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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

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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.

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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

Graft infiltrating cells within the renal cortex were counted in a 400-fold high power field (hpf) and expressed as mean ± standard deviation (SD) of ten hpfs per section. Four representative animals of each group (except iso control; n = 3) were analyzed for graft infiltrating cells by an independent investigator.

3.6. Gene expression examination with quantitative RT-PCR

Total RNA was isolated and purified using RNeasy Mini Kit (Qiagen, Germany) or Nucleospin II RNA Kit (Macherey-Nagel GmbH & Co. KG, Germany). The quantity and quality of RNA were determined using Infinity M200 (Tecan, Germany). Complementary DNA was synthesized using SuperScript® III Reverse Transcriptase (Invitrogen, Germany) or high capacity cDNA Reverse Transcription Kit (Applied Biosystems/Life Technologies GmbH, Germany).

Quantitative real-time PCR (qRT-PCR) for B-cell activating factor receptor (BAFFR) and interleukin-2 (IL2) was performed on a Thermocycler MX3000P (Stratagene, Germany) using SYBR Premix Ex Taq (Lonza, Switzerland) with the following primers: 5′-agc tca gtg gag ccc agt tc-3′ and 5′-ccg aag gag tcc agc aag agt-3′ for BAFFR, and QT00185360 (Qiagen, Germany) for IL2. Gene transcriptions were normalized to GAPDH (QT00199633; Qiagen). Amplification of tumor necrosis factor-alpha (TNFa) and interferon-gamma (IFNg) genes was performed on an Eppendorf realplex^2 Mastercycler (Eppendorf, Germany) and normalized to β-actin with the following primers: 5′-tcg agt gac aag ccc gta gc-3′, 5′-ctc agc cac tcc agc tgc tc-3′, probe 5′-cgt cgt agc aaa cca cca agc aga-3′ for TNFa, 5′-aac agt aaa gca aaa aag gat gca tt-3′, 5′-ttc att gac agc ttt gtg ctg g-3′, probe 5′-cgc caa gtt cga ggt gaa caa ccc-3′ for IFNg, 5′-gta caa cct cct tgc agc tcc t-3′, 5′-ttg tcg acg acg agc gc-3′, probe 5′-cgc cac cag ttc gcc atg gat-3′ for β-actin. The mRNA expression level was calculated by the ΔΔCt method in comparison to naive LEW rats (n = 3).

Representative samples of the renal cortex of each group were used: iso MSC i.a. n = 5, iso MSC i.v. n = 5 (except IL-2 n = 4), allo control n = 6, allo MSC i.a. n = 5, and allo MSC i.v. n = 5.

3.7. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 6.0b for Mac OS X (GraphPad Software, USA). Ordinary one-way ANOVA with Bonferroni correction and non-parametric Kruskal–Wallis test with Dunn's multiple comparisons test were used to compare multiple group means.

4. Results

4.1. Intraarterial isogeneic MSC application was well tolerated in the short term in an isotransplant model

In order to evaluate MSC effects on ischemia–reperfusion injury, we first tested the short term impact of MSC injection within 3 days.

Intraarterial application of MSCs was well tolerated. At day 3, the experiment was terminated and intragraft cytokine transcriptions were evaluated by real time PCR. The transcription rate of IFNg was non-significantly down-regulated compared to that of the control group (due to large data variations) while there was no difference in TNFa transcription (Fig. 1). The BAFFR transcription rate was very low in both the iso and the iso MSC i.a. groups at day 3. There were no significant differences between both groups (data not shown).

4.2. Long term follow up of MSC application in kidney transplantation revealed non-renal side effects of cell therapy

While in the iso control group animals successfully survived to 24 weeks, 3/6 animals scheduled for 24 weeks of survival after MSC i.a. treatment died 5–30 days post-transplantation. Following alternative i.v. MSC application, 3/6 animals died at 5–14 days. Three animals survived in the long term (>24 weeks) in both groups (Fig. 2a). Iso group deaths were unrelated to renal insufficiency since 5/6 animals that died before the experiment's end had a SCr level below 1 mg/dl (not shown). One iso MSC i.a. group animal suffered from pyelonephritis 30 days post-transplant and was sacrificed with a SCr level of 3.2 mg/dl. The other two animals in this group were sacrificed at day 5, prior to nephrectomy of the second kidney due to a severely impaired general condition. In the iso MSC i.v. group, one animal that died 11 days post-transplant was diagnosed with a lung infarction by histopathology.

