Interferon-γ Signaling Inhibition Ameliorates Angiotensin II–Induced Cardiac Damage

Lajos Markó,* Heda Kvakan,* Joon-Keun Park,* Fatimunnisa Qadri, Bastian Spallek, Katrina J. Binger, Edward P. Bowman, Markus Kleinewietfeld, Verena Fokuhl, Ralf Dechend, Dominik N. Müller

Abstract—Angiotensin (Ang) II induces vascular injury in part by activating innate and adaptive immunity; however, the mechanisms are unclear. We investigated the role of interferon (IFN)-γ and interleukin (IL)-23 signaling. We infused Ang II into IFN-γ receptor (IFN-γR) knockout mice and wild-type controls, as well as into mice treated with neutralizing antibodies against IL-23 receptor and IL-17A. Ang II–treated IFN-γR knockout mice exhibited reduced cardiac hypertrophy, reduced cardiac macrophage and T-cell infiltration, less fibrosis, and less arrhythmogenic electric remodeling independent of blood pressure changes. In contrast, IL-23 receptor antibody treatment did not reduce cardiac hypertrophy, fibrosis, or electric remodeling despite mildly reduced inflammation. IL-17A antibody treatment behaved similarly. In the kidney, IFN-γR deficiency reduced inflammation and tubulointerstitial damage and improved glomerular filtration rate. Nonetheless, albuminuria was increased compared with Ang II–treated wild-type controls. The glomeruli of Ang II–treated IFN-γR knockout mice exhibited fewer podocytes, less nephrin and synaptopodin staining, and impaired podocyte autophagy. Thus, IFN-γ blockade, but not IL-23 receptor antibody treatment, protects from Ang II–induced cardiac damage and electric remodeling. In the kidney, IFN-γ signaling acts in a cell type–specific manner. Glomerular filtration rate is preserved in the absence of the IFN-γR, whereas podocytes may require the IFN-γR in the presence of Ang II for normal integrity and function. (Hypertension. 2012;60:1430-1436.) ● Online Data Supplement

Key Words: angiotensin II ■ immune system ■ hypertension ■ arrhythmia ■ interferon-γ

Hypertension is the major cardiovascular disease risk factor, and angiotensin (Ang) II may mediate target-organ damage.1 Ang II inhibition and blockade may be protective beyond blood pressure reduction.2 Ang II–induced target-organ damage may largely be mediated by immunity.3 Other studies and we have showed that various anti-inflammatory and immunosuppressive interventions reduced Ang II–induced target-organ damage.4,5 The mechanisms are unclear; however, proinflammatory CD4+ T helper (Th) 1 immune responses could play a role. Th1 lymphocytes coordinate the immune response by direct cell-cell contact and by cytokine release. Recently, interleukin (IL)-17 producing Th17 cell and regulatory T-cell subsets have been added to the existing schema.6 Th1 cells expressing interferon (IFN-)γ, IL-2, and tumor necrosis factor (TNF)-α mediate a wide range of responses. In rats, Ang II induced IFN-γ production by splenocytes and T cells, indicating a shift toward the Th1 immune response. Angiotensin receptor 1 blockade ameliorated renal damage and Th subset imbalance, whereas blood pressure lowering with hydralazine did not.7 In renovascular hypertension, splenocytes from high Ang II apolipoprotein E−/− mice produced more IFN-γ than those from apolipoprotein E−/− mice with normal Ang II levels.8 Crowley et al9 showed that Ang II–induced Th1/Th2 imbalance is not limited to splenocytes and that an accumulation of renal CD4+ T lymphocytes and increased expression of IFN-γ and TNF-α occurs in Ang II–infused 129/SvEv mice. Immunosuppressive treatment reversed the phenotype without changing blood pressure.4 Guzik et al10 showed that T cells are activated to produce IFN-γ and TNF-α on Ang II infusion, although only blockade of TNF-α inhibited oxidative stress and blood pressure. Angiotensin receptor 1 blockers reduce IFN-γ production in human lymphocytes without a demonstrable effect on Th2 cytokines, suggesting a specific inhibitory effect on IFN-γ production.10

Th17 cells could play a crucial role in inflammation and host immunity. The generation of pathogenic Th17 cells is dependent on IL-23, whereas Th17 cells generated via transforming growth factor-β1 and IL-6 appear less pathogenic.11,12 IL-23 belongs to the IL-12 cytokine family and is composed of an IL-23p19 and an IL-12/23p40 subunit. Antigen-presenting cells secrete IL-23 that binds to a receptor complex composed of the IL-12/23p40-binding IL-12 receptor (R)-β1 subunit and the IL-23p19 binding IL-23R subunit.

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IL-23R is highly expressed on a subset of CD4+ T cells (Th17) and is expressed at low levels on naïve T cells, natural killer cells, dendritic cells, and macrophages. In p19 knockout mice, IL-23 does not affect Th1 polarization, as evidenced by an intact IFN-γ response. Because T lymphocytes seem to mediate Ang II–induced target-organ damage, we tested the hypothesis that IFN-γ and/or IL-23 are involved.

