CURRENT OPINION

Mesenchymal stromal cells for organ transplantation: different sources and unique characteristics?

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Purpose of review
In this review, recent findings on the effects of tissue and donor origin, culturing conditions and preconditioning regimens on the therapeutic effect of mesenchymal stem cells (MSC) in organ transplantation are discussed and the importance of understanding the characteristics of MSC for developing efficient therapy is stressed.

Recent findings
MSC research in organ transplantation is currently moving from safety-feasibility studies to efficacy studies and finding the optimal MSC for therapy is therefore highly relevant. Although sharing basic properties, there are subtle differences between MSC from different tissue sources that may affect their efficacy. Furthermore, the use of MSC from diseased organ recipients, donor or third party may affect their therapeutic effect. The importance of these differences in MSC properties may however be overshadowed by the impact of culture conditions on MSC. Culture conditions dramatically change the characteristics of MSC, and this situation can be exploited by exposing MSC to preconditioning treatment to bring about the desired properties in MSC. As MSC appear to be short-lived after infusion, the specific characteristics of MSC are mostly relevant for short-term interactions between MSC and host cells, which will subsequently take over the effects of MSC. The multiple effects of MSC are by no means unique, but the full spectrum of the effects in combination with their easy isolation and expansion make MSC a suitable cell type for therapy.

Summary
Tissue source, donor source and culture conditions affect the phenotypical and functional properties of MSC. The efficacy of MSC therapy will therefore depend on the source and manipulation of MSC.

Keywords
culture, mesenchymal stem cells, preconditioning, source

INTRODUCTION

Mesenchymal stem cells (MSC) are multipotent cells capable of differentiating in mesodermal lineages, in particular osteoblastic, adipogenic and chondrogenic lineages. They have, furthermore, immunomodulatory capacities and can inhibit the proliferation of activated lymphocytes, provide a survival signal to resting immune cells and stimulate the induction of regulatory macrophages and regulatory T cells. MSC are immunophenotypically characterized by the expression of a panel of markers, including CD29, CD44, CD73, CD90, CD105, CD166 and HLA class I, whereas they lack expression of HLA class II and co-stimulatory molecules CD80 and CD86. Under inflammatory conditions, the expression of HLA class II by MSC is induced and the expression of HLA class I is strongly increased. The properties of MSC have raised interest in the use of these cells for regenerative and immunomodulatory therapy and MSC may advance into a therapeutic option for transplant patients in years to come. MSC are present in all tissues, from the bone marrow to connective tissues and solid organs. Traditionally, MSC used for research and therapeutic purposes are isolated from the bone marrow, but other tissue sources may turn

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Curr Opin Organ Transplant 2014, 19:41–46

DOI:10.1097/MOT.0000000000000036

KEY POINTS

- Tissue-specific differences of MSC and in-vitro preconditioning may modulate the therapeutic effect of MSC in organ transplantation.
- MSC are short lived after infusion and their effects are likely to be mediated via host immune cells and allograft resident MSC.
- The effects of MSC are unlikely to be unique; once the complex molecular pathways that mediate the effects are elucidated methods to mimic the effects of MSC may be explored.

out to be of greater benefit as they harbor higher numbers of MSC or have better accessibility. The impact of tissue and donor origin, but also of cell culture conditions, on the properties of MSC is discussed in this review.

TISSUE SOURCE OF MESENCHYMAL STEM CELLS

MSC from different sources share many phenotypical and functional characteristics. There are, however, subtle differences in differentiation capacities and expression of specific cell surface markers. For instance, bone marrow MSC are negative for CD34, whereas adipose tissue MSC are positive for CD34 and CD36 early after isolation [1]. Even within tissues it is believed there are different MSC subsets. By using the cell surface markers CD146 and CD34, MSC of perivascular and nonperivascular origin can be identified in the stromal vascular fraction of digested adipose tissue [2,3]. The suitability of different MSC subsets for clinical application is only starting to be explored.

