TNF-alpha Produced in the Kidney Contributes to Angiotensin II-dependent Hypertension

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Supplemental Material

Supplemental Methods

Animals

IFN-γ KO (B6.129S7-Ifngr1^{tm1Agt}/J) mice were purchased from Jackson Laboratory and backcrossed to the 129/SvEv background as described in the main manuscript.

Tail-cuff blood pressure measurements

Systolic blood pressure levels were determined in conscious wild-type and IFN-γ-deficient mice by the noninvasive tail-cuff method after 2 weeks of daily training.[1] Data were recorded for 4 weeks including one week prior to the implantation of Ang II osmotic mini-pumps, and 3 weeks following Ang II infusion.

Acute pressor response experiments

Blood pressure was measured by carotid arterial catheterization as described previously.[2] After anesthetization, mice were given a bolus injection of Ang II, epinephrine, or saline control while mean arterial blood pressures were continuously recorded. Data were analyzed using the LabChart software (ADInstruments, Colorado Springs, CO).

Mouse kidney Transplantation

Transplantation of a single mouse kidney with bilateral native nephrectomy was performed as we have described previously.[3] The left native kidney was removed at the time of transplant, and the right native kidney was removed through a flank incision 3 days later such that all kidney function in the recipient was provided by the transplanted kidney. The adrenal gland and its blood supply were preserved during both native nephrectomies. Each group consisted of ≥6 mice. Telemetry catheters were implanted 7 days after the initial transplant, followed 10 days later by subcutaneous implantation of osmotic minipumps infusing Ang II (500 ng·kg^{-1}·min^{-1}; Sigma-Aldrich) .

Metabolic studies

After chronic infusion of Ang II for 4 weeks, the mice were placed in metabolic cages, and urine was collected for 24 h. Urinary concentrations of albumin and total nitric oxide (NO) were measured in individual samples using specific ELISAs for mouse albumin (Exocell), nephrin (Exocell), and nitrite and nitrate (Cayman Chemical), respectively, as

previously described.[4] Creatinine (Cr) concentrations were measured with a picric acid-based method by using a kit from Exocell. To quantitate food and water ingestion, uni-nephrectomized mice were placed into metabolic cages and provided a gel food (Nutragel, Bio-Serv) as the only source of water and nutrients for 1 week prior to and 2 weeks after initiation of chronic Ang II infusion.

Histological studies

Formalin-fixed kidney tissues were embedded in paraffin, sectioned, and stained with Masson's trichrome. All of the tissues were examined by two experienced pathologists masked to the experimental groups. The kidney sections were graded based on the presence and severity of abnormalities in glomeruli, tubules, vessels, and interstitium. As previously described, the severity of renal pathological abnormalities was graded using a semiquantitative scale, in which 0 represented no abnormalities, and 1+, 2+, 3+, and 4+ represented mild, moderate, moderately severe, and severe abnormalities, respectively. An overall histologic score for each kidney was obtained by adding together the compartmental scores.[1]

To assess T-lymphocyte infiltration in the kidneys, sections were stained with anti-CD3 (clone SP7, Laboratory Vision) according to the manufacturer's instructions. The severity of perivascular T-cell infiltrates was scored in a blinded fashion on the basis of a previously established method [5] by assigning renal vessels to the following tertiles: (1) mild, indicating 0 to 9 T cells, (2) moderate, indicating infiltrates containing 10 to 29 T cells, or (3) severe, indicating infiltrates with ≥30 T cells.

Quantification of gene expression

Kidneys were harvested after 28 days chronic infusion of Ang II, and total RNA was isolated by using an RNeasy Mini Kit per the manufacturer's instructions (Qiagen). The gene expression levels of renin, IL-1b, eNOS, and NGAL were determined by realtime RT-PCR as previously described.[6]

Statistics

The values within a group are expressed as Mean±SEM. For comparisons between groups, statistical significance was assessed using ANOVA followed by an unpaired *t*-test. For comparisons within groups, variables were analyzed by a paired *t*-test.

