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CELLULAR TRANSPLANTS (A PILEGGI, SECTION EDITOR)

New Steps in the Use of Mesenchymal Stem Cell in Solid Organ Transplantation

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Abstract Cell therapy with mesenchymal stem cells stands at the front line of new potential tolerance-inducing therapies in solid organ transplantation. Pre-clinical and in vitro models have shown very potent immunomodulatory effects of MSC, and the first clinical trials hold promising perspectives. On the other hand, new approaches around the therapeutic use of MSC are also under research. In this article, we review recent evidence on MSC immunomodulation and their clinical application. We further discuss and speculate about new lines of research in the field including new applications and alternatives or variations of the cell product.

Keywords Mesenchymal stem cells · Transplantation · Tolerance · B cells · Extracellular vesicles · Exosomes

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Introduction

Solid organ transplantation is a well-established treatment for end-stage failure of many organs. Along the 20th century, surgical, technical, and pharmacological advances have improved organ and patient survival. However, the improvements in short-term outcome do not pair long-term results which remain sub-optimal. Pharmacological research in transplantation is mainly dedicated to achieve a state of immune tolerance minimizing the toxic effects of the compounds used.

Among the new approaches to reach tolerance, cell therapy stands at the front line. Different cell types with regulatory functions are being studied; among which, we can find regulatory T cells, dendritic cells, or mesenchymal stem cells (MSC).

Herein, we review recent evidence on MSC immunomodulation and their clinical application. We further discuss and speculate about new lines of research in the field including new applications and alternatives or variations of the cell product.

Immunomodulatory MSC

Mesenchymal stem cells are heterogeneous cell population of stromal cells defined by the International Society of Cell Therapy (ISCT) by their adherence to plastic; their capacity to differentiate into osteoblasts, adipocytes, and chondroblasts; and by the expression of an array of surface markers including CD73, CD90, and CD105 and the lack of expression of hematopoietic lineage markers such as CD45, CD34, CD14 or CD11b, and HLA class II (in non-activated state) [1].

MSC can be easily and economically expanded from a relatively small amount of tissue sample being bone marrow, adipose tissue, and umbilical cord or placenta the most used.

However it's still under study whether the different sources of MSc (bone marrow, adipose tissue, umbilical cord and placenta among others) are equally suitable for therapeutic uses [2•]. MSC have stable phenotype in culture up to several passages without risk of maldifferentiation or immortalization [3], but most of the published studies for immunomodulatory and clinical therapeutic purposes utilized passages not higher than P4 or P5, and it is advised to use passages P1 or P2 for an increased success of the treatment [4].

MSC initially captured the interest of researchers in transplantation field due to their ability to suppress T lymphocyte proliferation [5], and since then, knowledge on interactions of MSC with T cells has been extensively acquired [6]. MSC can abrogate proliferation and activation of CD4 T cells and induce regulatory T cells [7]. Recently, it has been shown how MSC decrease differentiation of CD4 T cells into Th1 or Th17 by increasing the percentage of IL-10^{+}CD4^{+}CD25^{+}FoxP3^{+} regulatory T cells (Treg) [8•], mechanism supported by the ability of MSC to induce Th17 to Treg plasticity [9•].

The effect of MSC on CD8 T cells has also been studied. MSC can reduce the generation and proliferation of antigen-specific CD8 T cells [10], and interestingly, they are able to control alloreactive CD28^{-}CD8^{+} T cells that escape other immunosuppressive drugs like belatacept [11•]. However, allogeneic MSC appear to increase reactivity of effector CD8 T cells [12•, 13].

Beyond T Cell Immunomodulation: Effect on B Cells

While the effect of MSC with T cells has been extensively studied, less knowledge has been acquired on the interaction of MSC with other immune cell types like macrophages [14, 15], dendritic cells [16], NK cells (reviewed in [17]), and B cells (reviewed in [18]).

