to be involved include: modification of innate immune mediators; alteration of the development, migration and secretion properties of dendritic cells; induction of regulatory T-cell populations; and suppression of T-cell and B-cell responses (reviewed in detail in [17, 18]).

Other properties of MSCs such as promotion of adaptive immunity (as opposed to suppression) and homing to sites of inflammation make these cells suitable for use as a vaccine platform (reviewed in [19]) and as a vehicle for the delivery of anticancer drugs into tumors [20], respectively.

Also, MSCs were shown to ameliorate tissue damage in almost all of the major organs of the body: heart, brain, lung, liver, kidney, eye and skin [21-27]. Although the differentiation *in vitro* of MSCs into various mesoderm lineages has been well documented only a limited number of *in vivo* studies demonstrate a similar differentiation or the occurrence of other donor derived tissue cells. Some recent studies suggest that the latter may also be the result of heterotypic fusion between MSCs and cells in the target tissue [28-30].

The very low incidence of differentiated MSCs in the target tissue cannot account for the remarkable repairing effect observed. The now commonly held theory is that the therapeutic benefit is due—mainly—to a paracrine response: When cultured under hypoxic conditions MSCs secrete large quantities of bioactive molecules such as cytokines,

antioxidants, proangiogenic substances, trophic factors, and other proteins (reviewed in [31]). Injection of condition medium of the cultured cells into mice was found to mimic the beneficial effect of transplanted MSCs including enhancement of endogenous regeneration, prevention of apoptosis and augmentation of angiogenesis in the diseased tissues [32-34].

The therapeutic effect of immune-incompatible MSCs

We have divided the data analyzed in this review into two main categories. In the first the effect is associated with immune modulation; in the second the effect is associated with tissue repair. All described studies have been performed with culture-expanded cells.

Immune-incompatible MSCs for immune modulation

In the use of MSCs as immune modulators the cells are frequently infused systemically. It has been observed that intravenously administrated MSCs are trapped in the lungs and cleared from the circulation within minutes of infusion. A fraction of less than 0.1% of the inoculated cells leaves the lungs and home to other organs [35-37]. Nonetheless, a therapeutic benefit has been recorded with both syngeneic and allogeneic MSCs.

Intravenous administration of allogeneic MSCs attenuated inflammation in animals with experimental autoimmune encephalomyelitis [38], arthritis [39], lung injury [40] and prolonged skin graft survival [41]. The effect, as with syngeneic cells, is assumed to be due to a paracrine response and is often long lasting.

Other researchers observed, however, that transplantation of donor type MSCs after allogeneic BM transplantation in mice results in accelerated rejection of the allogeneic BM transplants, while recipient type MSCs enhanced their long-term engraftment [42]**.** Induction of memory T-cells specific to the allogeneic MSCs was demonstrated both in re-challenge experiments [43] and in naïve mice transplanted with allogeneic MSCs [42]**.** Formation of alloantibodies at levels sufficient to reduce survival of secondary injected allogeneic MSCs in naïve rats was also shown [44]**.**

Similarly, in rats grafted with an allogeneic kidney, pre-treatment of the recipient with kidney donor-type MSCs caused an enhanced humoral rejection with donor-specific IgG-antibodies levels significantly higher than those induced in rats pre-treated with recipient-type MSCs [45]. In all the above studies BM-derived MSCs obtained from adult donors were used. The inoculums varied from half to five million cells.

The BM and kidney transplantation studies above in particular argue against the notion that MSCs are intrinsically immune-privileged. The immune memory induced by the administration of immune-incompatible cells cautions against their sequential application.

MSCs application for enhancing tissue repair

The question whether tissue repair by MSCs requires the persistence of the cells in the injured tissue — in their naive or differentiated state— has not been conclusively answered yet. Most of the studies addressing the contribution of immune-incompatible MSCs to tissue regeneration do not report the fate of the transplanted cells; those that do, present conflicting results. A demonstration of long-term presence of grafted immune-incompatible MSCs or of their progeny in the target tissues will hold up the case for their non-anitigenicity.

Our analysis below will include the most frequently studied tissues in immune-competent laboratory animals: bone, skin and heart. The brain, being an immune privileged tissue, was not included.

Bone

The osteogenic differentiation potential of MSCs encourages their application in bone fractures and the repair of osseous defects. The efficacy of immune-incompatible MSCs for bone replacement—studied in various animal species—is inconsistent.

At 24-weeks after implantation, adipose tissue-derived allogeneic MSCs - delivered onto natural coral scaffolds and kept for 7 days on osteogenic differentiation medium - persisted and differentiated in a canine cranial bone-repair model, similarly to autologous cells. No evident systemic immune response in the hosts has been observed [46].

