Renal Denervation Exacerbates LPS- and Antibody-induced Acute Kidney Injury, but Protects from Pyelonephritis in Mice

Alexander M.C. Böhner,1,2 Alice M. Jacob,1 Christoph Heuser,1, Natascha E. Stumpf,1 Alexander Effland,3 Zeinab Abdullah,1 Catherine Meyer-Schwesiger,4 Sibylle von Vietinghoff,5 and Christian Kurts1,6

1 Institute for Molecular Medicine and Experimental Immunology, University Hospital of Bonn, Germany
2 Department of Radiation Oncology, University Hospital of Bonn, Germany
3 Institute for Applied Mathematics, University of Bonn, Germany
4 Institute for Cellular and Integrative Physiology, University Hospital Hamburg, Germany
5 Nephrology Section, University Hospital of Bonn, Germany
6 Department of Microbiology and Immunology, Doherty Institute for Infection and Immunity, Victoria, Australia

ABSTRACT

Background Renal denervation (RDN) is an invasive intervention to treat drug-resistant arterial hypertension. Its therapeutic value is contentious. Here we examined the effects of RDN on inflammatory and infectious kidney disease models in mice.

Methods Mice were unilaterally or bilaterally denervated, or sham operated, then three disease models were induced: nephrotoxic nephritis (NTN, a model for crescentic GN), pyelonephritis, and acute endotoxemic kidney injury (as a model for septic kidney injury). Analytical methods included measurement of renal glomerular filtration, proteinuria, flow cytometry of renal immune cells, immunofluorescence microscopy, and three-dimensional imaging of optically cleared kidney tissue by light-sheet fluorescence microscopy followed by algorithmic analysis.

Results Unilateral RDN increased glomerular filtration in denervated kidneys, but decreased it in the contralateral kidneys. In the NTN model, more nephritogenic antibodies were deposited in glomeruli of denervated kidneys, resulting in stronger inflammation and injury in denervated compared with contralateral nondenervated kidneys. Also, intravenously injected LPS increased neutrophil influx and inflammation in the denervated kidneys, both after unilateral and bilateral RDN. When we induced pyelonephritis in bilaterally denervated mice, both kidneys contained less bacteria and neutrophils. In unilaterally denervated mice, pyelonephritis was attenuated and intrarenal neutrophil numbers were lower in the denervated kidneys. The non-denervated contralateral kidneys harbored more bacteria, even compared with sham-operated mice, and showed the strongest influx of neutrophils.

Conclusions Our data suggest that the increased perfusion and filtration in denervated kidneys can profoundly influence concomitant inflammatory diseases. Renal deposition of circulating nephritic material is higher, and hence antibody- and endotoxin-induced kidney injury was aggravated in mice. Pyelonephritis was attenuated in denervated murine kidneys, because the higher glomerular filtration facilitated better flushing of bacteria with the urine, at the expense of contralateral, nondenervated kidneys after unilateral denervation.

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The kidney is densely innervated by sympathetic nerves that regulate glomerular perfusion and filtration. These nerves are positioned in the intima of the renal artery and extend into the glomerular tufts. Renal denervation (RDN) has been performed to treat therapy-resistant arterial hypertension since the 1940s, when no effective pharmacologic therapies were at hand. First-generation catheter-based radiofrequency ablation was widely used to target renal nerves. In 2014, a randomized study concluded that RDN does not significantly ameliorate blood pressure in patients with arterial hypertension, whereas drugs were effective in most patients. Interest in RDN for treatment purposes has recently been rekindled by the advent of improved second-generation radiofrequency ablation.

Renal sympathetic signals cause hemodynamic adjustments that result from changes in renal blood flow. Therefore, it is important to understand the effects of RDN on renal function and disease. The current study provides new insights into the role of renal denervation in inflammatory and infectious kidney diseases in mice.
in electrolyte and fluid retention, contributing to the maintenance of blood pressure, for example, in the case of fight-or-flight reactions. This is either achieved directly by autonomous vasoconstriction of the afferent arteries or indirectly by stimulating glomerular $\beta_1$-adrenergic receptors that induce renin production and release. In consequence, the renin-angiotensin-aldosterone system will reduce glomerular perfusion and filtration, and thereby retain fluid. RDN prevents these effects, and thereby reduces blood pressure. Glomerular perfusion and filtration are central parameters of kidney function and altered regulation after RDN might affect kidney diseases itself. However, the effects of denervation on the GFR in humans have not yet been definitively determined. In addition to hemodynamic changes, the autonomous nervous system can affect immune cells directly, not only in the kidney but in many organs. Little is known about the effects of RDN and its immunologic alterations on inflammatory kidney diseases.

