# REVIEW

# Mesenchymal stromal cells to promote solid organ transplantation tolerance

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**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

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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 immuno-regulatory 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.

## MESENCHYMAL STROMAL CELLS AND REGULATORY T CELL GENERATION

MSC are a heterogeneous population of adult, fibroblast-like multipotent cells characterized by their ability to differentiate into tissues of meso-dermal lineages, including adipocytes, chondro-cytes and osteocytes [17]. First identified and isolated from the bone marrow as plastic adherent cells [18], MSC are now isolated from a number of other sources including umbilical cord blood, adipose tissue and muscle [19,20]. The isolation of MSC by in-vitro expansion of plastic-adherent cells yields a heterogeneous cell population evidenced by the different morphology and functional potential. In order to create a consensus and more uniformly characterize these cells the International Society of Cellular Therapy proposed a standard set to define the identity of MSC [21]: adherence to plastic surfaces; potential to differentiate into osteocytes, adipocytes and chondrocytes under standard in-vitro differentiating conditions; and expression of CD105, CD73 and CD90 and must lack expression of CD45, CD34, CD14, CD11b, CD79a and HLA-DR.

Several in-vitro and in-vivo studies have documented the remarkable ability of MSC to polarize T cells toward a regulatory phenotype. In-vitro coincubation of human MSC with peripheral blood mononuclear cells or with purified CD4$^+$ T cells induced the differentiation of CD4$^+$ T cells into Foxp3-expressing Tregs [22–25], a process involving direct MSC contact with T cells followed by prostaglandin E$_2$ and transforming growth factor β-1 (TGFβ-1) expression [22,24]. Expanded Tregs potently suppressed the alloantigen-specific prolif-erative response in mixed-lymphocyte reaction (MLR) assay [23,24]. MSC induced a Treg phenotype (CD25$^{bright}$Foxp3$^+$CD127$^{low}$) both in naive CD3$^+$CD45RA$^+$ and in memory CD3$^+$CD45RO$^+$ T cells [26]. Other potential mechanisms of MSC-induced Treg generation include the release of soluble HLA-G5, a nonclassical HLA class I molecule [27] or of microvescicles [28]. MSC are also able to reprogram fully differentiated Th17 cells into Foxp3-expressing Tregs [29]. However, both the activation state of CD4$^+$ T cells and the cytokine milieu that MSC encounter dictate the ultimate cell outcome. Whereas the early addition of MSC to T cells cultured under Th1 and Th17 polarizing conditions exerted an extensive suppressive effect on all CD4$^+$ T-cell lineages, MSC added to already differentiated Th1/Th17 cells decreased IFNγ production by Th1 cells, but paradoxically increased proinflammatory interleukin 17 (IL-17) [30▪]. Moreover, MSC cultured in the presence of inflammatory cytokines secreted significant levels of IL-6, which, in addition to a spontaneous production of TGFβ supported retinoic acid-related orphan receptor γt expression and development of Th17 [31▪].

By exerting inhibitory effects on antigen presenting cells (APC), MSC can generate regulatory

APC with own Treg promoting activity. Dendritic cells cultured in the presence of MSC or conditioned medium expressed lower level of costimulatory molecules, hardly stimulated T-cell proliferation and efficiently generated Tregs through the release of TGFβ [32,33]. Tregs could also be expanded by macrophages polarized by MSC toward the M2 anti-inflammatory phenotype [34[]. 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 [35[]. These macrophages were in turn implicated in the generation of Tregs [35[].

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 [36[][]. Indeed, systemic infusion of either syngeneic or allogeneic murine bone marrow MSC in 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 [36[][].

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 [37], collagen-induced arthritis [38–40], diabetes [41–44], colitis [45] and autoimmune myasthenia gravis [46,47].

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 [48]. Subsequent studies in rodent models of heart [49–55], liver [56] islet [57–59,60[],61[]], kidney [62,63[]] and composite tissue [64[],65[]] allotransplantation confirmed the immunomodulatory potential of MSC in transplantation (Table 1 [48–59,60[],61[],62,63[]–

65[],66]). 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[]] or tolerogenic dendritic cells [51,53].

