Mesenchymal stromal cells to promote solid organ transplantation tolerance

Federica Casiraghi, Norberto Perico, and Giuseppe Remuzzi

Purpose of review
Mesenchymal stromal cells (MSCs) possess unique immunomodulatory features. MSCs dampen effector T-cell response while promoting the emergence of regulatory T cells. By skewing this balance, MSC could represent the ideal strategy for tolerance induction in organ transplantation. Here we review recent evidence on the efficacy of MSC-based therapy in experimental models of solid organ transplantation as well as the early clinical experiences in kidney transplantation.

Recent findings
MSC infusion in experimental models of solid organ transplantation resulted in a Treg-mediated tolerance. MSC also synergized with low-dose or transient pharmacological immunosuppression in inducing long-term graft survival indicating that these cells could allow safe minimization of maintenance drug therapy. Early results from clinical studies in kidney transplant recipients reported encouraging results on the immunoregulatory effect of MSC, although posttransplant MSC infusion could associate with acute graft dysfunction (engraftment syndrome).

Summary
Immunoregulatory functions of MSC are not fixed but rather the result of microenvironment they encounter in vivo. Further studies are needed to establish how and wherein these cells have to be administered and how they may function to safely modulate host immune response in vivo in clinical transplant setting.

Keywords
effector T cells, kidney transplantation, mesenchymal stromal cells, regulatory T cells, solid organ transplantation

INTRODUCTION
Since the first successful renal transplantation in Boston in 1954 [1], organ transplantation has made dramatic strides, evolving from an experimental procedure to standard of care in the treatment of patients with end-stage organ disease. Although powerful immunosuppressive drugs are undoubtedly the cornerstone of transplant success by preventing acute cellular rejection [2], they affect the function of all responding T cells irrespective of their antigen-specificity, rendering transplant recipients susceptible to life-threatening infections and malignancy [3,4]. In addition, life-long use of broad-spectrum pharmacological immunosuppression is associated with unwanted side effects, including accelerated cardiovascular disease, metabolic complications and with a direct toxic effect to transplanted tissues [3,4], eventually contributing to long-term graft loss, a common event in renal transplantation. Ideally, the induction of donor-specific tolerance would overcome these shortcomings, possibly allowing indefinite graft survival [5].

The immune system has evolved multiple mechanisms for controlling the effector adaptive immune response [6]. Transplantation of a major histocompatibility complex-incompatible graft triggers the activation of graft destructive effector T cells as well as protective regulatory T cells (Tregs); it is the balance of such opposing subsets that ultimately determines the fate of the allotransplant [5]. The most extensively studied populations of Tregs are the so-called naturally occurring CD4+CD25+Foxp3+ Treg that develop in the thymus [7,8] and the adaptive Tregs...
that are induced in the periphery in response to antigen stimulation under tolerogenic conditions [9]. Together, Tregs maintain tolerance to self-antigens and control excessive immune response to foreign antigens and may contribute to the induction and maintenance of tolerance to allografts [10,11].

Bone marrow-derived multipotent mesenchymal stromal cells (MSC) have emerged as a promising cell population for immunomodulatory therapy in transplantation given their unique immunoregulatory properties on both the adaptive [12] and innate [13**] immune cells. MSC are capable of suppressing T effector cells [14] including memory T cells [15,16], skewing T cells toward Foxp3⁺ Tregs with concurrent suppression of Th1, Th2 or Th17 responses [14]. The findings that MSC target effector/memory T cells and promote the development of Tregs have led to propose MSC as a novel, potentially suitable cell-based approach for tolerance induction in organ transplantation.

Here, we have reviewed recent evidence on the capability of MSC to skew the balance between T effector cells and Tregs as well as the safety and efficacy of MSC-based therapy in experimental models of solid organ transplantation and in early clinical experience.
Tregs could also be expanded in mouse Treg generation. This effect transduced by macrophages polarized by MSC toward the M2 anti-inflammatory phenotype. In the in-vitro setting of anti-CD3/anti-CD28 antibody T-cell stimulation, MSC promoted the differentiation of the monocyte fraction of peripheral blood mononuclear cells into IL-10-secreting M2 immunosuppressive macrophages via the induction of indoleamine 2,3-dioxygenase expression. These macrophages were in turn implicated in the generation of Tregs.

