5th Session: IRI and cells

Keynote lecture: Mesenchymal stromal cell therapy in ischemia/reperfusion injury : review of the experimental and clinical evidence
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O13 ADMINISTRATION OF THIRD-PARTY MESENCHYMAL STROMAL CELLS AT THE TIME OF KIDNEY TRANSPLANTATION: INTERIM SAFETY ANALYSIS AT ONE-YEAR FOLLOW-UP

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Introduction: Mesenchymal stromal cells (MSC) therapy has been suggested in kidney transplantation (KTx). We report on the 1-year follow-up of an open-label phase I trial using MSC at the time of KTx.
Patients and Methods: On postoperative day 3 (D3), third-party MSC (~2.0 $\times$ 10^6/kg) were administered to 7 non-immunized first-transplant recipients from deceased donors, under standard immunosuppression (Basiliximab, Tacrolimus, MMF and steroids). No HLA matching was required for MSC donors. In parallel, 7 comparable KTx recipients were included as controls. Informed consent was obtained from all participants.
Results: No hemodynamic or immune-allergic side-effect was noted at the time of MSC injection. Still, 1 patient with a history of ischemic heart disease had a NSTEMI ~3 h after MSC infusion. Ten months after KTx, 1 MSC patient had type B aortic dissection and STEMI. Four MSC patients had at least 1 opportunistic infection, whereas 3 controls had polyoma-BK viremia. Three MSC patients were affected by at least 1 (pulmonary) infection, whereas 3 controls had urinary infection. No MSC engraftment syndrome was observed. At D14, eGFR in MSC and control groups was 47.1 $\pm$ 6.8 and 39.7 $\pm$ 5.9 ml/min, respectively (p, 0.05). Nevertheless, eGFR in MSC and control groups at 1 year was 43.1 $\pm$ 17.8 and 53.9 $\pm$ 13.4 ml/min, respectively (p, 0.25). At 3-month protocol biopsy, borderline rejection (BR) was evidenced in 1 MSC patient. Later on, 1 BR and 1 AR were diagnosed at D240 and D330, respectively. No biopsy-proven AR was noted in controls. Three patients developed anti-HLA antibodies against MSC (n = 1) or shared kidney/MSC (n = 2) mismatches.
Conclusions: MSC infusion was safe in all patients except one. Incidence of opportunist and non-opportunist infections was similar in both MSC and control groups. No MSC engraftment syndrome was documented. No difference in eGFR was found at 1 year *post* KTx. Putative immunization against MSC was observed in 3 patients.

O14 CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM PORCINE ADIPOSE TISSUE AND THEIR EFFECTS ON KIDNEY GRAFT RECOVERY IN A PRECLINICAL PORCINE MODEL OF RENAL AUTO-TRANSPLANTATION MIMICKING THE NON-HEART-BEATING-DONOR CONDITIONS

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Introduction: Ischemia reperfusion (IR) is a key process involved in acute and chronic renal graft dysfunction. The objective of this study was to characterize mesenchymal stem cells from porcine adipose tissue (pASC) and their role in the graft function recovery in conditions mimicking Non-Heart-Beating-Donors (NHBD).
Materials and Methods: Morphology, proliferative capacities, phenotype by flow cytometry and the metabolic profile in Nuclear Magnetic Resonance (NMR) of porcine ASC (pASC) were determined. Their resistance to a sequence of hypoxia-reoxygenation (HR) was tested by analyzing their viability and metabolic profile in NMR. Feasibility, functional and histological outcomes of an autologous injection of 10^6 pASC/kg in the renal artery of 3 auto-transplants kidneys after 1 h of warm ischemia and 24 h of storage at 4°C in UW solution and contralateral nephrectomy were compared to a group of autotransplanted pigs without injection of pASC.
Results: The cell extraction technique was reproductible and allowed having sufficient pASC with the characteristics of mesenchymal stem cells. The metabolic profile in NMR of pASC was not changed with the passages, characterizing the stability of the cell lines. The cell viability after a sequence of HR exceeded 70%. The injection of 10^6 pASC/kg was practicable 15 days after removal of adipose tissue. The function recovery was significantly improved and the histological lesions were significantly reduced in the group treated by pASC.
Conclusion: Injection of pASC in renal graft artery at reperfusion of the grafts in a porcine model mimicking Non-Heart-Beating-Donors conditions could improve graft function recovery and limits tubular damages at day 7. These therapeutic potentials will be confirmed by further studies at the end of the follow-up at 3 months of 6 animals.