In this study, we performed RDN in mice and studied consequences for three kidney diseases: nephrotic nephritis, a mouse model of crescentic GN that initially depends on innate, and after 5 days on adaptive immune cells, pyelonephritis, which is cleared by neutrophils recruited into the kidney by chemokines produced by kidney dendritic cells (DCs), and endotoxin-induced kidney failure, which is mediated by recruited neutrophils and monocytes. We found that an RDN-induced increase in perfusion and urine production affected all these diseases, either positively or negatively, depending on disease-specific characteristics.

**MATERIAL AND METHODS**

**Mice**
Female 8–12-week-old C57BL/6 mice were bred and housed according to the guidelines of the animal facility of the University Clinic Bonn. All experiments were approved by the state authorities.

**Induction of RDN in Mice**
Mice were anesthetized with 40 $\mu$g/g bodyweight ketamine (Ketanest, Pfizer) and 8 $\mu$g/g bodyweight of xylazine (Sigma-Aldrich) intraperitoneally in PBS (ThermoFisher Scientific). Eyes were covered with dexpanthenol cream (Bepanthen Augen and Nasensalbe, Bayer Vital) to avoid dehydration. Mice were fixed on a heat mat to maintain body temperature and connected to an isoturance (Baxter) inflator. The extent of added isoturance depended on visual assessment of sufficient narcosis. The respective flank was disinfected with 70% (vol/vol) ethanol (Fischar). An incision approximately 5 mm long was established caudally to the lateral rib cage opening the peritoneal cavity. Kidneys were extracorporated while maintaining tissue perfusion. A 5–0 surgical suture (Ethicon) soaked in a mixture of 90% pure ethanol (Carl Roth) and 10% pure phenol (Sigma-Aldrich) (vol/vol) was used to encircle the respective renal artery for RDN-treated animals. Sham-operated animals received the same treatment, but the suture was not encircling the renal artery was soaked with PBS instead. Kidneys were reincorporated and the wound was sealed using 5 mm long was established caudally to the lateral rib cage opening the peritoneal cavity. Kidneys were extracorporated and the wound was sealed using two distinct interrupted sutures (Ethicon), one for the abdominal musculature and one for the skin. The wound was disinfected with povidone iodide solution (Betaisodona, Mundipharma) and infiltration of isoturance stopped. Animals were monitored until fully awake. In patients with unilateral RDN (uRDN) (or sham), only the left kidney was treated.

**Urine Collection and Analysis**
Quantitative collection of overall urine was performed using metabolic cages from Tecniplast for 12 hours. Albumin concentrations were measured using ELISA (ab207620, Abcam).

**Visualization and Analysis of Glomerular Filtration and Albuminuria in Individual Kidneys**
Mice were injected intravenously (iv) with 50 $\mu$g fluorescent 10 kDa-dextran-AF647 (Invitrogen, ThermoFisher Scientific) conjugates approximately 3.5 minutes before sacrifice tracing primary urine. To visualize albuminuria and glomeruli, animals initially received iv 5 $\mu$g CD31-AF647 (Biologend) antibodies 20 minutes before analysis, followed by Albumin-TexasRed (ThermoFisher Scientific), which was administered like the 10 kDa-dextran-AF647. Animals were sacrificed with CO$_2$ and perfused through the left cardiac ventricle with prewarmed 50 mM EDTA (Merck). Kidneys were fixed at 6°C in 4% paraformaldehyde (Merck) overnight. Samples were incubated in 99% ethanol (Merck) for 3 hours, then transferred into 99% ethyl-cinnamate (Sigma-Aldrich) according to established protocols. For primary urine depilation, kidneys were recorded with light-sheet fluorescence microscopy (LSFM) (LaVision Biotech) at 120×, longitudinal step-size 10 $\mu$m. The excitation laser emitted at 640 nm, emission filter was set to 680/30 nm. For glomerular and proteinuria detection, kidneys were recorded with LSFM at 24×, longitudinal step-size 10 $\mu$m. The excitation laser for albumin-TexasRed was 561 nm, excitation for CD31 was 640 nm. Emission filters were 620/60 nm (albumin-TexasRed) and 680/30 nm (CD31). Image series were processed with Fiji.