Bone marrow-derived mesenchymal stem cells

The bone marrow is the classical source of MSC. Although bone marrow aspirates contain multiple cell types, a simple plastic adherence selection procedure is usually sufficient to obtain pure MSC cultures. Much of the preclinical work with MSC has been carried out with bone marrow MSC. In recent years, the use of bone marrow-derived MSC in clinical transplantation has been demonstrated to be well tolerated and feasible [4–6]. The relatively large amount of data and experience is a great advantage for the use of bone marrow MSC, as opposed to using MSC from alternative tissue sources that may have different properties. Nevertheless, bone marrow may not be the ultimate source of MSC. The yield of MSC from the bone marrow is low [7], and MSC from the bone marrow do not proliferate as fast as MSC from other tissue sources [8]. Furthermore, bone marrow aspirations are invasive and it is unknown whether multiple bone marrow aspirations affect the MSC compartment in the bone marrow.

Adipose tissue-derived mesenchymal stem cells

Adipose tissue is an emerging source of MSC. It is easier accessible and can be collected with less burden than bone marrow. Although bone marrow and adipose tissue MSC share many properties, there are differences in gene expression profiles, secretion of growth factors and entry in senescence [9▪]. It is important to realize that there are different types of adipose tissue and that MSC from these tissues may have different properties. MSC isolated from abdominal and breast adipose tissue, for instance, show differences in fibroblast growth factor and receptor expression, suggesting a difference in their angiogenic potential [10]. Similar to bone marrow-derived MSC, infusion of adipose tissue MSC protects against ischemia-reperfusion injury of the kidney in murine models [11] by reducing oxidative stress and inhibiting the inflammatory response [12]. Adipose tissue MSC furthermore have comparable immunomodulatory capacity to bone marrow MSC and share the capacity of inducing *de novo* regulatory T cells [13]. Infusion of adipose tissue MSC in man is well tolerated [14], but no clinical studies in organ transplantation have been carried out up to date.

Mesenchymal stem cells from other tissue sources

MSC are present and can be isolated from multiple other tissues, such as the skin, muscle, kidney, dental pulp, spleen and heart. However, many of these tissues are not useful as a source for MSC for clinical therapy due to their poor accessibility. The blood would in this respect be the ideal source of MSC, but the presence of MSC in the circulation is questionable. Although animal studies have demonstrated the presence of MSC in the blood [15,16], in human this has proven problematic. The only report of circulating MSC in man comes from a study in hip fracture patients, in which disruption of the bone marrow could have been the cause of release of MSC in the circulation [17]. It has been demonstrated earlier that organ grafts contain donor MSC, which remain present and functional for multiple years after transplantation [18,19]. More recently, it

was shown that MSC reside in the liver and that liver MSC are mobilized in the perfusion fluid during the transplantation procedure [20]. Organ perfusates may therefore be a useful source of donor-derived MSC. Alternatively, the fetal membrane has been indicated as a rich source of functional MSC that are protective against ischemia-reperfusion-induced kidney injury [21]. The membrane is usually discarded as a waste product after delivery, and therefore obtainable in a noninvasive manner. In a similar manner, MSC can be obtained from umbilical cord blood [8]. The fetal membrane and cord blood are no practical sources for obtaining autologous MSC, but they may become useful as sources of allogeneic MSC.

