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Isogeneic MSC application in a rat model of acute renal allograft rejection modulates immune response but does not prolong allograft survival

M. Koch [a,*], A. Lehnhardt [a,b], X. Hu [a], B. Brunswig-Spickenheier [c], M. Stolk [d,e], V. Bröcker [f], M. Noriega [g], M. Seifert [d,e], C. Lange [c]

[a] Dept. of Hepatobiliary Surgery and Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[b] Pediatric Nephrology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[c] Clinic for Stem Cell Transplantation, Dept. of Cell and Gene Therapy, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[d] Institute of Medical Immunology, Charité Universitätsmedizin Berlin, Campus Virchow Klinikum, Berlin, Germany
[e] Berlin-Brandenburg Center for Regenerative Therapies, Charité Universitätsmedizin Berlin, Campus Virchow Klinikum, Berlin, Germany
[f] Department of Histopathology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
[g] Nephropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

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ABSTRACT

Application of mesenchymal stromal cells (MSCs) has been proposed for solid organ transplantation based on their potent immuno-modulatory effects *in vitro* and *in vivo*. We investigated the potential of MSCs to improve acceptance of kidney transplants in an MHC-incompatible rat model including isogeneic kidney transplantation (RTx) as control. MSCs were administered i.v. or i.a. at time of transplantation. No immunosuppression was applied. Renal function was monitored by serum-creatinine, histopathology, immunochemistry for graft infiltrating cells and expressions of inflammatory genes. We demonstrated the short-term beneficial effects of MSC injection. In the long term, however, MSC-related life-threatening/shortening events (thrombotic microangiopathy, infarctions, infections) were evident despite decreased T- and B-cell infiltration, lower interstitial inflammation and downregulated inflammatory genes particularly after i.a. MSC injection. We conclude that i.a. MSC administration provides efficient immunomodulation after allogeneic RTx, although timing and co-treatment strategies need further fine-tuning to develop the full potential of powerful cell therapy in solid organ transplantation.

1. Introduction

Clinical allograft acceptance comes at the price of life-long drug-based immunosuppression, significantly reducing the overall well-being of transplant patients. Therefore, research into alternative treatment approaches is warranted to decrease the need for immunosuppressive medication, improve long-term graft survival, and ideally induce tolerance. Several components of the immune system leading to vascular, glomerular, and tubular injuries are involved in the highly complex pathophysiology of acute renal allograft rejection. Cell-based therapies seem to influence multiple pathophysiological mechanisms, while pharmacological interventions often target only one aspect.

MSCs are of special therapeutic interest because of their capacity to enhance tissue repair by secreting bioactive molecules that (a) inhibit apoptosis and limit the extent of damage or injury; (b) inhibit fibrosis or scarring at sites of injury; (c) protect the microvasculature and stimulate angiogenesis to improve perfusion; and (d) stimulate the mitogenesis of tissue-intrinsic progenitor cells [1–4]. Additionally, MSCs may play specific roles as modulators in the maintenance of peripheral and transplantation tolerance, autoimmunity, tumor evasion, as well as fetal–maternal tolerance [4,5]. MSCs influence all components of the immune system as shown for T-, B-, natural killer- (NK-), monocytic and dendritic cells *in vitro* and *in vivo* [6,7].

In vivo MSCs were effective when infused before the onset of inflammatory processes, but were also effective when administered at the peak of disease, suggesting amelioration of active inflammatory processes. Consequently, MSC-based therapy may be effective in preventing anti-donor reactivity and reducing active rejection in organ transplantation [8]. Particularly interferon-gamma (IFNg) and tumor necrosis factor-alpha (TNFa) are powerful inducers of immunosuppressive activity supposedly via the upregulation of indoleamine 2,3-dioxygenase and prostaglandin E2 respectively [9]. The requirement of a pro-inflammatory environment for MSC activation into effective anti-inflammatory cells was recently shown by Waterman et al. [10]. Danger signals released following most tissue pathologies lead to the secretion of immune modulating

Abbreviations: MSCs, mesenchymal stromal cells; RTx, renal transplantation; SCr, serum creatinine; Hpf, high power field; TMA, thrombotic microangiopathy; HUS, hemolytic uremic syndrome.

* Corresponding author at: University Medical Center Hamburg-Eppendorf, Dept. of Hepatobiliary Surgery and Transplantation, Martinistraße 52, 20246 Hamburg, Germany. Tel.: +49 40 7410 56136; fax: +49 40 7410 43431.
E-mail address: martina.koch@uke.de (M. Koch).

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factors and subsequently to a polarization of MSCs toward an anti-inflammatory MSC2 or pro-inflammatory MSC1 phenotype.

We showed in rodents with ischemic acute kidney injury that infusion of bone marrow (BM)-derived MSCs was protective and accelerated tissue repair and return of renal function [11,12]. The beneficial effects of MSCs in rodents were recently confirmed in a phase I clinical trial [13].

In solid organ transplantation, several studies in experimental models suggest that MSCs have immunomodulatory capacities but the impact on graft survival is controversial [14,15]. In humans, very limited studies are available. The group of Tan et al. [16] showed positive effects of MSCs as induction therapy in living related kidney transplantation (n = 106). Remuzzi's group presented negative effects when MSCs were given after RTx (n = 2) but no side effects when given before RTx (n = 2) [17,18].

2. Objective

Due to their properties that enhance tissue repair and modulate immune response, MSCs are of interest in the adjuvant management of ischemia–reperfusion injury, transplant rejection and chronic allograft nephropathy. The aim of our study was to evaluate the impact of isogeneic MSCs in different applications (i.a. and i.v.) at the time of transplant on allograft rejection in a fully MHC mismatched rat renal transplant model.