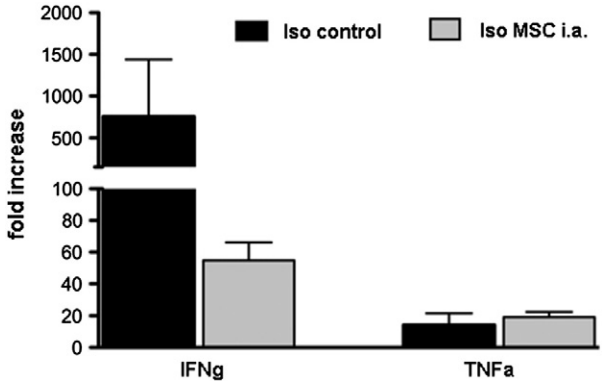

Fig. 1. Effects of intraarterial MSC application. Intragraft mRNA expression levels at day 3 (mean ± SEM) after isogeneic kidney transplantation with MSC injection (iso MSC i.a., n = 5) or without (iso control, n = 3) are shown as mean fold increase of the target genes IFNg and TNFa in the test samples compared to the values in the kidneys of 3 naive rats ($p > 0.05$).

The long-term surviving iso MSC group animals showed no signs of renal insufficiency. SCr level was 0.5 mg/dl in all animals 24 weeks post transplantation and similar to the iso control group. Mean U-albumin excretion was not increased in the iso MSC i.a. (n = 3, mean 70 mg/dl; range 46–77 mg/dl) and iso MSC i.v. (n = 3, mean 54 mg/dl; range 24–94 mg/dl) groups compared to that in the iso control group (n = 3, mean 91 mg/dl; range 14–174 mg/dl).

4.3. MSC application in allogeneic kidney transplantation led to improved renal function at day 7 post-transplantation, but also led to decreased recipient survival

After transplantation of a fully MHC mismatched kidney (allo control), all animals developed severe renal insufficiency within 30 days. 6/8 animals had to be sacrificed within 30 days. After MSC i.a. and i.v. injection, the day 7 SCr level was lower in the treated animals compared to that in the allo control group (mean SCr level 2.9 ± 1.1 mg/dl vs.

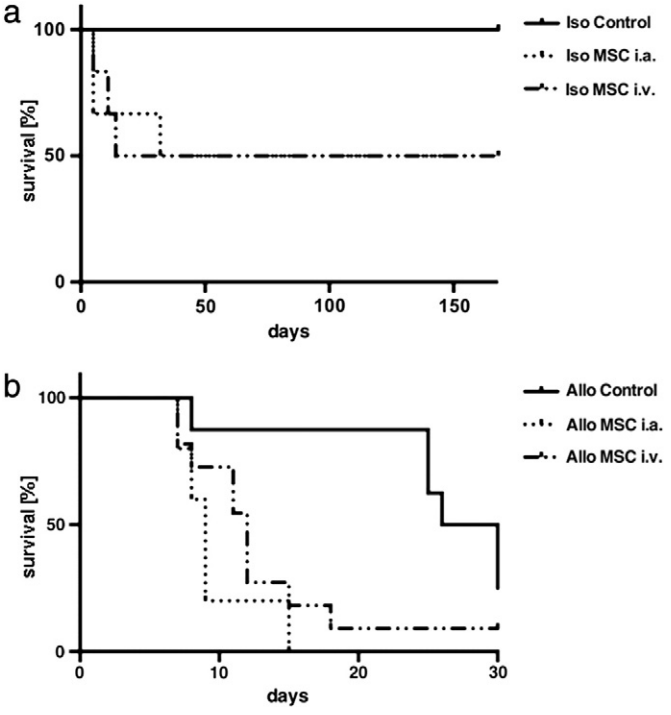

Fig. 2. Survival of animals after kidney transplantation. The Kaplan–Meier survival curves are shown for (a) the isogeneic and (b) the allogeneic kidney transplanted animals without (iso/allo control groups) or with intravenous/intraarterial MSC injections (iso/allo MSC i.a. or i.v.). *:There is a significant survival difference between control and both MSC groups ($p < 0.05$).