O15 CONTRIBUTION OF GD T-CELL SUBSETS AND IL-17A ACTIVATION TO RENAL ISCHEMIA REPERFUSION INJURY IN MICE

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Background: Ischemia reperfusion injury (IRI) contributes to acute kidney injury (AKI) and to delayed graft function (DGF) after kidney transplantation. After initial activation of myeloid cells in the first 48 h after IRI, T-cells invading the renal tissue are relevant producers of the pro-inflammatory mediator IL-17A. In this project, we evaluated the role of T-cell subsets (ab versus gd T-cells) and IL-17A on inflammation and fibrosis induced by ischemia reperfusion injury in mice.
Methods: IRI was induced by unilateral clamping of the renal pedicle for 45 min and mice were sacrificed after 7 days when infiltrating T-cells were dominant. In a second model IRI induced delayed graft function (DGF) was studied after allogenic transplantation (ktx) and again leukocyte composition was studied at day 7 and compared to IRI alone. T-cell receptor (TCR-gd) and IL-17A deficient and wildtype (WT) control mice were tested in the IRI model as well. FACS analysis, histology and immunohistochemistry for inflammation and fibrosis as well as qPCR was done.
Results: IRI and ktx resulted in substantial T-cell infiltration but the distribution of T-cell subsets were different. In IRI ab T-cell infiltrates were 2.5 fold higher compared to gd T-cells whereas in the combination of IRI with ktx ab T-cell infiltrates were about 8 fold higher compared to gd T-cell infiltrates. The gd T-cells contributed substantially to elevated IL-17A production. Surprisingly, gd T-cells and IL-17A deficient mice were not protected from IRI and showed progressive renal fibrosis similar to WT mice. In both mouse strains (TCR-gd and IL-17A deficient mice) IL-17A production of gd T-cells was totally abrogated in ex vivo T-cell stimulation with PMA/ionomycin.
Conclusion: Surprisingly, neither gd T-cell nor IL-17A deficiency attenuated IRI, inflammation or tissue fibrosis.

O16 ENDOTHELIAL MICROPARTICLES RELEASED BY ACTIVATED PROTEIN C EXERT A CYTOPROTECTIVE EFFECT ON BETA CELLS: INTEREST IN ISLET PANCREATIC TRANSPLANTATION

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During islet transplantation, Ischemia/reperfusion leads to inflammation and graft loss. Early events combine endothelial damage, the local recruitment of leukocytes and activation of coagulation. The Activated Protein C (APC) limits thrombin generation and exerts endothelial cytoprotection by targeting the Protease Activated Receptors (PARs). In blood flow, procoagulant microparticles (MP) shed from the plasma membrane of activated cells are cellular effectors. APC-treated endothelial cells (EC) release MP bearing protein C receptor. This study characterized the MP shed by APC-treated EC or β-cells and compared their effects on β target cells submitted to oxidative stress.

Rat β cells (Rinm5f) and porcine coronary artery EC were treated with 2–70 nM APC (Xigris®) for 24 h. Washed MP isolated from EC supernatant were applied for 6 h to Rinm5f, before addition of 100 μM H202. After 24 h, apoptosis was assessed by hypodiploïde DNA staining, secreted insulin by ELISA; expression of glycosylated PAR, endothelial NO synthase (eNOS) and annexin1 (A-1) by Western blot. APC activity was measured using a chromogenic substrate, MP concentration by prothrombinase assay.

APC enhanced APC activity at both cell surfaces (rinm5f: 2.4, EC: 1.4-fold $p < 0.001$) whilst apoptosis remained low (4 $\pm$ 0.9%, 3.2 $\pm$ 0.5%). APC activated PAR1 in both cells, and up-regulated the expression of A1 and eNOS, mainly in EC. APC (>20 μM) enhanced MP release from EC and Rinm5F (by 32% and 28%) with a 6-fold rise in MP-borne APC activity ($p < 0.001$). MP from APC-treated β-cells had no cytoprotective effect. Conversely, MP from EC (>10 nM eq Phosphatidylserine) reduced the apoptosis of H_2O_2 treated β-cells (5 $\pm$ 1% vs. 21 $\pm$ 1%, $p < 0.001$), and restored insulin secretion (10 ng/ml vs. 0.8 ng/ml, n = 3) whereas APC alone remained inactive (up-to 70 nM).

MP from APC-treated EC protect β cells against oxidative stress. They may prove a promising therapeutic tool in the protection of transplanted pancreatic islets.