Supplemental MATERIALS AND METHODS

Animal Models

Surgical and imaging experiments were approved by a governmental-committee on animal welfare and were performed in accordance with national animal protection guidelines. Male Lewis–Brown-Norway (LBN) and Lewis (LEW) rats (200–270 g body weight (BW), Charles River, Sulzfeld, Germany) with free access to standard rat chow (Ssniff, Soest, Germany) and tap water were used. Surgeries were performed under anaesthesia with ketamine 100 mg/kg body weight intra peritoneal (i.p.) and xylazine 5 mg/kg BW i.p. (Xylazin, Ketamin, CEVA Tiergesundheit, Düsseldorf, Germany). Further doses of ketamine were injected as needed.

Transplantation was simultaneously performed by two investigators as published before *(19)*, *(20)*. In short, the left kidney including ureter, renal artery, a piece of aorta and renal vein were transferred into the recipient. Kidneys from age- and weight-matched LBN were transplanted into LEW (aTX). Transplantations were performed immediately after bilateral nephrectomy of the recipient. While the total operation time of the recipient did not exceed 90 min, the ischemia time of the graft was always shorter than 40 min. Grafts were recovered on postoperative day (POD)1, 4 or 7 after transplantation. The chosen aTX model leads to histological and functional changes typical for acute rejection *(19)*, *(20)*. We reported functional (e.g., polyuria, increased fractional excretion of Na^+ and K^+, changes in protein excretion pattern) and histological signs of AR occurring clearly on POD4 before *(19)*, *(20)*. As in this AR model without immunosuppression graft necrosis starts only few days beyond POD4 we decided to investigate changes until POD4. Syngeneically transplanted rats (LBN to LBN, sTX) and aTX treated with cyclosporine A since transplantation (CSA-POD0, 5 mg/kg BW/day i.p.) without AR served as controls. As acute tubular necrosis (ATN) and acute cyclosporine toxicity (CSA-TOX) are common differential diagnoses of AR, these groups were also included. ATN (as ischemia/reperfusion injury) and acute CSA toxicity were

induced as published before *(16)*, *(21)*. For ischemia/reperfusion injury, left renal artery was dissected as in TX-groups and ligated for 40 min using a microvascular clamp. The right kidney was removed. After clamp release, the returning of original surface colour of the kidneys was confirmed visually. For acute CSA-induced nephrotoxicity, rats received 50 mg/kg CSA (Sandimmun, Novartis, Nürnberg, Germany) i.p. for two days. To monitor therapy response, aTX was performed and the recipients received CSA (CSA-POD 4, 5 mg/kg/day i.p.) starting on POD4 after development of AR as confirmed by PET analysis. Blood samples for analyses of creatinine and BUN were collected by tail vein puncture. Creatinine (enzymatic measurement) and urea (urease-GLDH-method) were analyzed on a Roche Diagnostic analyzer (Modular P, Roche Diagnostics, Mannheim, Germany).

Image acquisition - PET

^{18}F-FDG was produced in a clinical routine setup on site using an RDS 111 cyclotron (CTI, Knoxville, TE, USA). Image acquisition of non-fasting subjects was performed as described before (16). In short, ^{18}F-FDG uptake was calculated from a whole body acquisition of 30 min length 3 h after injection of 30 MBq ^{18}F-FDG in 100 µl 0.9% NaCl solution into a tail vein of non-anaesthetised rats. Thereafter, the catheter was purged with additional 900 µl 0.9% NaCl solution. Rats remained in a restrainer without anaesthesia until start of the scan and were hydrated by i.v. injection of 1 ml of 0.9% NaCl solution hourly. To minimize tracer uptake in the kidneys caused by renal excretion of ^{18}F-FDG acquisitions started 3 h after ^{18}F-FDG injection. During acquisition, rats were anaesthetized with oxygen/isoflurane inhalation (2% isoflurane, 0.7 l/min oxygen) and body temperature was maintained at physiological values by a heating pad. PET scans were performed with a high-resolution multi wire chamber-based animal PET camera (quadHIDAC, Oxford Positron Systems Ltd, Oxford, UK)(22). To delineate the kidney contours the ^{18}F-FDG-PET acquisition was followed by i.v. injection of 5

MBq ^{18}F-fluoride without moving the rat in the scanner and another PET acquisition of 15 min length.

Analysis of PET images and quantitative evaluation

Images were reconstructed into an image volume of 280 x 120 mm and a voxel size of 0.8 x 0.8 x 0.8 mm, using a list-mode based resolution recovery reconstruction algorithm with no attenuation and scatter correction applied (23). A renal parenchyma volume of interest (VOI) was manually traced around the kidneys on 12 reconstructed images 2 min after ^{18}F-fluoride injection. This VOI was projected onto the ^{18}F-FDG images. The renal pelvis was carefully excluded from the VOI to ensure that no renally excreted activity was included. Mean ^{18}F-FDG uptake of the renal parenchyma was calculated by the ratio of total counts and volume.

Autoradiography

To validate the ^{18}F-FDG-PET uptake animals were sacrificed and kidneys were harvested immediately after ^{18}F-FDG-scanning on POD4 or POD7. High-resolution autoradiography (μ-imager, Biospace Measures, Paris, France) was performed as published before (16). In short, kidneys were snap-frozen, sliced into 10 μm thick cryosections and activity was measured in a mid-coronary renal slice for 3h.