**Conventional and Immunofluorescence Microscopy**
For conventional microscopy, paraffin-embedded samples were sectioned into 5

**Significance Statement**

The advent of second-generation radiofrequency ablation has renewed interest in renal denervation (RDN) to treat patients with drug-resistant arterial hypertension. In this study, we demonstrate that RDN-induced alterations in glomerular perfusion and filtration have hitherto unknown consequences for inflammatory kidney diseases. Denervated kidneys are more susceptible to immune complex GN or endotoxin-induced renal inflammation. In contrast, the improved GFR of denervated kidneys provides relative protection against pyelonephritis, at the cost of higher susceptibility of the contralateral kidney. These effects need to be kept in mind when RDN is considered in patients with hypertension and other conditions. Our findings suggest that unilateral RDN might allow enriching drugs in one kidney.
µm slices and stained using periodic acid–Schiff. Immunofluorescence was performed by incubating 6–7 µm thick cryosections with 1:1000 diluted antibodies (Abcam ab150177 or Abcam ab112) using established protocols. For the quantification of nephrototoxic sheep serum (NTS) deposition, at least five distinct random areas in the kidney cortex were imaged and analyzed for determining the mean fluorescence of anti-sheep-IgG for an individual kidney cortex.

**Flow Cytometry**

FACS analyses were performed on kidney homogenates digested with DNase and collagenase in RPMI 1640 for 30 minutes. Erythrocytes were lysed with RBC buffer for 12 minutes and incubated with 1:400 diluted antibodies following established protocols and gating strategies. Pregating was performed on live CD45+ single cells. Leukocyte population were identified as follows: Ly6G+ CD11b+ neutrophils, MHC II+ CD11c+ F4/80+ CD103+ classic type 1 DCs (cDC1s), MHC II+ CD11c+ F4/80+ CD103+ CD11b+ (resident cDC2s), MHC II+ CD11c+ F4/80+ CD103+ CD11b++ classic DCs (inflammatory cDC2s), MHC II+ CD11c+ F4/80+ macrophages (MØs), CD3e+ γδ-TCR+ γδ T cells, NK1.1+ natural killer cells. Cells were measured on a BD Canto II flow cytometer and analyzed using FlowJo Software.

**Disease Models**

Nephrototoxic serum nephritis (NTN) was induced by intraperitoneal administration of 10 µl/g bodyweight of in-house-produced NTS, as previously described. For pyelonephritis, mice were anesthetized using 40 µg/g bodyweight ketamine and 8 µg/g bodyweight of xylazine intraperitoneally, and dexamethasone cream was applied on the eyes as mentioned above. Uropathogenic Escherichia coli O6 (strain 536, UPEC) was grown in Lysogeny broth medium (Carl Roth). Bladders were filled completely using elastic tubes (REF391349, BD Neoflon-26GA). The complete procedure was repeated after 3 hours, as described previously.

For LPS-mediated acute kidney injury, 5 µg/g bodyweight LPS (E. coli O55: B5) was administered iv in 100 µl sterile PBS. Animals were analyzed 24 hours after injection.