The main goal of the use of MSC in SOT (as well as of any new therapeutic compound to be used in transplantation) is to obtain operationally tolerant patients. This requires a refined and personalized therapy that minimizes the alloreactive immune response directed against the donor without compromising the regulatory immune cell populations, as well as the basic surveillance immune system to avoid the occurrence of infections and malignancies. Tregs have been the focus of study for many years to achieve this tolerant state among transplantation patients, but recent reports suggest a key role of B cells rather than T cells in this process in kidney transplantation, since it was observed in a cohort of spontaneous tolerant patients [19–21]. These studies conclude that a proper balance between IL-10-producing B cells and antibody-producing B cells, favorable to the first, could create a favorable environment to tolerance maintenance.

In this context, new knowledge acquired on the role of MSC on B cell activation and plasticity has an increased interest. Previous reports showed opposed effects of MSC on B cell activation, although the discrepancies might be due to different stimulation and an incomplete knowledge on B cell biology [18]. Recent reports show how lupus mice that received MSC injections have significantly decreased numbers of B cells, plasma cells, and circulating BAFF levels [22]; Peng and colleagues observed an altered pattern of B cells in kidney transplantation [23] and in graft-versus-host disease (GvHD) [24] patients treated with MSC. GvHD patients improved after treatment, and that effect was correlated with increased numbers of IL-10-producing CD5^{+} B cells [25••]. In vitro, MSC have been proven capable of reducing B cell proliferation and differentiation in a T cell-dependent manner [26•], although our own results substituting T cells for a T cell mimicking stimulation indicate that the effect on B cell differentiation to plasmablasts is T cell independent [27]. In this same study, we also show a direct induction of regulatory B cells (CD19^{+}CD24^{high}CD38^{high}IL-10^{+}) by MSC, in tune to what Peng and colleagues had observed in vivo in patients [25••].

MSC in Transplantation

Evidence on the immunomodulatory capacity of MSC toward more regulatory phenotypes in vitro and in preclinical models gave green light to the trial of MSC in clinical kidney transplantation [28].

Clinical Trials in Solid Organ Transplantation

Several clinical trials have been performed in solid organ transplantation (SOT) using MSC since 2011, with satisfying results regarding safety and good perspectives on efficacy [23, 29–33]. The details and comparison has been extensively reviewed by Pileggi et al. [34]. Injected MSC peritransplantation have proven safe [23, 29, 30, 32] and comparable to induction therapy with basiliximab when combined with low-dose cyclosporine inhibitors (CNI) [30], with some hints on promotion of long-term allograft survival with increase in the frequency of Tregs [29] and show an increase of CD27^{+} B cells [23]. Two out of six biopsy proven rejection patients treated with MSC reduced interstitial fibrosis and tubular atrophy (IFTA) score and induced donor-specific hyporesponsiveness while maintaining third-party response [31]. However, it is important to take into account that three out of six patients showed CMV or BK infections after the treatment. The clinical trials performed plea in favor of the immunomodulatory capacity of MSC observed in in vitro and in vivo models, but the effects observed are not as strong as expected. Whether it is due to an inadequate timing, immunosuppressive regime or patient type will need to be studied in future trials.

While the results obtained from the trials hold promising potential, the scarce number of patients and heterogeneity in the designs limits the possibility of drawing conclusions.

MSC as B Cell-Directed Therapy in Transplantation

If the aim in SOT is to achieve tolerance, attention should be focused in obtaining potential tolerant like individuals or to modulate those post-transplantation to become tolerant. As said, in kidney transplantation, this is linked to a B cell compartment modulation that involves an increased percentage of IL-10-producing B cells along with a decrease in plasmablasts or plasma cells.

Furthermore, the analysis of immunosuppressive regime in a cohort of operationally tolerant patients showed no difference in maintenance therapy, but a substantial lower number from those that become tolerant had received induction therapy (48 versus 78.3 % in the stable group and 96.3 % in the rejection group) [35]. As reviewed in Stolp et al. [36] these data and the fact that a trial of comparison between rituximab (anti-CD20) and daclizumab (anti-CD25) as induction therapy was stopped due to the high incidence of acute cellular rejection in the rituximab groups [37], suggests that a stable B cell population is required for the induction of long-term tolerance. Whether MSC could represent a good induction treatment to obtain this stable B cell population before using other B cell treatments remains to be tested, although this could be the key behind the positive outcome in Tan et al. [30].