1.7 ± 1.2 mg/dl in the allo i.a. group and 1.3 ± 0.4 mg/dl in the allo i.v. group). As shown in Fig. 3, the difference between the allo control and allo MSC i.v. groups was statistically significant.

While the allo control group showed a median survival time of 28 days (range: 8–>30 days), statistically significant reductions in median survival were observed for the allo MSC i.a. and i.v. groups with 9 days (range: 7–15 days) and 12 days (range: 7–>30 days), respectively (Fig. 2b).

4.4. MSC injection resulted in reduced lymphocyte infiltration in grafts

To investigate the mechanism of allograft rejection, we evaluated the infiltration of B-cells, T-cells, macrophages/monocytes, CD8 + cells and NK-cells in the harvested kidney.

In all iso groups the infiltration of cells was mild (Fig. 4). The gentle increase of ED1 + cells in the iso MSC i.a. and i.v. groups was not statistically significant, but the number of ED1 + cells was higher in the kidney of animals sacrificed within the first 2 weeks of RTx (animals surviving 24 weeks: 5 ± 4 cells; days 5–14: 25 ± 16 cells). There were no significant differences for T- and B-cells between the three iso groups.

In the allo groups, massive cell infiltrates were found (Figs. 4, S1). All cells analyzed were significantly increased compared to the iso groups. Macrophages/monocytes were the dominant cell infiltrate in all allo groups, with levels not differing significantly between groups. NK-cells and CD8 + cells increased in all allo groups, without significant influence of MSC application (Fig. 4).

4.5. Histopathological evaluation revealed reduced interstitial infiltration, TMA and infections in the grafts

To analyze the reason of graft failure and animal deaths, we next evaluated renal and lung morphologies.

All 6 animals of the allo control group showed histological characteristics of acute rejection and chronic lesions (interstitial fibrosis and tubular atrophy) according to Banff criteria. Prominent interstitial inflammation (ai) (Banff grades 2–3) was accompanied by glomerulitis (ag) and glomerulopathy (cg) in all animals (Fig. 5a).

Kidneys from the iso groups lacked histological signs of rejection. One of the 4 iso MSC i.a. animals was revealed to have suffered from pyelonephritis, when the animal was sacrificed after 4 weeks. In the iso MSC i.v. group, one animal sacrificed on day 11 post-transplantation displayed lung infarction and in another, glomerular thrombotic microangiopathy (TMA) was demonstrated on day 14 after RTx (Fig. 5b).

In the allo MSC groups, interstitial inflammation was less prominent compared to the allo control group (ai 0–1 vs. ai 2–3). In the allo MSC i.v. group infarctions in the kidney grafts were diagnosed in 4/7 kidneys (Fig. 5c). Also, 4/7 kidneys revealed histological characteristics of infection (pyelonephritis, abscesses), and TMA was present in two grafts. In the allo MSC i.a. group 3/5 animals were diagnosed with pyelonephritis (Fig. 5d). These unusual histological features were not observed without MSC application.

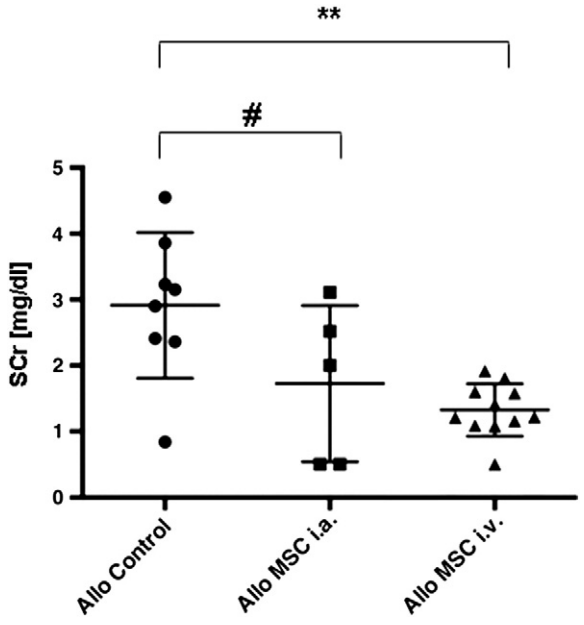

Fig. 3. Intravenous MSC injection improved renal function in allogeneic kidney transplanted animals. Serum creatinine (SCr) level 7 days post-transplantation was significantly lower in the animals treated intravenously with MSCs (i.v.) but not intraarterially (i.a.) compared to allo controls. Shown are the mean ± SD. $^{**}p < 0.005$, $\#p > 0.05$ (not significant).