References

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3. Crowley SD, Gurley SB, Oliverio MI, Pazmino AK, Griffiths R, Flannery PJ, Spurney RF, Kim HS, Smithies O, Le TH, Coffman TM. Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. *J Clin Invest*. 2005;115:1092-1099.
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5. Muller DN, Shagdarsuren E, Park JK, Dechend R, Mervaala E, Hampich F, Fiebeler A, Ju X, Finckenberg P, Theuer J, Viedt C, Kreuzer J, Heidecke H, Haller H, Zenke M, Luft FC. Immunosuppressive treatment protects against angiotensin ii-induced renal damage. *Am J Pathol*. 2002;161:1679-1693.
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S1

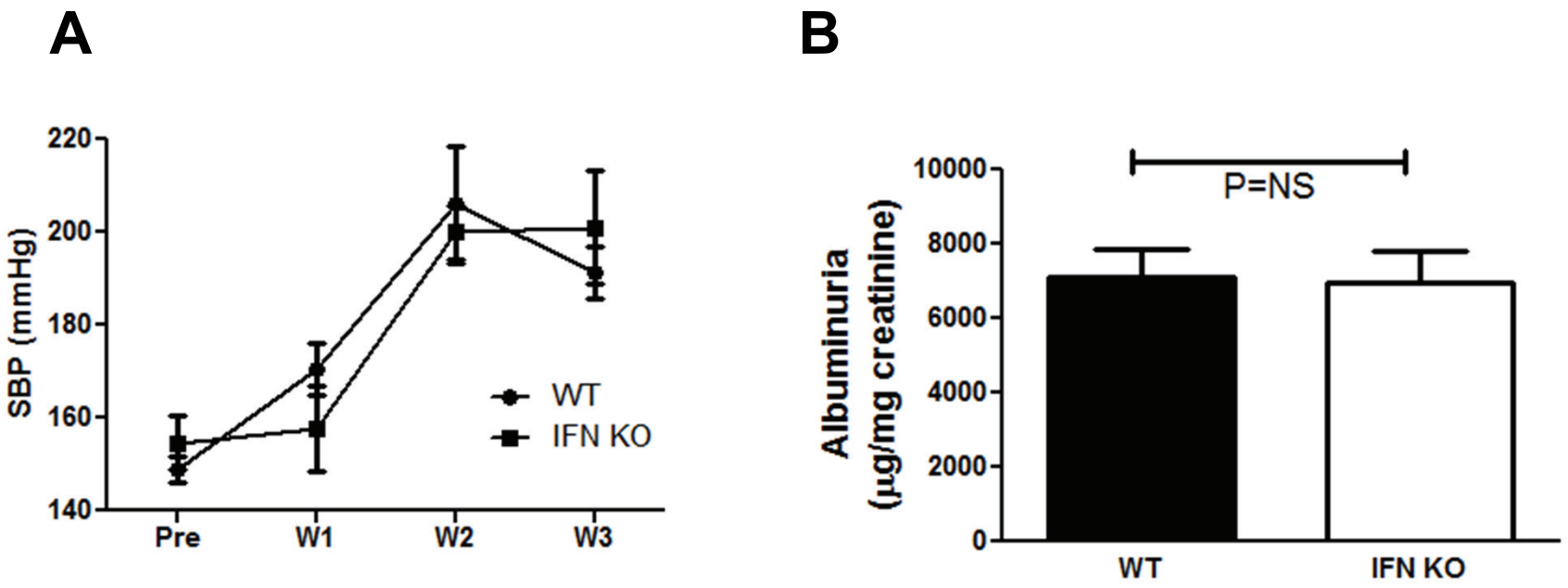

Figure S1. IFN-γ-deficient mice have preserved hypertensive and albuminuric responses to chronic Ang II infusion. A, Systolic blood pressures measured by tailcuff in wild-type (WT, circles), and IFN-γ-deficient (KO, squares) cohorts at baseline ("pre") and during 3 weeks of Ang II infusion. n = 9 per group. B, Urinary albumin excretion (μg/mg creatinine) in the experimental groups after 25 days of Ang II.