**RESULTS AND DISCUSSION**

We first studied the effects of uRDN in kidneys under nondisease conditions. Success of denervation was verified 3 days after uRDN by showing a reduction of tyrosine-hydroxylase–positive fibers via immunofluorescence microscopy (Figure 1A, Supplemental Figure 1). Then 3 or 7 days after uRDN, kidneys of unilaterally denervated mice were analyzed for their immunologic status, morphology, and urinary production. No alterations between denervated and contralateral control kidneys were observed for CD45+ cells, Ly6G+ CD11b+ neutrophils, MHC II+ CD11c+ mononuclear phagocytes, MHC II+ CD11c+ F4/80+ DCs, MHC II+ CD11c+ F4/80+ MØs, and CD3e+ γδ-TCR+ γδ T cells (Figure 1B, Supplemental Figure 2). Absolute numbers of these immune cell subsets were not changed in denervated kidneys. Gross and microscopic tissue morphology was also unaltered by RDN treatment (Figure 1, C and D). Overall urine production was unchanged 3 and 7 days after uRDN (Figure 1E). We next analyzed glomerular filtration separately for both kidneys, by injecting freely filtrable fluorescent 10 kDa dextran, followed by optical tissue clearing and LSFM. Computer-aided analysis (Figure 1F) of the images (Figure 1, G and H) revealed higher glomerular filtration in denervated kidneys relative to contralateral control kidneys 3 and 7 days after uRDN (Figure 1), which can be explained by higher filtration pressure due to the loss of sympathetic constrictive effect of afferent glomerular arterioles. This is in line with previous studies showing that RDN increases glomerular filtration and urine production.

To study the consequences of these hemodynamic alterations on inflammatory kidney diseases, we used the NTN model, where NTS binds to renal cortical antigens and triggers an intrarenal inflammation, driven initially by innate immune cells including MØs, γδ T cells, and neutrophils, and after day 4 by DCs, CD4+ T cells, and MØs. We injected a moderate NTS dose that had been previously titrated in nondenervated mice to trigger moderate inflammation, but neither proteinuria nor kidney failure. Injection of NTS into mice with uRDN resulted in significantly more leukocytes in denervated kidneys on day 1 and day 3 after disease induction compared with contralateral control kidneys (Figure 2A), with the highest relative increase seen for Ly6G+ CD11b+ neutrophils and for γδ T cells (Figure 2B), which have been reported to attract neutrophils in the early phase of NTN. cDC1s and natural killer cells were increased as well, whereas no differences were observed for MØs (Figure 2B). cDC2 expressing normal CD11b levels, that is, the kidney-resident DCs that constitute >95% of kidney DCs, were less abundant (Figure 2B), consistent with their inflammation-induced exit to the draining lymph node. Recently recruited cDC2, recognizable by expression of high CD11b levels, were increased by approximately 36% (Figure 2B).

Notably, only mice after uRDN showed significant proteinuria in our experimental setting (Figure 2C). To identify the kidney from which the albumin in the urine had originated, we injected fluorescent albumin as a tracer and imaged the explanted kidneys by optical tissue clearing and LSFM. Much more intrarenal albumin was detected in the denervated kidney (Figure 2, D and E, Supplemental Figure 3). Immune fluorescence microscopy of kidney sections stained for sheep IgG revealed that 5.2-fold more nephrotoxic IgG had been deposited in denervated kidneys relative to contralateral controls (Figure 2, F to H). Of note, the anti-CD31 antibody used to label
endothelial cells in glomeruli (Figure 2, D and E) was preferentially detected in the denervated kidney (Supplemental Figure 4A), which can either be explained by higher CD31 expression during inflammation38 or by higher deposition of this antibody due to higher glomerular perfusion, or both. Analysis of three-dimensional image stacks showed a higher glomerular volume in the denervated kidney (Supplemental Figure 4B), indicating stronger glomerular swelling. In summary, the higher perfusion of the denervated kidneys allowed more NTS to be deposited, resulting in stronger inflammation and more severe kidney damage.

Endotoxemia can cause renal damage by stimulating injurious immune cells, such as neutrophils.39 We speculated that the higher perfusion of denervated kidneys might also increase susceptibility to circulating LPS. Indeed, after intravenous LPS injection, more CD45+ immune cells, especially neutrophils, but not mononuclear phagocytes, were detected in the denervated compared with contralateral kidney (Figure 3, A to C). The mean expression levels of CD11b and CD11c were higher in mononuclear phagocytes (Figure 3, D and E) in the uRDN kidneys, consistent with higher activation of these cells.20,31,36 The neutrophils in denervated kidneys expressed more CD11b as well, indicative of amplified neutrophil activation (Figure 3F).40

RDN in patients with arterial hypertension is usually performed bilaterally.