The role of macrophages in MSC-induced Tregs has been recently confirmed in vivo in mouse models of fibrillin-mutated systemic sclerosis and experimental colitis. Indeed, systemic infusion of either syngeneic or allogeneic murine bone marrow MSC to these mice-induced transient T-cell apoptosis via the FasL–Fas pathway, which triggered macrophages to produce high levels of TGFβ in the peripheral blood, eventually enhancing CD4+CD25+Foxp3+ Treg generation. This effect translated into the amelioration of the disease phenotypes.

The polarization of T cells toward a Treg phenotype with a concomitant decrease in Th1/Th17 development has been also shown to be associated with MSC immunomodulatory effect in other experimental models of autoimmune and inflammatory diseases such as systemic lupus erythematosus, collagen-induced arthritis, diabetes, colitis, and autoimmune myasthenia gravis. Together these in-vitro and in-vivo studies indicate the ability of MSC to modulate the immune response to antigens mainly by promoting the generation of T cells with regulatory phenotype and possibly lowering the availability of Th1/Th17 effector cells.

MESENCHYMAL STROMAL CELLS IN EXPERIMENTAL MODELS OF SOLID ORGAN TRANSPLANTATION

Almost a decade has elapsed since the first study reporting the capability of MSC to prolong survival of skin graft in nonhuman primates. Subsequent studies in rodent models of heart, liver, islet, kidney, and composite tissue allotransplantation confirmed the immunomodulatory potential of MSC in transplantation (Table 1). Of note, long-term graft acceptance achieved after MSC infusion alone or in association with low-dose immunosuppressive drugs was found to be related to the expansion of Tregs [52,53,56,60,61,62,63–65]. There is also evidence that Treg depletion abrogated the MSC effect of inducing long-term graft acceptance [62,63], highlighting that MSC-mediated tolerance is maintained by Tregs. Regulaty T-cells isolated from long-term survival mice were antigen-specific [52].

We recently demonstrated that the timing of MSC infusion in respect to solid organ transplantation is one of the main factors affecting MSC capability to expand Tregs and prolong graft survival [63]. Murine MSC given to mice pretransplantation localized preferentially into lymphoid organs where allowed early expansion of Tregs, eventually leading to immune tolerance to subsequent kidney allografts. At variance, MSC infused posttransplant localized preferentially into the kidney graft with very low expansion of Tregs [63]. Intragraft MSC localization associated with acute graft dysfunction, intragraft neutrophil recruitment and C3 deposition and poor graft survival [63]. Similarly, the migration of MSC into recipient lymphoid tissues have been shown to be critical for MSC immunomodulatory effects in autoimmune encephalomyelitis, autoimmune enteropathy, diabetes and graft-versus-host disease, supporting the concept that MSC need to interact with immune cells in sites of initial effector T-cell priming in order to effectively exert immunomodulation.

Most of the experimental studies with MSC in organ transplantation have been performed without any additional pharmacological immunosuppressive therapy. However, in the perspective of translating cell-based MSC therapy to clinical transplant programs, it is critical to evaluate the possible negative impact of currently used anti-rejection drugs on MSC-induced Treg generation and function and eventually graft survival.

Data on the effect of cyclosporine (CsA) on MSC-induced immunoregulation are controversial [55,66] (Table 1). There is experimental and clinical evidence that calcineurin inhibitors (CNI), by blocking IL-2 expression in T cells, prevent both Treg development and homeostasis [71], although at low-dose these drugs may expand Tregs in both the periphery and the allografts [72].

In a mouse model of in-vivo MLR, CsA inhibited the MSC-mediated suppression of CD4+ T-cell proliferation [54]. At variance, other in-vitro studies have documented the CsA did not interfere with MSC-mediated Treg generation [23] and that MSC...
Table 1. Effect of bone marrow-derived mesenchymal stromal cells on graft survival in experimental models of solid organ transplantation