4.6. Intra-arterial MSC injection resulted in reduced inflammation-associated gene expression

The immunological processes in transplanted isogeneic and allogeneic kidneys under MSC treatment were further characterized by analysis of TNFa, IL2, IFNg and BAFFR transcriptions. In the grafted kidneys of the allo control group, cytokines IL2 and IFNg were significantly upregulated compared to the iso control group indicating ongoing inflammation and T-cell reactivity (Fig. 6). Additionally, BAFFR was significantly upregulated, indicating B-cell compartment involvement in rejection processes. As shown in Fig. 6, IFNg, IL2 and BAFFR transcriptions were comparable between the allo control and allo MSC i.v. groups suggesting an insufficient local effect in kidney grafts after systemic MSC administration. In contrast, these three values were significantly decreased in the allo MSC i.a. group compared to the allo control group ($p < 0.05$), approaching levels for iso-transplanted kidneys. For TNFa, the expression levels of both the allo control and allo MSC groups were similar and only marginally increased compared to those of the iso MSC groups without statistical differences.

5. Discussion

Long-term results of organ transplantation remain unsatisfactory, mainly because of chronic rejection and complications associated with immunosuppressive medications [26,27]. Therefore new treatment regimens are required to improve the long-term survival of transplanted organs and reduce the side effects of immunosuppression. The application of MSCs has reduced ischemia–reperfusion injury in experimental models and clinical studies. For organ transplantation, the reported results are controversial. The aim of this study was to modulate the immune response in a rat allogeneic kidney graft model comprising isolated but complete MHC incompatibility (LEW.1U to LEW).

We first evaluated whether MSCs influence ischemia–reperfusion injury by using an iso transplant model. MSCs were injected in the suprarenal aorta after graft reperfusion and rats were sacrificed at day 3. De Martino et al. [28] confirmed the protective effects of MSCs injected i.a. in kidney transplantation in reducing inflammatory response and conserving organ function within a 7 day observation period. Concordantly, we demonstrated that i.a. application was feasible and safe within the first 3 days in our model. IFNg transcription was clearly but non-significantly downregulated in animals with MSC application, suggesting immune-modulatory capacities.

These data encouraged us to evaluate extended MSC effects in the same iso transplant model. Surprisingly, many animals died after intraarterial cell injection. Two animals were sacrificed due to severely impaired general condition before contralateral nephrectomy (day 5), with one retrospectively diagnosed with pyelonephritis. It cannot be excluded that MSCs caused ischemia of the kidney or other abdominal organs since cells reach the capillary system directly via i.a. application [11,12].

To avoid such side effects, an iso-transplanted group with i.v. MSC injection was generated. It has been described that cells are captured in the lung after i.v. injection [29]. Therefore, death by lung infarction of one iso MSC i.v. rat could be explained by the trapping of MSCs in the lung. However, TMA was unexpectedly diagnosed in the kidney graft of another animal of the iso MSC i.v. group. Such occurrences are observed in hemolytic uremic syndrome (HUS) or disseminated intravascular coagulopathy. In our transplant model it is unlikely that this histological finding can be explained by HUS but it might be coagulopathy associated.

An even higher number of unexpected deaths occurred in the allo MSC groups. While in the allo control group, most animals survived for >25 days, the experiment had to be terminated before day 15 in all animals of the allo MSC i.a. group. While SCr level was lower on day 7 post RTx and at harvest compared to the allo control group (1.6 vs. 2.1 mg/dl), animals of the MSC groups lost >20% of body weight (data not shown) and had to be sacrificed. Histopathological evaluation of the organs harvested revealed unexpected diagnoses. MSC application i.a. led to granulocyte accumulation and histological indications of nephritis in 3/5 animals. Bacteria were occasionally found in some