Figure 1. Immunologic and physiologic consequences of uRDN for the healthy kidney. (A) Expression of tyrosine hydroxylase (TH) in glomeruli of contralateral control (c. ctrl.) kidneys compared with previously denervated kidneys (uRDN) measured by immunofluorescence microscopy 3 days after denervation. (B) Ratios of immune cell counts of treated kidneys relative to contralateral kidneys measured by flow cytometry 3 days after denervation. Each data point represents the mean ratio of all animals comprising the respective group. White points depict sham-treated animals, red points display values for RDN-treated animals. CD45+ leukocytes (CD45), Ly6G+CD11b+ neutrophil granulocytes (PMNs), MHC II+ CD11c+ mononuclear phagocytes (MPS), MHC II+CD11c+F4/80+ DCs (DCs), MHC II+CD11c+F4/80+ MØs, CD3+ gd-TCR+ gd T cells. (C) and (D) Representative periodic acid–Schiff stained sections of a contralateral control (C) or denervated (D) kidney 3 days after RDN. Scale bar 100 μm. (E) Total urine production collected 3 or 7 days after uRDN in metabolic cages for 12 hours. (F) Ratios of urine production of treated relative to contralateral kidneys 3 or 7 days after uRDN measured by quantification of AF647 labeled intratubular 10 kDa dextran with LSFM. Each data point represents three individual three-dimensional (3D) samples. (G) and (H) Two-dimensional histologic images of intratubular 10 kDa-dextran tracer in contralateral control (G) or denervated (H) kidney of a representative specimen imaged by LSFM in the coronary plane. Animals were sacrificed approximately 180 seconds after tracer injection. The color-code on the right side indicates tracer deposition. Scale bar 200 μm. Experiments were performed with at least three mice per group. Error bars show SEM. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
It is theoretically possible that uRDN and bRDN might relay distinct proinflammatory signals via afferent neuronal to the central nervous system.\textsuperscript{41,42} We therefore performed bilateral denervation (bRDN) and studied consequences after LPS injection. In this setting, the phagocyte influx into both kidneys was increased as in denervated kidneys after
Figure 3. Consequences of uRDN and bRDN for LPS-induced AKI. (A)–(C) Number of leukocytes (A), MHC II⁺ CD11c⁺ mononuclear phagocytes (B), and neutrophils (C) of c. ctrl. kidneys compared with previously uRDN, 1 day after LPS injection. (D)–(F) Geometric mean fluorescence intensity of CD11c (D) or CD11b in renal MHC II⁺ CD11c⁺ mononuclear phagocytes (E) or of CD11b in renal neutrophils (F) comparing c. ctrl. kidneys to previously denervated kidneys (uRDN), 1 day after LPS injection. (G)–(I) Number of leukocytes (G), MHC II⁺ CD11c⁺ mononuclear phagocytes (H), and neutrophils (I) of ctrl. kidneys compared with previously bRDN animals, 1d after LPS injection. (J)–(L) Geometric mean fluorescence intensity of CD11c (J) or CD11b in renal MHC II⁺ CD11c⁺ mononuclear phagocytes (K) or of CD11b in renal neutrophils (L) of ctrl. kidneys compared with previously bRDN animals, 1d after LPS injection. Experiments were performed with at least four kidneys per group. Error bars show SEM. P values were calculated using paired t-tests in graphs (A)–(F) and unpaired t-test in graphs (G)–(L). *P<0.05; **P<0.01. Data shown were obtained using flow cytometry of 5% of the kidney after digestion of half a kidney. Single cells were further gated on living cells by exclusion of DAPI+ dead cells.
Figure 4. Consequences of bRDN and uRDN for the clearance of pyelonephritis. (A) CFUs per kidney 1 day after urinary tract infection (UTI) induction in bRDN-treated mice quantified by plating in Lysogeny broth (LB) agar. (B) Neutrophils per kidney in the mice from (A), measured by flow cytometry. (C) CFUs per gram kidney of the mice from (C), quantified by plating in LB agar medium, showing uninfected mice (healthy), sham-operated and uRDN-treated mice. (D) Ratio of CFU between sham- or uRDN-treated kidneys and the respective contralateral control-kidneys of the mice from (C). (E) Sum of the CFU of both kidneys of in sham- and uRDN-treated mice of the mice from (C). (F) Neutrophils per gram kidney at d1 after UTI induction in the mice from (C). Error bars show SEM. P values were calculated using paired t-tests in all graphs shown in this figure. Grubbs’ test was applied to identify outliers with P=0.05. *P<0.05. In total, one outlier pair was removed.