S2

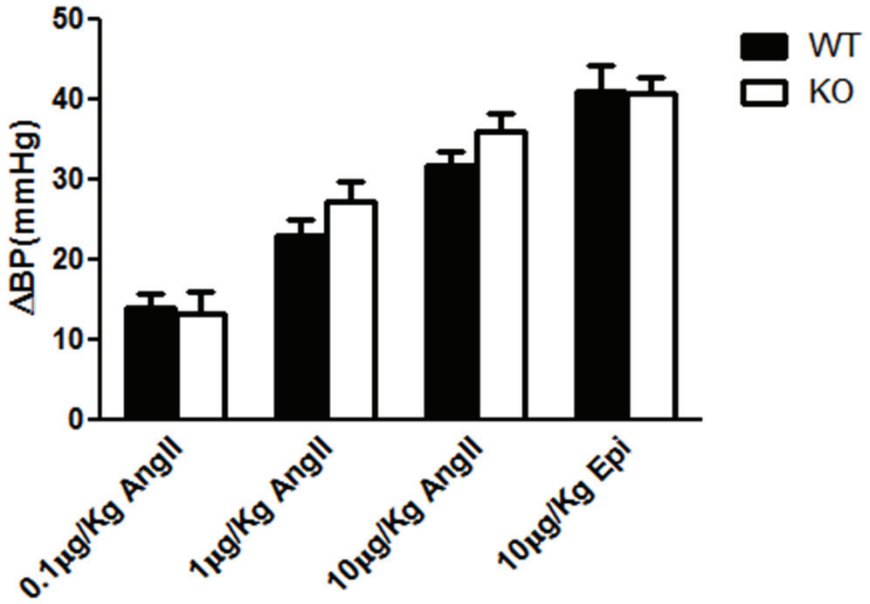

Figure S2. TNF-α-deficiency does not impact the acute pressor response to Ang II. Changes in mean arterial blood pressure in mice under anesthesia injected with increasing doses of Ang II or a single dose of epinephrine. N ≥ 6 per group.

S3

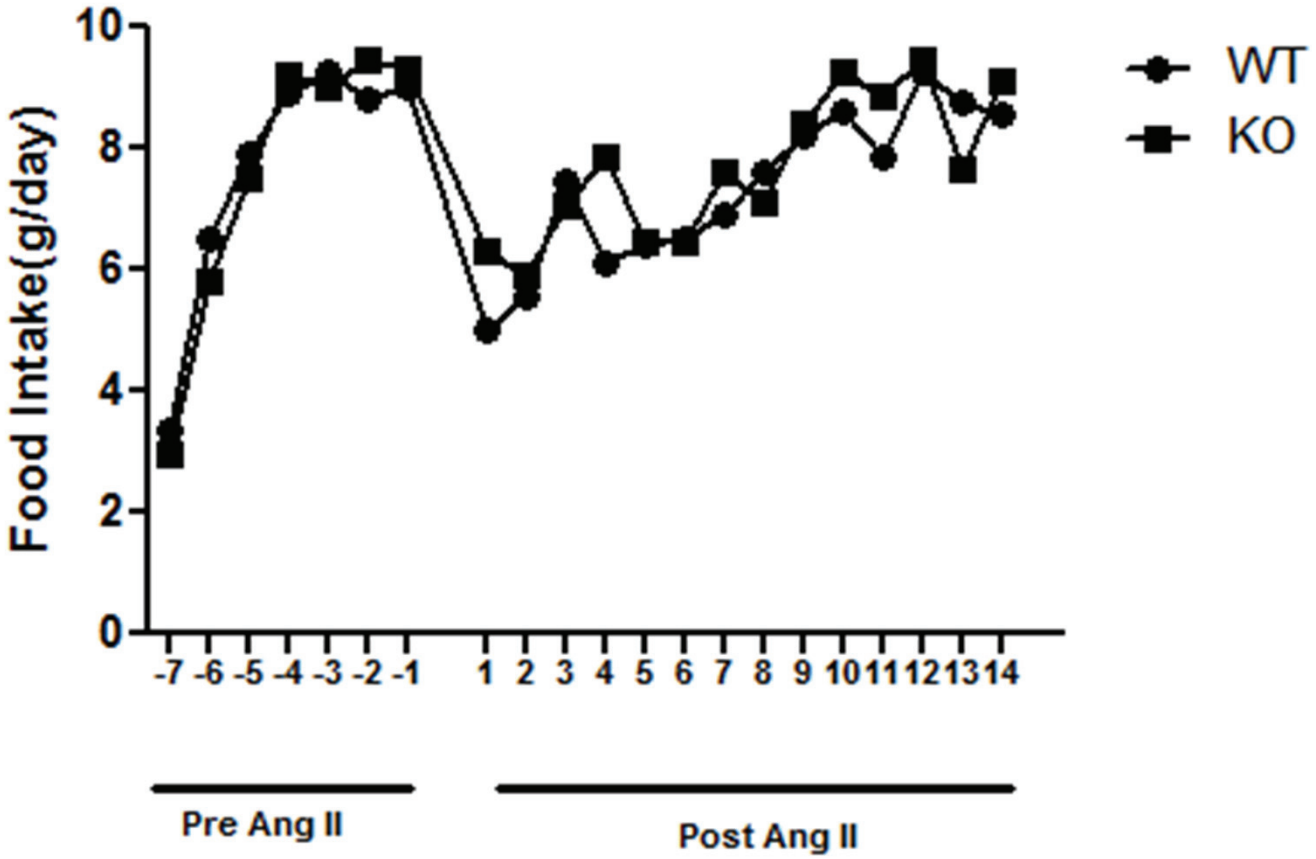

Figure S3. *TNF WT* and *KO* mice ingest similar levels of food and water in hypertension model. Uni-nephrectomized mice were placed into metabolic cages and provided a gel food as only source of water and nutrients for 1 week prior to and 2 weeks after initiation of chronic Ang II infusion. During period when blood pressures in Ang II-infused *TNF WT* and *KO* groups diverged, food and water ingestion ("food intake") was virtually identical in the 2 groups. N ≥ 7 per group.