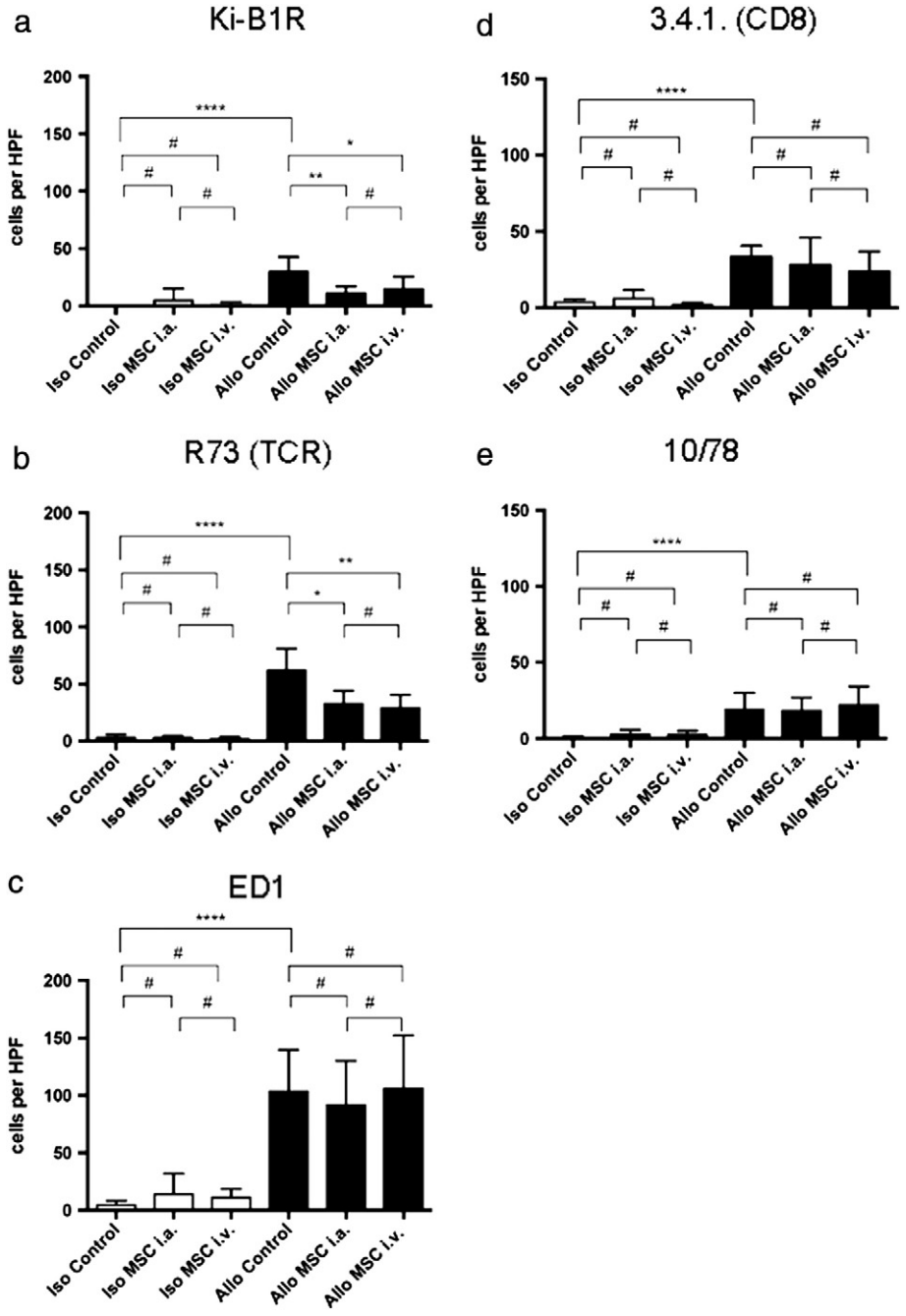

Fig. 4. MSC injection predominantly led to macrophage infiltration in allo-transplanted animals. Graft infiltrating (a) B-cells (KiB1), (b) T-cells (R73), (c) macrophages/monocytes (ED1), (d) CD8 + cells (3.4.1.) and (e) NK (10/78) cells in the kidney at the time of death of the animals. Cells were counted in 10 high power fields by an investigator blinded to the treatment groups (iso control n = 3, all other groups n = 4). Shown are the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{****}p < 0.0001$, $\#p > 0.05$ (not significant).