3. Materials and methods

3.1. Mesenchymal stromal cells (MSCs)

In this experimental setting we used isogeneic MSCs. Bone marrow was procured from Lewis (LEW) rats (recipient strain) by flushing femurs and tibiae. Cells were resuspended in DMEM/Hams-F12 medium supplemented with 20% preselected fetal bovine serum (both from Biochrom, Germany) and 2 mol/l L-glutamine (Gibco, Germany) and seeded in tissue culture flasks (Greiner, Germany). Plastic adherent cells were grown to near confluency, passaged and stored in liquid nitrogen as passages 3–4 and used as a working cell bank. Expanded MSCs were characterized for their phenotype using flow cytometry and differentiation capability into adipogenic, osteogenic and chondrogenic lineages as described previously [19,20]. Cells of passages 7–9 were used throughout all transplantation experiments. No antibiotics were used for cell expansion to avoid sublevel microbial contamination. Regular testing for mycoplasma was performed.

3.2. Kidney transplantation

Animal experiments were approved by the local ethical committee (No. 49/09) and performed according to local and EU guidelines. Male LEW rats (LEW, $RT1^l$) (Charles River, Germany) received a kidney graft from weight and age matched LEW.1U rats (LEW.1U, $RT1^u$) (Zentrales Tierlabor, Medizinische Hochschule Hannover, Germany). Sharing the same genetic background, donor and recipient differed completely in MHC haplotypes, resulting in MHC class I (RT1.A and RT1.C) as well as MHC class II (RT1.B/D) incompatibilities [21].

Life sustaining RTx was performed as previously described [22]. Graft ischemia time was limited to 30 min. The left kidney of the recipient was removed during transplantation whereas the right kidney was excised 5–7 days post-transplantation. However, in day 3 controls, both native kidneys were removed during transplant.

3.3. Experimental groups

3.3.1. Control groups

Rats received a kidney graft from either the same strain (isogeneic control (iso control) group; LEW → LEW, n = 6) or a fully MHC-mismatched congeneic strain (allogeneic control (allo control) group; LEW.1U → LEW, n = 8). While 3/6 animals in the iso control group were sacrificed at day 3, 3 animals were designated for harvest at 24 weeks. In the allo control group, the experiment was terminated at day 30.

3.3.2. Intervention groups

To evaluate the tolerability of MSC injection into the suprarenal aorta (i.a.) directly following transplant surgery, isogeneic kidney transplantation was performed. Animals were sacrificed at day 3 post-transplant (iso MSC i.a. day 3, n = 5) or 24 weeks after transplant (iso MSC i.a., n = 6) to monitor long-term effects.

To evaluate a more clinically relevant application mode, MSCs were injected intravenously after transplantation (iso MSC i.v., n = 6). This group was to survive for 24 weeks.

Both application forms were applied in allogeneic transplantation: allo MSC i.a. (n = 5) and allo MSC i.v. (n = 11). The experiment was terminated at day 30, matching the allo control group. No immunosuppressants were used in any experiments. Each animal received 1.5×10^6 MSC with a viability of >97% determined with Trypan blue. Experimental groups were summarized in Table 1.

3.4. Renal function assays

Body weight and graft function were monitored weekly for iso groups and in alternate days for the allo groups. Serum-creatinine (SCr) level was analyzed with Reflovet Plus (Roche Diagnostics, Switzerland; detection limit 0.5 mg/dl). Urine (U)-albumin concentrations (collected after 24 h in metabolic cages every other week) were quantified by competitive ELISA specific for rat albumin (Nephrat II, Exocell Inc., USA).

3.5. Histopathology

Morphological studies were performed by light microscopy. Kidney grafts and lungs were fixed in buffered formalin. Paraffin sections were stained with hematoxylin–eosin, periodic acid-Schiff and Masson's trichrome and evaluated according to the Banff working classification [23,24] by a pathologist blinded to the experimental groups.

For immunohistochemistry on frozen sections, the following mAbs were used: R73 (rat TCR constant determinant; BioLegend, Germany), ED1 (rat tissue macrophages, monocytes and dendritic cells), 10/78 (CD161, NK cells) (both from Serotec, Germany), Ki-B1R (rat pan B-cell marker; Dianova, Germany), and 3.4.1 (CD8, BD Biosciences, Germany). Single staining techniques were performed as described previously [25]. Briefly, 5 μm sections were blocked, incubated with primary antibody, washed and treated with peroxidase-coupled rat-anti-mouse IgG (Dianova, Germany). Peroxidase activity was visualized with 3-amino-9-ethyl-carbazole. Sections were counterstained with Mayer's hemalaun (Merck, Germany).

Table 1
Experimental groups used in the transplantation setting.

Group	n	Donor/recipient	Treatment	Scheduled termination of experiment
Iso control	3	LEW/LEW	None	Day 3
	3			Week 24
Iso MSC i.a.	5	LEW/LEW	1.5×10^6 MSC i.a.	Day 3
	6			Week 24
Iso MSC i.v.	6	LEW/LEW	1.5×10^6 MSC i.v.	Week 24
Allo control	8	LEW.1U/LEW	None	Day 30
Allo MSC i.a.	5	LEW.1U/LEW	1.5×10^6 MSC i.a.	Day 30
Allo MSC i.v.	11	LEW.1U/LEW	1.5×10^6 MSC i.v.	Day 30