uRDN (Figure 3, G–L), indicating that uRDN and bRDN exerted similar proinflammatory effects in the kidney. These proinflammatory effects are consistent with higher glomerular perfusion leading to elevated LPS-induced neutrophil recruitment. Alternatively, the increased neutrophil numbers in denervated kidneys might result from impaired neuronal regulation of the intrarenal immune system. To distinguish between these possibilities, we induced pyelonephritis in denervated mice, by using our protocol of intraurethral inoculation with UPEC.24 Both kidneys of bRDN mice contained fewer bacterial CFU than kidneys of sham-operated mice after 1 day (Figure 4A).

Importantly, the numbers of neutrophils, the principal immune effectors against pyelonephritis,43 were reduced (Figure 4B), arguing against immunostimulatory effects of denervation. Instead, it was consistent with a stronger flow of primary urine that improved physical displacement of invading bacteria, and thereby reduced the infectious stimulus for neutrophil recruitment. Indeed, mice produced more urine after bRDN (1.00±0.53 ml/12 hours) than after sham surgery (0.78±0.42 ml/12 hours uRDN) or uRDN (0.58±0.37 ml/12 hours) (Figure 1F).

To further corroborate this interpretation, we also infected mice after uRDN. Their denervated kidneys contained far fewer CFU than contralateral kidneys, whereas kidneys in sham-operated mice showed intermediate CFU numbers that did not differ significantly (Figure 4, C and D). The sum of the CFUs in both kidneys was comparable in uRDN and sham-treated animals (Figure 4E). Neutrophil numbers were 40% reduced in denervated compared with contralateral kidneys (Figure 4F, Supplemental Figure 5), again arguing against immunostimulation by denervation. Importantly, the contralateral kidneys contained more UPEC than kidneys of sham-operated mice (Figure 4C). This can be explained by a reduced glomerular filtration in contralateral kidneys (Figure 1, F to G) to compensate for the higher urine
production by denervated kidneys,\textsuperscript{14} which incapacitated bacterial flushing in contralateral kidneys profoundly. Finally, the ratio of neutrophils per CFU in a kidney was higher in denervated kidneys compared with contralateral controls (Supplemental Figure 6), suggesting that the higher perfusion of denervated kidneys allows for better recruitment of neutrophils from the circulation. This may represent a second mechanism how denervation improved the defense against pyelonephritis.

In summary, we found that denervated kidneys are more susceptible to LPS- and antibody-induced injury, whereas they were less so for pyelonephritis. RDN and the ensuing amplification of glomerular filtration and perfusion affected inflammatory kidney diseases in previously undescribed ways. Thus, our findings suggest the use of RDN to treat arterial hypertension may predispose individuals to immune complex GN, or drug- or endotoxin-induced organ damage or failure. In contrast, unilateral denervation might be therapeutically used to direct intravenously applied drugs preferentially into one particular kidney, for example, in the context of neoplastic diseases that cannot be treated surgically. bRDN might theoretically protect kidneys with reflux from pyelonephritis. Unilateral denervation is not advisable in this condition because of the increased susceptibility of the contralateral kidney.

For chronic diseases such as arterial hypertension, it has to be kept in mind that denervation by chemical substances is reversible, and nerves grow back into the kidney, although it is unclear whether the full extent of innervation is achieved.\textsuperscript{44–46} For currently performed second-generation radiofrequency ablation, it has not been clarified yet whether sympathetic fibers can respawn into the kidney. Finally, our findings may also be relevant for kidney transplantation, because the graft is naturally denervated, at least initially. Hence, the graft may be relatively protected against pyelonephritis, but may be more susceptible to circulating toxins, antibodies, or leukocytes driving graft rejection.

DISCLOSURES

All authors have nothing to disclose.

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SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2021010110/-/DCSupplemental.

Supplemental Figure 1. Verification of denervation using immunofluorescence.

Supplemental Figure 2. Enumeration of leukocyte subsets after RDN.

Supplemental Figure 3. Control kidneys analyzed with LSFM.