DONOR SOURCE OF MESENCHYMAL STEM CELLS

The tissue source of MSC is directional for making the choice of using MSC of autologous, donor or 3rd party origin for therapy. Functionality is another consideration. The in-vitro immunosuppressive effect of MSC has been demonstrated to be independent of HLA subtype and from this perspective the use of allogeneic MSC for immunomodulatory therapy is a therapeutic option. Allogeneic cells may, however, induce an immune response. Although MSC are considered to be low immunogeneic, they can be recognized by the adaptive immune system [22]. The use of autologous MSC avoids recognition of the cells by the adaptive immune system, but alternatively patient-derived MSC may show reduced functionality compared with MSC of healthy individuals. Two studies have investigated the phenotypical and functional characteristics of bone marrow-derived and adipose tissue-derived MSC from end-stage renal disease patients and both concluded that there were no differences between patient MSC and MSC from matched healthy individuals [23▪,24▪]. These results justify the use of autologous MSC in clinical trials. From a logistic, economic and patient burden point of view, however, the use of third party MSC has advantages over the use of autologous cells. A study by Eggenhofer *et al.* [25▪▪] demonstrated that third party multipotent adult progenitor cells can tolerize rats against allogeneic heart grafts. Furthermore, heart grafts from tolerized rats could be re-transplanted without immune suppression in a secondary host. This finding demonstrates that the use of third party MSC is feasible, well tolerated and effective in organ transplantation. A study using a model of autoimmune thyroiditis confirmed the efficacy of allogeneic MSC and showed no difference in the effect of autologous and allogeneic MSC on disease resolution [26]. They did observe, however, the occurrence of humoral responses in the animals treated with allogeneic MSC. Schu *et al.* [27▪] made similar observations and found furthermore that rat MSC treated with IFN-γ were susceptible for cytotoxic lysis by $CD8^+$ T cells. The question whether MSC of allogeneic origin are as effective in a human outbred population battered with viral infections as in infection-free inbred laboratory animals will need further investigation in coming years. Experience in the setting of hematopoietic stem cell transplantation, in which third party MSC were successfully used to treat graft versus host disease without side-effects [28], suggests that the use of third party MSC in transplant patients is a realistic possibility. Finally, in transplantation, there is the option to use MSC of donor origin. A small clinical trial in kidney transplant recipients demonstrated that the use of donor-derived MSC is well tolerated and feasible and may be effective in allowing tapering of immunosuppressive drugs [29].

IMPORTANCE OF CULTURE AND PRECONDITIONING

Identifying MSC with the most optimal properties for clinical application is the subject of a great number of studies. The relevance of tissue-specific differences between MSC for immunomodulatory therapy in organ transplantation may, however, be limited when using culture-expanded MSC. Standard culture conditions have a major impact on MSC phenotype and function. Although freshly isolated MSC have a diameter of 5–6 μm, cultured MSC typically have a diameter of 25–30 μm. The volumes of cultured and noncultured MSC therefore vary more than 100-fold. Furthermore, in contrast with MSC *in vivo*, the proliferation apparatus of cultured MSC is highly activated and the expression of MSC surface markers is increased in culture. The impact of culture on the expression of chemokine receptors is unknown. Cultured and noncultured MSC may therefore be home to different sites and have different interplays with the immune system. Both cultured and noncultured adipose tissue-derived MSC have been demonstrated to be effective in ameliorating ischemia reperfusion injury of the kidney [30]. Furthermore, noncultured adipose tissue MSC have been successfully tested in models of acute myocardial infarction [31] and cisplatin-induced kidney injury [32▪], but so far the efficacy of nonmanipulated MSC in transplant models is unknown.

It is known for some time that the manipulation of culture conditions can modulate the function of MSC. In the presence of IFN-γ, MSC upregulate

indoleamine 2,3-dioxygenase (IDO) and thereby increase their immunosuppressive potency [33]. Improved immunosuppressive capacity of MSC can also be induced by the inflammatory conditions found in nephritis kidneys [34] or by using pharmacological compounds such as *S*-nitroso-*N*-acetylpenicillamine [35]. Avoiding pharmacological interference, variation of oxygen levels may modulate the immunosuppressive capacity of MSC [36] and glucose levels have been shown to improve the ability of MSC to repair the infarcted myocardium [37].

INTERACTION WITH IMMUNE CELLS AND TISSUE RESIDENT MESENCHYMAL STEM CELLS

Ironically, although a lot of effort is put in optimizing MSC preparations for immunomodulatory therapy, the evidence for sustained engraftment of intravenously infused MSC remains inconclusive. Some studies demonstrated the distribution of labeled MSC to various tissue sites, among them to sites of injury [38–40]. Other studies, however, suggest that MSC have only a limited life time after infusion. Within 24 h after intravenous infusion of syngeneic MSC in mice, viable MSC can no longer be detected at any tissue site, not even in ischemic liver [41▪]. A study on autopsy material of graft versus host disease patients demonstrated a low frequency of donor MSC DNA in lungs and lymph nodes of a number of patients, and the detection of donor DNA was negatively correlated with time from infusion to sample collection [42▪▪].