Similarly, adipose tissue-derived allogeneic MSCs implanted in scaffolds composed of tricalcium phospate and collagen I and cultured for 48 hours in stroma medium, were reported to accelerate posterior lumbar spinal fusion in rats as efficiently as syngeneic cells. The levels of inflammatory cell infiltration in the lesions observed with both cell types were significantly lower than those in the control rats implanted with scaffolds only [47].

In contrast, in a study of femoral bone repair in immune-competent rats, transplanted fetal human MSCs seeded onto macroporous poly-ε-0-caprolactone tri-calcium phosphate scaffolds and pre-differentiated during two weeks, could not be recovered. The level of T cells in the injured site was clearly elevated compared to the controls [48].

Also, when loaded onto hydroxyapatite ceramic scaffolds and implanted subcutaneously in rats, new bone formation as well as high alkaline phosphatase activity was recorded with syngeneic but not with allogeneic BM-derived MSCs. When the latter were grafted into immune-suppressed (FK506 treated) rats the cells survived and differentiated along the osteogenic lineage, suggesting their rejection in the immune-competent host [49].

Addressing the question whether MSCs differentiation alters their immunological status Niemeyer et al. [50] observed that in a xenotransplantation model *ex vivo* osteogenic differentiated human BM-derived MSCs cultivated on mineralized collagen were rapidly eliminated by the host's immune system. In contrast, undifferentiated cells (seeded onto the same scaffolds) persisted for 8 weeks. Anti-donor reactive lymphocytes and macrophages were present in significantly higher numbers in mice treated with the differentiated cells compared to mice receiving the naïve cells.

A possible explanation for the different outcomes in the studies discussed above may lie in the nature of the scaffolds employed by the different groups. It is conceivable that MSC-surface proteins associated with immune recognitions are modulated by some biomaterials but not by others.

Skin

MSCs have been employed for treatment of experimental surgical skin wounds and found to enhance the healing. Though the therapeutic effect of MSCs is assumed to be mediated by bioactive molecules some evidence is provided for differentiation of the applied cells in the skin (reviewed in [51]). Using excisional wounds in mice Wu et al. [52] demonstrated persistence at day 28 of 2.5% of the one million green fluorescence protein (GFP) labeled allogeneic BM-derived MSCs, originally injected around the wound bed. Analysis at day 14 showed that about 50% of the GFP-positive cells expressed the keratinocyte-specific protein keratin—indicating differentiation.

After local application of one million allogeneic or syngeneic GFP-positive BM-derived MSCs in full-thickness skin excisional wounds, the same group [53] reported equal cell engraftment (up to 28 days) and enhanced wound closure. The level of infiltrated T cells was in both cases low compared to allogeneic implants of cultured fibroblast, suggesting the absence of an immune response against the MSCs. The fibroblasts that served as negative controls failed to enhance repair, and disappeared much faster.

In contrast to damaged skin, healthy skin of immune-competent mice seems not to tolerate implanted immune-incompatible MSCs. Evidence is provided by Eliopulos et al. [54] who found rejection of unmodified BM-derived allogeneic MSCs (one million per mouse) and long-term survival of BM-derived sygeneic MSCs upon subcutaneous implantation. The recorded presence of cellular

inflammatory infiltrates at the delivery site of the allogeneic MSCs is consistent with an active rejection process. Likewise, la Garza et al. [55] reported significantly shorter survival of human BM-derived MSCs injected subcutaneously (half a million per mouse) in immune-competent mice than in immune-deficient mice.

Heart

Quevedo et al. [56] showed in female swine with chronic ischemic cardiomyopathy that allogeneic MSCs restore cardiac function. In this model 200 million of male BM-derived allogeneic MSCs or placebo (vehicle) were administrated transendocardial at 12 weeks after myocardial infarction. Twelve weeks after transplantation male donor cells with markers of cardiac, vascular muscle, and endothelial lineages (trilineage) were present at the injured site. Quantification of these interesting findings was not provided. Similar results were reported by Amado et al. [57] who implanted 10 million allogeneic BM-derived MSCs intramyocardially at 3 days after myocardial-infarction, also using pigs. These authors reported retention of about 50% of the injected allogeneic cells at 8 weeks.

In contrast, in rats, trilineage differentiation of allogeneic MSCs following implantation of three million BM-derived cells into infarcted myocardia (3 weeks after damage induction) was found to cause a switch of the originally non-immunogenic MSCs to an immunogeneic phenotype that triggered their rejection by the host. At 5 weeks the allogeneic cells had been eliminated and anti-donor specific antibodies, reacting only with the differentiated but not with the naïve allogeneic MSCs were present in the blood. Functional benefit was lost within 3 months. Animals in the control group, similarly treated with syngeneic cells, maintained the donor cells as well as the restored cardiac function as long as 6 months after treatment [58].