<table>
<thead>
<tr>
<th>Model</th>
<th>MSC source</th>
<th>Dose</th>
<th>Timing (tx = day 0)</th>
<th>Immunosuppression tx = day 0</th>
<th>Graft survival (days)</th>
<th>Treg expansion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin tx in baboons</td>
<td>Third party</td>
<td>$1.2 \times 10^7$</td>
<td>Days 0 and +3</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Heart tx in rats</td>
<td>Donor</td>
<td>$12 \times 10^6$</td>
<td>Days −7 and 0</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Heart tx in rats</td>
<td>Donor</td>
<td>$2 \times 10^6$</td>
<td>Days −7, 0, +1, +2, +3</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Heart tx in mice</td>
<td>Donor</td>
<td>$0.5 \times 10^6$</td>
<td>Days −7 and −1</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Syngeneic</td>
<td>Days 0 and +3</td>
<td>10</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Islet tx in mice</td>
<td>Syngeneic</td>
<td>$4 \times 10^6$</td>
<td>Days 0</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>30</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Heart tx in rats</td>
<td>Syngeneic</td>
<td>$3 \times 10^6$</td>
<td>Days 0</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Islet tx in mice</td>
<td>Donor</td>
<td>$1 \times 10^6$</td>
<td>Days −3, −2 and 0</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>16</td>
<td>&gt;28</td>
</tr>
<tr>
<td>Liver tx in rats</td>
<td>Syngeneic</td>
<td>$2 \times 10^6$</td>
<td>Days 0, +1, +2, +3, +1, +2, +3</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Donor</td>
<td>$1 \times 10^6$</td>
<td>Days −8, −12, +16</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>21</td>
<td>47</td>
</tr>
<tr>
<td>Kidney tx in mice</td>
<td>Donor</td>
<td>$1 \times 10^6$</td>
<td>Day +1</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>31</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Kidney tx in mice</td>
<td>Syngeneic</td>
<td>$0.5 \times 10^6$</td>
<td>Days −7 and −1</td>
<td>None</td>
<td>Days 0 and +3</td>
<td>10</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Heart tx in rats</td>
<td>Donor</td>
<td>$2 \times 10^6$</td>
<td>Day−4</td>
<td>MMF (2.0 mg/kg·day from day 0 to +7)</td>
<td>Days 0 and +3</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Third party</td>
<td></td>
<td></td>
<td></td>
<td>Syngeneic</td>
<td>Days 0 and +3</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Heart tx in mice</td>
<td>Donor</td>
<td>$0.5 \times 10^6$</td>
<td>Day−4</td>
<td>MMF (1.60 mg/kg·day from day 0 to +7)</td>
<td>Days 0 and +3</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Heart tx in mice</td>
<td>Donor</td>
<td>$1 \times 10^6$</td>
<td>Day +1</td>
<td>Rapamycin (2 mg/kg·day from day 0 to +13)</td>
<td>Days 0 and +3</td>
<td>7.5</td>
<td>14</td>
</tr>
<tr>
<td>Heart tx in rats</td>
<td>Donor</td>
<td>$5 \times 10^6$</td>
<td>Days 0 and +3</td>
<td>CsA (0.5 mg/kg·day from day +5 to +9)</td>
<td>Days 0 and +3</td>
<td>9</td>
<td>8.8</td>
</tr>
<tr>
<td>Syngeneic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Days 0 and +3</td>
<td>8.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Islet tx in rats</td>
<td>Syngeneic</td>
<td>$3 \times 10^6$</td>
<td>Day 0</td>
<td>CsA (1.0 mg/kg·day from day 0 to +20)</td>
<td>Days 0 and +3</td>
<td>7</td>
<td>&gt;51</td>
</tr>
<tr>
<td></td>
<td>Donor</td>
<td>$2 \times 10^6$</td>
<td>Day 0</td>
<td>CsA (5 mg/kg·day from day 0 to +14)</td>
<td>Days 0 and +3</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Islet tx in rats</td>
<td>Syngeneic</td>
<td>$2 \times 10^6$</td>
<td>Day 0</td>
<td>CsA (5 mg/kg·day from day 0 to +14)</td>
<td>Days 0 and +3</td>
<td>5</td>
<td>7.8</td>
</tr>
<tr>
<td>Third party</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Days 0 and +3</td>
<td>5</td>
<td>3.7</td>
</tr>
<tr>
<td>Heart-lung tx in rats</td>
<td>Donor</td>
<td>$5 \times 10^6$</td>
<td>Day 0</td>
<td>CsA (0.5 mg/kg·day −1)</td>
<td>Days 0 and +3</td>
<td>3</td>
<td>14.5</td>
</tr>
<tr>
<td>Hind-limb tx in swine</td>
<td>Third party</td>
<td>$10 \times 10^7$</td>
<td>Days +1, +7, +14, +21</td>
<td>Irradiation + CsA (10 mg/kg per day from day 0 to +14; 5 mg/kg per day from day +14 to +28)</td>
<td>Days 0 and +3</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Hemi-facial tx in swine</td>
<td>Third party</td>
<td>$2.5 \times 10^7$</td>
<td>Days −1, +1, +3, +7, +14, +21</td>
<td>CsA (0.1 mg/kg·day from day 0 to +14; 5 mg/kg per day from day +14 to +28)</td>
<td>Days 0 and +3</td>
<td>9</td>
<td>34</td>
</tr>
</tbody>
</table>