MSC group kidney grafts, and were absent in control groups. Interestingly, interstitial inflammation was lower in both allo MSC groups compared to the allo control group according to the Banff criteria.

Most confusing were the results of the allo MSC i.v. group. While in the iso MSC i.v. group, the long-term surviving animals did not show any pathology, several kidney grafts of the allo MSC i.v. group revealed infarct zones, granulocyte infiltration and signs of infections. Interestingly, MSC treatment was identified as a risk factor for pneumonia-related death after stem cell transplantation in patients [30]. The strong immune-suppressive effect of MSCs might explain the infections in both i.a. and i.v. MSC-injected animals since no infections were found in animals that were not injected with MSCs in our experiments.

Additionally, two animals were diagnosed with TMA, as was observed for one iso MSC i.v. group animal. This unexpected histological finding together with the sudden death of several animals in the iso and allo MSC groups indicates that MSC injection, especially i.v., might induce fatal disseminated intravascular coagulopathy. Recently, Moll et al. [31] reported innate immune attack elicited by extended culture-expanded human MSCs. This "instant blood-mediated inflammatory reaction" led to arterial microinfarcts, complement/coagulation cascade activation, activated platelets binding to cells, and clot infiltration by neutrophils and monocytes, eventually leading to cell destruction. Concordantly, a graft dysfunction associated with intragraft neutrophil and complement accumulation was reported in the first two patients treated with autologous MSCs after allogeneic kidney transplantation [18]. The same group also revealed the pro-inflammatory effects of MSCs administered i.v. post-transplantation in a mouse model [32] with increased expression of IL6 and TNFa and complement C3 accumulation

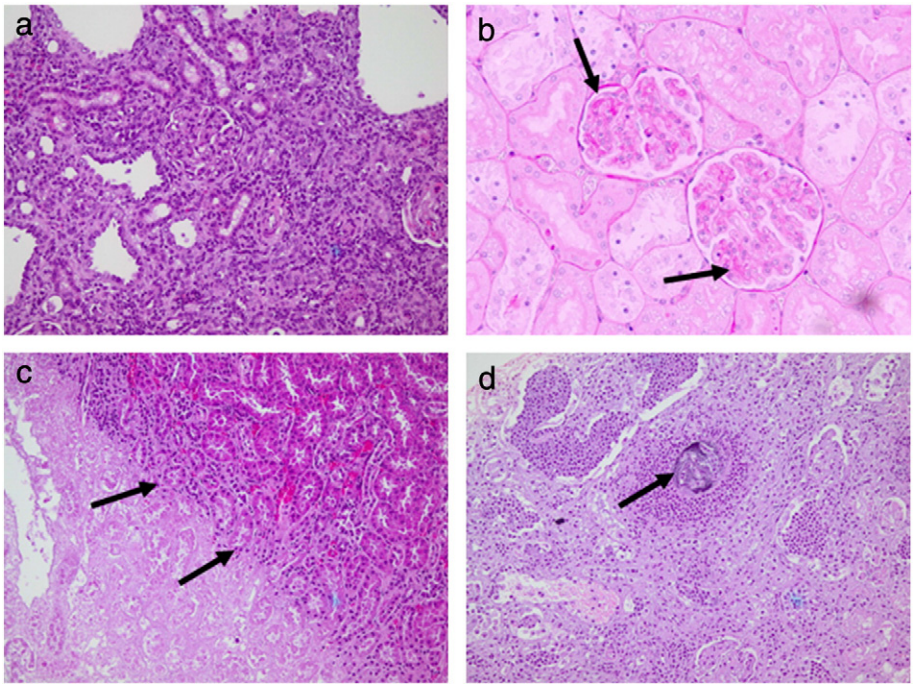

Fig. 5. Side effects of MSC injections in kidney transplantation. (a) Representative picture of rejection in an animal of the allo control group at day 30 post-transplantation (Banff-classification: i3, t1, g2, v0, ptc3, ci3, ct3, cg1). (b) Thrombotic microangiopathy (TMA) in the glomerulum of a kidney from the iso MSC i.v. group (arrows mark thrombotic material in the glomerular capillaries). (c) Renal infarction seen in the allo MSC i.v. group. Arrows mark the demarcation line. (d) Accumulation of granulocytes and bacteria (arrow) in a representative graft of the allo MSC i.a. group. Magnification ×200 for a, c and d, and ×400 for b.