Histology

Portions of kidneys were snap-frozen and fixed in 4% formaldehyde in PBS. Histological changes (glomerulitis, tubulitis, endothelialitis, and infiltration) were examined by light microscopy in paraffin-embedded tissue with PAS staining. We quantified the renal infiltration according to the ti-score (total interstitial inflammation score, analysis of 10 fields of view (FOV, 350 x 250 μm each) in the renal cortex, supplemental table 1), recently added

to the Banff classification (24). The cortex was chosen, because the extent of medullary inflammation does not reflect the degree of AR (25).

Real time PCR

Expression profiles of selected marker genes for infiltrating cells were validated by real time PCR which was performed using SYBR Green PCR Master Mix or TaqMan Universal PCR Master Mix on an ABI PRISM 7700 Sequence Detection System. Specific primer pairs were used (supplemental table 2). All instruments and reagents were purchased by Applied Biosystems (Darmstadt, Germany). Relative gene expression values were evaluated with the $2^{-\Delta\Delta Ct}$ method using GAPDH as housekeeping gene (26).

Statistics

Laboratory data was compared by ANOVA with a Bonferroni multiple comparisons test. Data is presented as mean values ± SEM (n = number of rats, samples, or experiments). Significance was inferred at the $P<0.05$ level.

Supplemental table 1: ti-score

Group	ti-score	Quantitative criteria for cellular interstitial inflammation
sTX (5), CSA POD0 (5), ATN (5), CSA TOX (5), aTX POD1 (5),	ti0	No or trivial interstitial inflammation (<10% of parenchyma)
CSA POD4 (2)	ti1	10–25% of parenchyma inflamed
CSA POD4 (3), aTX POD4 (5)	ti2	26–50% of parenchyma inflamed
aTX POD4 (4)	ti3	>50% of parenchyma inflamed

Kidneys were analyzed on POD1 (aTX POD1), POD4 (sTX, CSA POD0, CSA TOX, ATN, aTX POD4) and POD7 (CSA POD4), respectively; number of samples in brackets, ti-score according to (24); POD: postoperative day; sTX: syngeneically transplanted; aTX: allogeneically transplanted; CSA POD0: allogeneically transplanted and treated with cyclosporine A [5 mg/kg/day] i.p. since POD0; CSA POD4: allogeneically transplanted and treated with cyclosporine A [5 mg/kg/day] i.p. since POD4; ATN: acute tubular necrosis; CSA TOX: acute cyclosporine toxicity [50 mg/kg/day]for 2days i.p.

Supplemental Table 2: Sequences of primers used for real time PCR

Gene	Primer ID	Primer Sequence	Accession No.
CD4	Sense	TGTGTCAGGTGCCGGCACCAACAG	NM_012705
	Antisense	GTGGGGCCCAGGCCTCATATG	
CD8α	Sense	AGGGAATGGGATTGGGCTTCGC	NM_031538
	Antisense	CTCTGAAGGTCTGGGCTTGAC	
CD8ß	Sense	GCTTGACATGTGGCCTCATTAC	NM_031539
	Antisense	CGTGGGCGCGGATCATTTGTG	
CD20	Sense	CTGCCTTCTTCCAGAAGCTCG	NM_001107578.1
	Antisense	GGGAAGATACTCCACTTAGCTC	
CD40	Sense	GACACTGTTTGTACCTGCAAGG	NM_134360
	Antisense	TCCGACCGGGCAGGGTTGGCAG	
CD56	Sense	CTGACATGTGAAGCCTCCGGAG	NM_031521.1
	Antisense	CTCTTGCTTCTCTGGTCGAGTC	
CD66a	Sense	GTGAAGCCCGGAACCCAGCG	NM_001033860.1
	Antisense	GTCTGCATGGCAGGAGAGGTTG	
CD79a	Sense	AACTTGGGTGAGGAGGCCGTC	XM_001077003.1
	Antisense	CGGGGAAGATCAGCTTGCCC	
CD80	Sense	CGTTTGCCTGGGCAGGATCTG	NM_012926
	Antisense	GCTGCTTCCACAGGCCCTATG	
CD163	Sense	AGATGCTTCTGTGAAGTGCCTC	XM_232342
	Antisense	GTAAGCCGTTGGGTCTGTCGT	
Caspase 3	Sense	GCA TGC CAG AAG ATA CCA GTG G	NM_012922
	Antisense	GCG CGT ACA GTT TCA GCA TGG C	
Ficolin B	Sense	GAACCAATGAGCTGCGGGTGG	NM_053634
	Antisense	GTTTTGGGAAGTCAGGGAGTCAC	
Foxp3	Sense	GTGGTGCAGTCTCTGGAGCAGC	XM_001064182
	Antisense	CAGGAGCTCTTGTCCACTGAGGC	
GAPDH	Sense	CATCAACGACCCCTTCATT	NM_017008
	Antisense	ACTCCACGACATACTCAGCAC	
Granzyme B	Sense	GCGTATAATTCTAAGACAATCTCC	NM_138517
	Antisense	CCCATTGGGCCCAGCTTTCCC	

Supplemental table 3: The four outcomes in a contingency table.

		acute rejection		
		positive (n)	**negative (n)**	n
FDG-PET	**positive (n)**	24	17	41
(FDG cut-off value: 0.275% ID)	**negative (n)**	3	65	68
n		27	82	109

ID: injected dose; true positive rate: 0.89 (24 out of 27); false positive rate: 0.21 (17 out of 82)