CsA, cyclosporin A; IS, immunosuppression; MMF, mycophenolate mofetil; MSC, mesenchymal stromal cells; nd, not evaluated; tx, transplant.
synergized with CsA in inhibiting T lymphocyte activity [73]. The combination of MSC and sub-therapeutic doses of CsA exerted a synergistic immunosuppressive effect, which translated into long-term graft acceptance of islet allografts [58,60]. In rat islet allograft models MSC and low-dose CsA induced early expansion of IL-10 producing CD11b cells, which mediated T-cell hyporesponsiveness and allowed long-term Foxp3 Tregs expansion in lymph nodes and in the graft [60]. Moreover, in swine the combination of multiple infusions of allogeneic MSC with short-term CsA immunosuppression achieved indefinite graft survival of hind-limb transplants [64] and prolonged the survival of a semi-facial transplant [65]. In both studies long-term surviving animals showed increased levels of Foxp3 Tregs in the periphery and in the graft [64,65].

On the contrary, mammalian target-of-rapamycin inhibitors have been consistently shown to sustain Treg expansion in vitro and in vivo in animal models and kidney transplant recipients [74]. In an experimental model of heart transplantation in mice rapamycin synergized with MSC in inducing Treg-mediated tolerance [53]. Similarly, in the same model in rats, mycophenolate combined with donor MSC induced long-term graft acceptance [51,54].

Altogether these results indicate that in experimental models MSC infusion synergized with low-dose or transient immunosuppressive drug treatment in inducing long-term graft acceptance, indicating that these cells allow safe minimization of maintenance pharmacological antirejection therapy.

**MESENCHYMAL STROMAL CELLS IN KIDNEY TRANSPLANTATION IN HUMANS**

There are few protocols of MSC-based therapy in organ transplantation (www.clinicaltrials.gov). Actually, clinical trials on the use of MSC in kidney and liver transplantation are being performed in our center in Bergamo, Italy (NCT00752479), in Leiden, The Netherlands (NCT00734396), in Liege, Belgium (NCT01429038) and in China (NCT00659620). So far only results from the Italian and Chinese experiences with MSC in living-donor kidney transplant recipients have been published. Our protocol is aimed at characterizing the safety and tolerability of peritransplant MSC infusion and to verify whether MSC, by skewing Treg/Teff balance allow creating a protolerogenic environment. We initially started with two living-related donor kidney recipients who were given ex-vivo expanded, autologous, bone marrow-derived MSC at day 7 posttransplant, after induction therapy with basiliximab/low-dose thymoglobulin [75]. MSC infusion did promote on long-term a protolerogenic environment characterized by lower memory/effector CD8+ T cells, expansion of CD4+ Tregs and reduction of donor-specific CD8+ T-cell cytotoxicity, compared with control kidney transplant recipients given the same induction therapy but not MSC. However, few days after cell infusion, both MSC-treated patients developed acute renal insufficiency. Histological and immunohistochemical analysis of graft infiltrating cells did exclude an acute cellular or humoral rejection, but intragraft recruitment of neutrophils together with MSC, as well as complement C3 deposition were observed [75].

It was hypothesized that the subclinical inflammatory environment of the graft in the few days postsurgery could have favoured the prevalent intragraft recruitment and activation of the infused MSC promoting a proinflammatory milieu with eventual acute renal dysfunction (engraftment syndrome), as reported by others with combined kidney and bone marrow transplantation [76]. This hypothesis has been confirmed back into a murine kidney transplant model showing that MSC administration before (day-1) but not few days after kidney transplantation avoided the acute deterioration of graft function, while maintaining the immunomodulatory effect of MSC [63].

The Chinese group performed a single-site prospective, randomized study aimed at comparing the risk-benefit profile of bone marrow-derived autologous MSC infusion (at kidney reperfusion and 2 weeks later) versus induction therapy with the anti-IL-2 receptor antibody basiliximab in living-related donor kidney transplants [77]. MSC treatment resulted in lower incidence of acute rejection at 6 months posttransplant, decreased risk of opportunistic infection and better estimated renal function. The investigators concluded that MSC may replace basiliximab induction therapy, allowing the use of lower than conventional CNI maintenance doses without compromising patient safety and graft outcome. However, lower acute rejection rate and better renal function documented at 6 months after transplantation were transient and not confirmed at 1 year. The study has been criticized in a recent letter [78]. Unfortunately, this study did not report any attempt to in-depth assess the in-vivo effects of MSC on host immune system, especially on Treg and effector T-cell function by any immunological tests, which are mandatory for an innovative cell therapy still in its infancy before
moving it to routine clinical application for transplant programs.