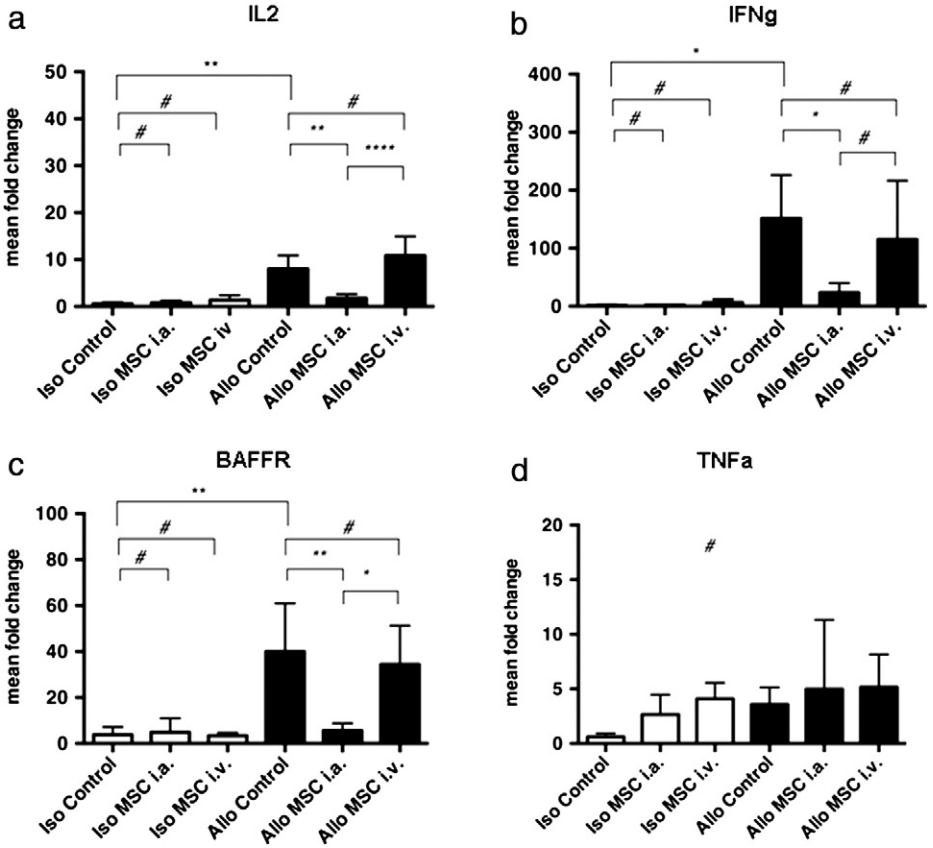

Fig. 6. Comparative gene expressions in transplanted kidneys of MSC treated rats. Shown are the values of intragraft transcription of (a) IL2, (b) INFg, (c) BAFFR and (d) TNFa in all experimental groups compared to normal kidneys. Shown are the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{****}p < 0.0001$, $\#p > 0.05$ (not significant).

in grafts. Thrombotic complications might explain the sudden deaths of some animals, but it remains unclear whether effects were caused by immunoreactions toward culture-expanded MSCs or were inherent to the strain and model used. Altogether, TMA has been observed in 3 of 15 animals (20%) after MSC i.v. injection but was not observed in any animal of the i.a. groups. From 28 animals treated with MSCs only 7 survived in the long term until the scheduled end of the experiment. Therefore we postulate that MSCs indeed caused the observed side effects, also because cell preparations were exchanged between two laboratories involved in this study with identical results.

Nevertheless, several immune modulatory effects were observed in our model. Kidney function at one week post-transplant and at harvest was improved by MSC treatment. B- and T-cell infiltrates in MSC-treated allogeneic transplants clearly were reduced compared to allogeneic transplants without cell therapy. It cannot be ruled out, that the differing time points of graft harvest after transplant impacted the graft infiltrating cells. Remarkably, cells of the innate immune system (macrophages/monocytes, NK cells) were unchanged between the allo groups while B- and T-cell infiltrates were reduced in the MSC groups.