The potential rapid disappearance of MSC after infusion does not exclude their therapeutic effect as even while MSC may only be briefly present, they rescue hematopoiesis in lethally irradiated mice [43]. MSC may be recognized and cleared by the innate immune system because of their cell culture-modulated phenotype. The in-vivo effects of MSC that are observed in several transplant models can be explained by the interaction between MSC and cells of the immune system and the transfer of immunomodulatory effects from MSC to other cell types before MSC are cleared. Even though MSC may be short-lived, tissue-specific differences and culture-induced preconditioning of MSC may become relevant for the brief interaction between MSC and other immune cell types, such as macrophages and regulatory T cells. MSC are known to directly induce the generation of regulatory T cells and, in addition, a study in murine demonstrated that splenectomy abolished the therapeutic effect of MSC on acute kidney injury by inhibiting the formation of regulatory T cells, suggesting that MSC induce regulatory T cells through interactions with splenocytes [44]. MSC can also turn activated macrophages in a regulatory phenotype [45] and macrophages that phagocytose dead MSC obtain regulatory properties [46▪]. This situation indicates that the clearing up process of MSC in itself is an immunomodulatory mechanism of MSC therapy. Specific properties of MSC preparations are likely to affect the response of macrophages to MSC.

Next to their effect on immune cells, administration of MSC may affect tissue resident MSC. Infusion of MSC leads to a systemic cytokine response [47] and this response may reach MSC that are present in tissues, including in organ transplants. For instance, the pancreas is home to MSC that can be exploited for islet neogenesis [48] and targeting of these MSC may modulate their regenerative and immunomodulatory properties. MSC in lung allografts are indicated to be involved in inflammation-induced fibrogenesis [49]. The function of these MSC may be modulated by MSC therapy to bring about their immunomodulatory properties and suppress their fibrotic properties.

UNIQUENESS OF THE MESENCHYMAL STEM CELLS EFFECT

MSC are defined by a number of phenotypical and functional characteristics. As reviewed above, MSC exploit these characteristics to bring about their regenerative and immunomodulatory effects by directly targeting host cells and by targeting cells indirectly via cellular intermediates. Subtle differences between MSC of various tissues and preconditioning regimens will refine the interaction between MSC and host cells. The full spectrum of molecules and signaling pathways that mediate the effects of MSC therapy is currently unknown. It is unlikely that the mechanisms mediating the effect of MSC are specific and are not utilized by other cell types to mediate the same effects. *In vitro*, for instance, IDO is important for the immunomodulatory effects of MSC, but fibroblasts can also express IDO and, like MSC, can inhibit T-cell proliferation and expand regulatory T cells [50]. Proximal tubular epithelial cells can inhibit the proliferation of autologous T and B lymphocytes, partly via the programmed death ligand 1 pathway [51], thereby mimicking the effect of MSC. There are few direct comparisons between the in-vivo immunomodulatory effects of MSC and other cell types. A study in an ischemic skin model in the rabbit demonstrated similar effects of MSC and dermal fibroblasts on wound healing [52]. Particular aspects of the effect of MSC therapy may therefore be replaced by other cells or pharmacological compounds. However, replacement of the whole spectrum of MSC therapy

will need far more detailed knowledge on the mechanisms of MSC treatment. Furthermore, as MSC are relatively easy to access, isolate and expand, finding a more efficient cell type will be very challenging.

CONCLUSION

Tissue source, donor-origin and culture conditions have an impact on the properties of MSC and can affect their interaction with host immune and tissue resident cells. MSC with different properties may have different effects on the outcome of organ transplantation. Future research should be aimed at dissecting out those properties of MSC that are instrumental for their therapeutic effect. These studies can then lead to optimization of MSC therapy by generating an MSC product that possesses these properties.

Acknowledgements

None.

Conflicts of interest

The authors have no conflicts of interest to declare.

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