CONCLUSION

Cell therapy with MSC in solid organ transplantation has undoubtedly a great potential. However, although initial preclinical and early clinical results appear promising, moving the concept of MSC-based therapy forward toward clinical application should be critically assessed. We have to be aware that, so far, our knowledge about MSC is too scarce for embarking in large clinical trials and there remain many open questions both on the risk and the real benefit of these cells. Further studies are needed to establish how and where these cells have to be administered and how they may function to modulate host immune response in vivo in clinical transplant setting.

Rather than studying thousands of patients without enough attempt to safety issues and mechanistic/immunomodulatory pathways it seems preferable in our opinion in this kind of studies to proceed in few patients, however, very intensively investigated. Issues like source, dose, timing of administration, in-vivo localization, interaction with immunosuppressive drugs, whether these cells have to be used for prevention of acute rejection or for tolerance induction have not been addressed in this field and more explorative studies are required before embarking in formal clinical trials.

Acknowledgements

The Authors are member of the Mesenchymal Stem Cells in Solid Organ Transplantation (MISOT) study group, www.misot.de.

Conflicts of interest

This study has been partially supported by grants from Fondazione ART per la Ricerca sui Trapianti (Milan, Italy). Grant support was received from Superpig Program, project co-financed by Lombardy Region through the ‘Fund for promoting institutional agreements’ The authors of this manuscript have no conflict of interest to disclose.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as: ● of special interest ● ● of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 116).


The study showed how the activation status of T cells as well as the cytokine milieu that MSC encounter dictate the effect of MSC on Th17 cells.
MSC and transplantation tolerance

Casiraghi et al.


32. These studies showed how the activation status of T cells as well as the cytokine milieu that MSC encounter dictate the effect of MSC on Th17 cells.


34. These studies describe how MSC induce macrophages to differentiate toward an anti-inflammatory M2 phenotype.


36. These studies describe how MSC induce macrophages to differentiate toward an anti-inflammatory M2 phenotype.


38. This study demonstrates a previously unrecognized MSC-mediated therapeutic mechanism by which MSC use FAS to regulate MCP-1 secretion for T cell recruitment and subsequent use FAS to induce T-cell apoptosis. Macrophages subsequently remove apoptotic debris to release TGFβ, leading to upregulation of Tregs and, ultimately, immune tolerance.


54. These studies showed how the activation status of T cells as well as the cytokine milieu that MSC encounter dictate the effect of MSC on Th17 cells.


61. This study shows that in a murine transplantation model, posttransplant MSC infusion causes premature graft dysfunction and fails to prolong graft survival, whereas preemptive MSC infusion induces a significant prolongation of kidney graft survival by a regulatory T-cell dependent mechanism.


64. These studies show that in mesenchymal stem cells, regulatory T-cell generation and kidney allograft survival is correlated with T-cell regulation in a swine hind-limb model. Plast Reconstr Surg 2011; 127:569–579.

65. In composite tissue allograft transplantation models in large animals the combination of allogeneic MSC with short-term immunosuppression prolonged graft survival and was associated with increased levels of Foxp3+ Tregs in the periphery and in the graft.


67. In composite tissue allograft transplantation models in large animals the combination of allogeneic MSC with short-term immunosuppression prolonged graft survival and was associated with increased levels of Foxp3+ Tregs in the periphery and in the graft.


71. Ezquer F, Ezquer M, Contador D, et al. The antiinflammatory effect of mesenchymal stem cells is unrelated to their transdifferentiation potential but to their capacity to restore Th1/Th2 balance and to modify the pancreatic microenvironment. Stem Cells 2012; 30:1664–1674.

72. This study shows that the antiinflammatory effect of MSC was correlated to their engraftment into secondary lymphoid organs associated with reduction of auto-reactive T cells together with an increase in Treg cells.


77. Tan J, Wu W, Xu X, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. JAMA 2012; 307:1169–1177. This study reports results of a prospective, randomized study with autologous mesenchymal stem cells versus induction therapy with the anti IL-2 receptor antibody in living-related kidney transplants.

78. Riella LV, Chandraker A. Stem cell therapy in kidney transplantation. JAMA 2012; 308:130.