In the isogeneic setting, MSCs seem to lack pro-inflammatory effects since cell infiltrates were not significantly increased compared to the control group. Furthermore, neither IFNg and IL2 as markers of T-cell activity nor BAFFR as a marker for infiltrating B-cells was upregulated in the isogeneic kidney grafts after MSC application. Non-significant increases in the less specific inflammatory cytokine TNFa occurred for all groups compared to the iso control.

Recalling similar numbers of graft infiltrating cells in both allo MSC groups, it is remarkable that a statistically significant difference existed in cytokine transcription profiles for T- and B-cells between allo MSC i.a. and i.v. groups. While the allo MSC i.v. group was comparable to the allo control group, the i.a. administration of MSCs had a significant impact on IL2, IFNg and BAFFR transcription in the grafts. The difference between the allo MSC i.a. and i.v. groups might be explained by the fact that MSCs were captured in the lung after i.v. injection [33] and therefore cannot locally influence the kidney graft and graft infiltrating cells. Indeed, De Martino et al. [28] concluded that the immune-modulating effects of MSCs strictly depend on the high concentrations at the immunogenic injury site. Nevertheless, the improved kidney function at day 7 in the allo MSC groups and the decreased T- and B-cell infiltrates in the allo MSC grafts compared to the allo control grafts indicate an impact of MSC administered i.v. and i.a. on allograft rejection.

Additionally the time point of MSC application appears critical. Casiraghi et al. demonstrated in a mouse model that post-transplant MSC i.v. infusion caused premature graft dysfunction and failed to prolong graft survival [32]. In contrast, pre-transplant MSC infusion induced a significant prolongation of kidney graft survival by a Treg-dependent mechanism. Very recently, a clinical study with 2 patients supported the mouse data [17]. Similar results were published by Popp et al. [34] and Eggenhofer et al. [35] in a heterotopic heart transplant model with concurrent mycophenolate mofetil immunosuppression. However, these findings may not apply to kidney transplantation as a heterotopically transplanted heart does not fulfill life-sustaining activity like a transplanted kidney. Furthermore, microvascular damage like glomerular lesions may not be investigated using a heterotopic heart model. Indeed, using the identical protocol for kidney transplantation, enhanced humoral immune responses verified by intragraft B cell infiltration and complement factor C4d deposits were revealed [20]. Pre-treatment with either isogeneic or allogeneic MSCs resulted in higher degrees of kidney cortex damage and elevated SCr levels. Complement deposits measured in this study may have been caused by either humoral rejection or non-specific coagulopathy. Obviously, the time point, administration route and concurrent immunosuppression must be optimized for each manner of solid organ transplantation and experimental model. In our study we have deliberately chosen the MSC application after RTx because this is the most probable mode of clinical application in deceased donor organ transplantation. Several groups provided evidence for positive effects of MSC administration after RTx. Franquesa et al. showed reduction of interstitial fibrosis and tubular atrophy [36] in a rat model. However, in this low responder model (F344 to LEW) the MSCs were injected 11 weeks after RTx. In humans, this strategy of delayed MSC infusion (day 7) resulted in an increase of SCr level in two patients [18]. On the contrary, Tan et al. reported reduced acute rejection and faster recovery of renal function [16] in living donor RTx injecting MSCs after reperfusion and 2 weeks later. Reinders et al. revealed systemic immunosuppression through MSCs [37] which were given months after RTx. Interestingly, three of the 6 MSC treated patients developed opportunistic viral infections. Although these results do not unambiguously support the idea that MSCs are most effective in highly inflammatory situations, i.e. shortly after the kidney transplantation, it suggests new strategies for successful long-term improvements after organ transplantation using MSCs.

We conclude from our study that the mechanism of action of isogeneic MSCs in a kidney transplant model is not completely understood. MSC therapy improved kidney function in the first week after transplantation and reduced T-cell mediated inflammation and local T- and B-cell infiltration especially when applied i.a. There is no doubt that MSCs influence the immune system, but this might be counteracted by negative not yet fully explained effects in alloimmunity. Due to several unexpected results including renal and lung infarction, TMA and infectious complications after MSC application, caution is advised regarding MSC application in allogeneic organ transplantation.

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Disclosure

All authors of this manuscript have no conflicts of interest to disclose.

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