Supplemental Figure 4. Consequences of uRDN for CD31 deposition and glomerular volume.

Supplemental Figure 5. Reduced leukocyte influx into uninervated denervated kidneys during Pyelonephritis.

Supplemental Figure 6. Ratio between neutrophils and UPEC CFU per kidney at d1 after UTI induction.

REFERENCES


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1Institute for Molecular Medicine and Experimental Immunology, University Hospital of Bonn, Rheinische Friedrich-Wilhelms University, Bonn, Germany.
2Clinics for Radiology and Radiation Oncology, University Hospital of Bonn, Rheinische Friedrich-Wilhelms University, Bonn, Germany
3Institute for Applied Mathematics, Rheinische Friedrich-Wilhelms University, Bonn, Germany.
4Institute for Cellular and Integrative Physiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
5Nephrology Section, Medical Clinic 1, University Hospital Bonn, Rheinische Friedrich-Wilhelms University, Bonn, Germany.
6Department of Microbiology and Immunology, Doherty Institute for Infection and Immunity, University of Melbourne, Victoria, Australia

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Suppl. Fig. 1: Verification of denervation using immunofluorescence.

Representative images of kidney cryo-sections stained with anti-Tyrosine Hydroxylase (TH) to confirm effect of RDN. A) Control kidney. B) RDN-treated kidney three days after treatment. Color-scheme for TH signal intensity on the right side. Scale bar is 20µm for both images.
Suppl. Fig. 2: Enumeration of leukocyte subsets after renal denervation.

Black bars indicate contralateral kidneys from unilaterally denervated mice after 3 days, white bars indicate kidneys from sham-operated mice after 3 days, red bars indicate denervated kidneys from unilaterally denervated animals after 3 days, red hatched bars indicate denervated kidneys from unilaterally denervated animals after 7 days, red dotted bars indicate kidneys from bilaterally denervated animals after 3 days. Error bars indicate S.E.M. Experiment was performed with at least 5 animals per group in three individual experiments.

Data shown were obtained using flow cytometry of 5% of the kidney after digestion of half a kidney. Single cells were further gated on living cells by exclusion of DAPI* dead cells.
Suppl. Fig. 3: Control kidneys analyzed with LSFM.

A, B) Virtual 3D figures of optically cleared kidneys of an unchallenged uRDN-treated control kidney (A) 3 days after denervation and a sham-treated kidney (B) 3 days after NTN induction. Virtual coronary sections of 200µm thickness. Vasculature stained with CD31-AF647 in cyan. Dysfiltrated albumin-TxRed in red, scale bar is 2mm.
Suppl. Fig. 4: Consequences of uRDN for CD31 deposition and glomerular volume.

A) Geometric mean fluorescence intensity of 2000 glomeruli per kidney 3 days after uRDN. Mean and S.E.M. with gray lines on top of data points. P-value was calculated using unpaired t-test. **** P<0.0001

B) Glomerular volumes of 2000 glomeruli 3 days after denervation. Boxes show the 95% CI. P-value was calculated using unpaired t-test. **** P<0.0001

Kidneys intravitally stained for CD31 were optically cleared and imaged with LSFM. Glomeruli were segmented by CD31 signal intensity and investigated using a macro in Fiji.
Suppl. Fig. 5: Reduced leukocyte influx into uRDN kidneys compared to their contralateral controls (c. ctrl.) during pyelonephritis.

Experiment was performed with 10 mice per group. Error bars show S.E.M. P-values were calculated using paired t-test. * P<0.05; ** P<0.01; *** P<0.001. Data shown was obtained using flow cytometry of 5% of the cells after digestion of half a kidney. Single cells were further gated on living cells by exclusion of DAPI+ dead cells.
Suppl. Fig. 6: Ratio between neutrophils and UPECs (CFU) per kidney at d1 after UTI induction.

First panel represents the ratio of healthy uninfected kidneys (here not applicable). The second panel (sham) compares the contralateral control (c. ctrl.) kidneys to the sham operated kidneys. The third panel (RDN) compares the contralateral control (c. ctrl.) kidneys to the denervated (uRDN) kidneys.

Experiments were performed with at 7 mice per group. Error bars show S.E.M. P-values were calculated using paired t-tests.