The potential of MSC to inhibit allo-specific antibody production [38] and B cell proliferation and differentiation [26•] opens a door to the therapeutic use of MSC to treat antibody-mediated rejection or even as a pre-treatment for sensitized patients.

However, care should be taken in the choice of the MSC source as repeated injections of allogeneic MSC might increase MSC specific antibody titers [39].

Moving Toward New Products

Although MSC therapy is proving safe and efficient in the treatment of a number of diseases, some concerns are still being raised due to their heterogeneity, size, culture conditions, etc. New approaches are being developed to overcome those issues and generate a refined product based on MSC properties.

Pre-Activated MSC

Pre-treatment of MSC with IFN-γ has been shown to potentiate their immunomodulatory, regenerative, and homing ability [40]. IFN-γ pre-activation of MSC upregulates IDO activity in MSC [41], which is a key mechanism of T cell responses suppression by accumulation of the tryptophan metabolite kyneurine. IDO may act directly inhibiting T cell response or indirectly by inducing Tregs and tolerogenic dendritic cells [42]. MSC also upregulate PD-L1 expression on their surface when pre-treated with IFN-γ. PD-L1 is a co-inhibitory molecule that inhibits T cell proliferation and function [43], although it has been also demonstrated that it is not a key mechanism of action of MSC [44].

However, as the pre-activation with IFN-γ also upregulates HLA class I and class II molecules on MSC surface, potential risks are also associated to this pre-treatment. IFN-γ pre-treated bone marrow MSC and to a lesser degree adipose derived MSC induce alloantigen-specific cytolytic CD8 response as reported in vivo and in vitro [13, 45].

MSC also express in their surface toll-like receptors (TLR), and it has been shown that ligation of different TLR have different effects on MSC. It has been proposed that TLR4 priming induces a more inflammatory MSC phenotype characterized by IL6 and IL8 secretion, while TLR3 ligation induces an anti-inflammatory response in MSC by secretion of IP-10, IL-1RA, PGE2, and IDO [46]. Moreover, TLR3-primed MSC bind more leukocytes than those with TLR4 activation [47] and only allogeneic TLR4-activated MSC lead to T cell activation, but not the ones TLR3-primed [46].

One of the characteristics that define a MSC is their plastic adherence; however, some interest has been grown around the idea of expanding them in non-adherent conditions to obtain a cell population more closely resembling the freshly isolated cells [48]. MSC grown in spheroids have a smaller size (about 4 times smaller than the adherent cultured MSC) giving some advantage for therapeutic purposes, as they will get less trapped in the lungs [49]. This change in the culture condition not only changes the size but also the secretion profile of MSC. The authors report an increased secretion of prostaglandin E_2 (PGE_2), tumor necrosis factor-α induced protein 6 (TSG-6) and stanniocalcin1 (STC-1), that would make the MSC more efficient in reducing inflammation.

Defined Phenotype

As the knowledge on MSC mechanism of action and the interest in their therapeutic use increase the need for a defined phenotype that could eventually become a homogenic and easily isolated cell product increases. Some effort has been put in the identification of a unique cell-surface marker that facilitates the isolation and identification of the MSC as extensively reviewed by Lv et al. [50]. The authors propose that the best MSC marker would be the one that identifies MSC with both clonogenic and tripotency potential. Hereby, we present a summary of the most studied markers.

CD271 has been identified as a marker for CFU-forming MSC, as the isolated $CD271^-$ cells show no clonogenic capacity [51]. $CD271^+$ MSC display enhanced anti-proliferative

potential compared to whole plastic adherent MSC, although not all the CD271$^+$ clones had tripotent differentiation potential [52].