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. Regulatory 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 [67], autoimmune enteropathy [68], diabetes [69[]] and graft-versus-host disease [70], 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**

| Model | MSC source | Dose | Timing (tx=day 0) | Immunosuppression tx=day 0 | Graft survival (days) None | MSC | MSC + IS | Treg expansion | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Skin tx in baboons | Third party | $1.2 \times 10^7$ | Days 0 and +3 | None | 7 | 11 | | nd | [48] |
| Heart tx in rats | Donor | $12 \times 10^6$ | Days −7 and 0 | None | 6 | 23 | | nd | [50] |
| Heart tx in rats | Donor | $2 \times 10^6$ | Days−7, 0,+1,+2,+3 | None | 6 | 12 | | nd | [49] |
| Heart tx in mice | Donor | $0.5 \times 10^6$ | Days−7 and−1 | None | 10 | 40 | | YES | [52] |
| | Syngeneic | | | None | 10 | >60 | | | |
| Islet tx in mice | Syngeneic | $4 \times 10^6$ | Day 0 | None | 30 | >90 | | nd | [57] |
| Islet tx in mice | Syngeneic | $3 \times 10^6$ | Day0 | None | 16 | 38 | | YES | [61] |
| Islet tx in mice | Donor | $1 \times 10^6$ | Days −3, −2 and 0 | None | 16 | >28 | | nd | [59] |
| Liver tx in rats | Syngeneic | $2 \times 10^6$ | Days 0,+1,+2,+3, +8,+12,+16 | None | 21 | 45 | | YES | [56] |
| | Donor | | | None | 21 | 47 | | | |
| | Third-party | | | None | 21 | 57 | | | |
| Kidney tx in mice | Donor | $1 \times 10^6$ | Day+1 | None | 31 | >100 | | YES | [62] |
| Kidney tx in mice | Syngeneic | $0.5 \times 10^6$ | Days−7 and−1 | None | 10 | >60 | | YES | [63] |
| Heart tx in rats | Donor | $2 \times 10^6$ | Day−4 | MMF (20 mg/kg$^{-1}$ day from day 0 to +7) | 8 | 6 | >100 | NO | [51] |
| | Third party | | | | | | 20 | | |
| | Syngeneic | | | | | | 20 | | |
| Heart tx in mice | Donor | $0.5 \times 10^6$ | Day−4 | MMF (160 mg/kg$^{-1}$ day from day 0 to +7) | 8 | 7 | 32 | nd | [54] |
| Heart tx in mice | Donor | $1 \times 10^6$ | Day+1 | Rapamycin (2 mg/kg$^{-1}$ day from day 0 to +13) | 7.5 | 14 | >100 | YES | [53] |
| Heart tx in rats | Donor | $5 \times 10^6$ | Days 0 and +3 | CsA (0.5 mg/kg$^{-1}$ day from day +5 to +9) | 9 | 8.8 | 10 | nd | [55] |
| | Syngeneic | | | | | 8.6 | 10.4 | | |
| Islet tx in rats | Syngeneic | $3 \times 10^6$ | Day0 | CsA (10 mg/kg$^{-1}$ day from day 0 to +20) | 7 | | >51 | NO | [58] |
| | Donor | | | | 7 | | 11 | | |
| Islet tx in rats | Syngeneic | $2 \times 10^6$ | Day0 | CsA (5 mg/kg$^{-1}$ day from day 0 to +14) | 5 | 7.8 | 89 | YES | [60] |
| | Third-party | | | | 5 | 3.7 | 13.7 | | |
| Heart-lung tx in rats | Donor | $5 \times 10^6$ | Day0 | CsA (0.5 mg/kgat day − 1) | 3 | 14.5 | 18 | nd | [66] |
| Hind-limb tx in swine | Third-party | $10 \times 10^7$ | Days+1,+7,+14,+21 | Irradiation + CsA (10 mg/kg per day from day 0 to +14; 5 mg/kg per day from day +14 to +28) | 10 | 25 | >120 | YES | [64] |
| Hemi-facial tx in swine | Third-party | $2.5 \times 10^7$ | Days−1,+1,+3,+7, +14,+21 | CsA (10 mg/kg$^{-}$day from day 0 to +14; 5 mg/kg per day from day +14 to +28) | 9 | 34 | 70 | YES | [65] |

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 hemi-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

### Conflicts of interest

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 116).

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