S4

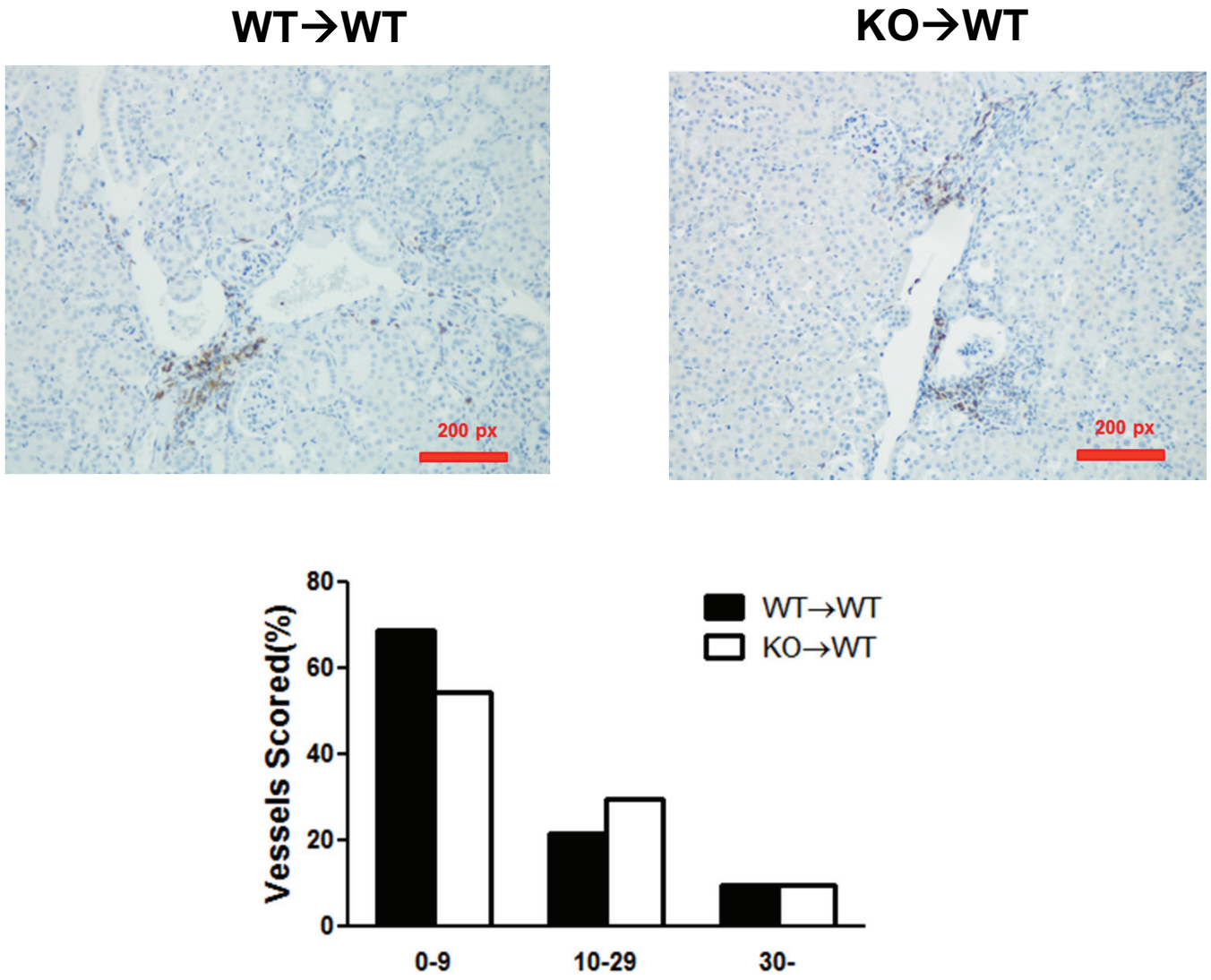

Figure S4. Renal T cell infiltration in *TNF* transplant groups during Ang II-dependent hypertension. Following 4 weeks of Ang II-induced hypertension, kidney sections from *TNF* transplant groups were stained with anti-CD3. Numbers of $CD3^+$ T cells surrounding the renal vessels were scored in blinded fashion. On top, representative images with T cells stained brown. On bottom, proportion of renal vessels from the groups surrounded by 0 to 9, 10 to 29, or ≥30 CD3 T cells, respectively.