STRO-1 has been also studied as a MSC marker; however, it is not suitable for all the sources of MSC (not for adipose tissue, umbilical cord, and peripheral blood-derived MSC). STRO-1$^+$ MSC have shown increased arteriogenesis in a model of cardiac injury [53], but STRO-1$^-$ and not STRO-1$^+$ supported better engraftment of hematopoietic stem cells in a NOD/SCID mouse model [54].

CD146 shows greater CFU enrichment capacity than CD90, STRO-1, or CD133 [55]. CD146 is also a good marker for identification of better hematopoietic supporter MSC [56], and interestingly, is a marker found in almost all the sources of MSC.

Microvesicles

Recent studies have suggested that the beneficial effect of MSC is attributed to their secretion of immunomodulatory cytokines and trophic factors [57]. The paracrine hypothesis introduces a radically different dimension to their therapeutic applications. Besides soluble factors, extracellular vesicles (EV) have been described as a new mechanism of cell-to-cell communication. EV are lipid bilayer particles coming from the cells and excreted to the extracellular medium. The term EV includes exosomes, microvesicles, and apoptotic bodies [58]. EV may interact with target cells by surface-expressed ligands, transfer surface receptors, delivery proteins, mRNA, and bioactive lipids [59]. Recently, MSC were found to secrete active EV, which would mediate the signature effects of MSC [60, 61•, 62, 63], although there is some controversy regarding their full efficiency [64••].

The EV from MSC have gained interest for their therapeutic application in a number of diseases associated with immunological and inflammatory disorders. In kidney transplantation, the study carry out by Gatti et al. [60] shows that the effects of MSC on AKI model can be mediated by EV. Furthermore, they propose that the favorable effects are a consequence of horizontal transfer of genetic information by EV.

Despite not having enough information about the effectiveness of MSC-EV, one MSC-derived EV product has been used clinically [65]. In this first attempt, the administration of escalating doses of EV derived from donor bone marrow MSC into a patient suffering from severe therapy-refractory cutaneous and intestinal graft-versus-host disease grade IV was found to be well tolerated and led to a significant and sustainable decrease of symptoms. However, the lack of controls in this study makes difficult to establish good conclusions about the EV role in the success of this case.

In general, the research to date indicates that MSC-derived EV potentially has significant clinical utility. The advantages of EV-based cell-free therapies in contrast to cell-based therapies in transplantation medicine can be generally easier to manufacture and prima facie safer, as they are non-viable and will not form tumors. By replacing the administration of live cells with their secreted EV, many of the safety concerns and limitations associated with the transplantation of viable replicating cells could be mitigated.

Discussion and Conclusions

The use of MSC in transplantation has been extensively explored over the past 10 years in pre-clinical and in vitro models raising big expectations on their therapeutic potential in the field, and the first clinical trials with MSC in kidney transplantation brought some promising results regarding safety and efficacy. However, the scarce number of patients treated and the diversity of applications between trials make difficult to get proper conclusions from the data obtained so far. In this regard, the International Society of Transplantation (ISCT) has proposed guidelines for the evaluation of the immunomodulatory properties of MSC before and after treatment [66] that will help in the future to compare between results from different trials.

Exciting results in the interactions between MSC and B cells and in parallel on the role of B cells in transplantation tolerance/rejection balance are opening new doors to explore new uses of MSC in transplantation. Other fields of research, mainly autoimmune diseases and GvHD, have better explored the effect of MSC on B cell activation and differentiation in vivo, and this knowledge is of potential interest applied to the SOT.

New products derived from MSC are also being explored based on the need to obtain a more refined product with the same or higher therapeutic efficacy but with less disadvantages associated mainly to their culturing artifacts and heterogeneity. A more defined cell product, if equally potent, might bring more solid and homogeneous therapeutic results. In this regard, microvesicles stand at the front line of the alternatives to the use of basic MSC.

MSC have been a main focus of study for many researchers in transplantation, and the field moved fast with interesting milestones being achieved. Better defined trials and standardized read-outs should answer whether MSC are future or past.

Compliance with Ethics Guidelines

Conflict of Interest Marcella Franquesa, Ana Merino, and Josep M Grinyó declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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