The fate of human MSCs in the infracted myocardium was compared between immune-competent and immune-deficient Nude rats. In this experiment one to two million of adult human BM-derived MSCs were injected into the myocardium surrounding the infracted scare either directly or three days after ligation of the left anterior descending artery. No human MSCs were retrieved in the immune-competent rats and an intense cellular immune response (primarily macrophages) was observed at the injection site. In the nude rat the MSCs recovered at 6 weeks after transplantation (frequency not specified) did not express cardiomyocte differentiation markers [59].

The data from the cardiac injury experiments do not provide an unambiguous answer to the question whether mesenchymal stem cell (MSC) engraftment is required for a therapeutic benefit. The experiments with swines showed an impressive functional repair following allogeneic MSCs injection and persistence of the grafted cells beyond the period normally required for allogeneic rejection. Unfortunately, controls with autologous MSCs were not carried out. In the rat studies where these controls were included the syngeneic MSCs performed better than the allogeneic cells.

Further of interest are the conflicting findings regarding immunogenicity of differentiated adult BM-derived MSCs. Some studies show long-term persistence of trilinage differentiated MSCs [56, 57] while others find that differentiation triggers immune rejection; this was observed in the heart [58] as well as the bone [50]. The mechanism underlying rejection of differentiated MSCs is not clear. It was suggested that this might be due to a switch in surface-MHC molecule composition during MSC differentiation as observed for cells transplanted in the heart. This, however, could not be confirmed for MSCs in regenerating bones.

prospectives

The current progress in genetic engineering envisages the development of genuine non-immunogenic MSCs through transduction with recombinant genes encoding viral immune-evasion proteins (immunoevasins). Effective down-regulation of MHC class I molecules present on the surface of MHC class II negative human MSCs has been accomplished

recently by our group through the use of human cytomegalovirus (HCMV) US11 protein. The generated MHC class I negative cells were not rejected upon implantation in NK cell-depleted but otherwise immune-competent mice [55]. Two other studies using human MSCs expressing US11 or US6 proteins and pig MSCs expressing US2 and US3 proteins reported similar down-regulation of cell-surface MHC class I molecules and a corresponding therapeutic benefit of liver regeneration in pre-immune fetal sheep and of osteogenesis in pigs [60, 61].

This novel approach highlights the potential of viral immunoevasins to permanently modify MSCs. The vulnerability of MHC class I negative cells to NK cell activity calls for a approach involving the use of multiple immunoevasins to protect human MSCs against destruction by both cytotoxic T lymphocytes and by NK cells.

The therapeutic efficacy of immunoevasin-modulated MSCs will have to be determined in future studies, examining their long-term survival in tissues after single and multiple administrations.

CONCLUSIONS

Pre-clinical studies and completed clinical trials have shown that treatment with autologous or allogeneic MSCs do not carry any significant adverse effects [62]. The majority of clinical studies that examined the efficacy of MSCs transplantation were conducted with autologous cells. MSCs of unrelated donors were used for treatment of GvHD or for enhancement of hematopoietic stem cell engraftment seem promising but are too limited to be conclusive (reviewed in [63, 64]).

Results from clinical studies confirm those of experimental animal models in that both show that MSCs infused intravenously disappear rapidly from the circulation. The anti-inflammatory effect attained by both self and non-self MSCs has been suggested to be due to bioactive molecules secreted by the MSCs shortly after their infusion. The extent of their effect is not yet fully known. Recent evidence from *in vitro* experiments shows that high-level production of these molecules is initiated by a contact-dependent cross talk between MSCs and target cells [65]. It has also been demonstrated *in vitro* that some immune-regulatory pathways require contact between MSCs and immune cells [66]. How MSCs trapped in the lungs could fit into such a mechanism remains unsolved [67].

The pre-clinical data suggest further that in cases of treatment with allogeneic MSCs an anti-inflammatory effect is not prevented by the recipient's primary immune response against the injected cells but there is evidence that a faster secondary response does annihilate this effect. The therapeutic efficacy of allogeneic cells in the treatment of inflammation-associated disorders may therefore be similar to that of autologous cells provided it is applied once only.

Other than with autologous/syngeneic MSCs, the findings in the pre-clinical literature hardly justify yet the use of allogeneic cells for tissue repair or replacement. Further research is needed for a better understanding of the immune status of MSCs and the implications of the recipient's immune response for their therapeutic potential.

The question whether a long-term presence of MSCs in the injured tissue is required for a therapeutic benefit, which is of particular interest for treatment with allogeneic cells, should be answered by quantitative evaluations of their presence and viability during the entire period of the manifested effect.

In cases of local delivery a distinction should be made between different target tissues as the immune-privileged status may, in some cases, be a property of the anatomical site rather than of the implanted cells. And most importantly, comparison between self and non-self MSCs is required in all such studies.

Finally, successful genetic engineering of genuine immune-resistant MSCs may be expected to broaden the MSC-based therapeutic applications as well as their general availability.

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