Methods

IFN-γ receptor 1 gene-deleted (IFN-γR KO)14 and 129S6/SvEvTac (wild-type [WT]) control mice, as well as NMRI mice (all aged 9 weeks; TaconicArtemis GmbH, Cologne, Germany) were treated with neutralizing IL-23R antibody (gift from Merck Research Laboratories), with respective IgG1 control antibody or IFN-17A antibodies, and with isotype IgG1 control antibodies (both from BD Systems) were used after due regulatory approval. Subcutaneous micro-osmotic pumps delivered Ang II (1.44 mg/kg per day) for 2 weeks. Antibodies were given intraperitoneally on day 1 and 1 week after Ang II pump implantation at the following concentrations: 30 mg/kg (IL-23R, clone 21A4 or IgG1, clone 27F11) and 200 μg per injection (IL-17A, clone 18H10 or IgG1, clone R3-34). The concentration and dosage protocol were chosen based on earlier studies. On day 12, mice were placed in metabolic cages for 24 hours and urine was collected. After 2 weeks, animals were euthanized, and blood and organs were collected. We measured urinary albumin with ELISA (Celltrend, Luckenwalde, Germany) and glomerular filtration rate with fluorescein isothiocyanate–inulin clearance15 (online-only Data Supplement). Mean arterial blood pressure (MAP) was measured by radiotelemetry. Cardiac hypertrophy was assessed by calculating the heart weight:body weight ratio and by means of echocardiography and electrocardiography were performed (online-only Data Supplement). Left ventricle of the heart and total kidney RNA extraction and/or IL-23R are involved.

Results

Before Ang II, both IFN-γR KO mice and WT mice were normotensive. Ang II infusion elevated MAP steadily by 50 to 60 mm Hg in both groups (Figure 1A). Cardiac hypertrophy was reduced in Ang II–treated IFN-γR KO mice compared with Ang II–treated WT mice (Figure 1B and Figure S1A in the online-only Data Supplement). Ang II infusion induced brain natriuretic peptide expression in the left ventricle of WT and IFN-γR KO hearts, whereas Ang II infusion increased modulatory calcineurin-interacting protein 1 only in WT hearts but not in IFN-γR KO. The finding suggests that calcineurin activity was higher in Ang II–infused WT hearts compared with Ang II–infused IFN-γR KO mice hearts (Figure S1B). Cardiac hypertrophy was accompanied by remodeling and excess accumulation of extracellular matrix. Fibronectin and collagen I in the heart after chronic Ang II infusion was reduced in IFN-γR KO mice compared with WT mice (Figure 1C). Semi-quantification confirmed the histological results (Figure S1C).

Neutralizing IL-23R antibodies did not affect the development of hypertension (Figure 1D), cardiac hypertrophy (Figure 1E and Figure S2A), or the hypertrophy marker brain natriuretic peptide, but modestly increased modulatory calcineurin-interacting protein 1 (Figure S2B). IL-23R antibodies also did not affect cardiac fibronectin and collagen I expression (Figure 1F). Semi-quantification was confirmatory (Figure S2C). We also performed a study with antibodies against the Th17 signature cytokine IL-17A. Blocking IL-17A alone did not reduce blood pressure and cardiac hypertrophy (Figure S3A through S3C). Also, brain natriuretic peptide and modulatory calcineurin-interacting protein 1 did not show any differences (Figure S3D). We observed a strong anti-inflammatory response blocking IFN-γ signaling (Figure 1G), whereas blocking IL-23 signaling slightly decreased cardiac cell infiltration (Figure 1H), and anti-IL-17A treatment was not effective (Figure S3E).

In contrast to IFN-γ, its receptor is also expressed in endothelial cells. To elucidate a putative mechanism for the reduced cell infiltration, we studied the effect of IFN-γR signaling blockade and IL-23R signaling blockade on the endothelial expression of the adhesion molecules, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. Whereas macrophage and T-cell infiltration were largely reduced in Ang II–infused IFN-γR KO mice, the endothelium of these mice showed weak intercellular adhesion molecule-1 or vascular cell adhesion molecule-1 expression compared with Ang II–infused WT mice (Figure 1I). In line with the only moderately reduced cell infiltration after IL-23R inhibition, these mice showed a higher intercellular adhesion molecule-1 or vascular cell adhesion molecule-1 expression measured by a greater number of affected vessels, as well as a higher intercellular adhesion molecule-1 or vascular cell adhesion molecule-1 expression in the respective endothelium (Figure 1J).

In Ang II–treated WT mice, reproducible nonsustained ventricular arrhythmias were induced in 86% of all animals, whereas Ang II–treated IFN-γR KO mice showed a lesser 25% induction rate (Figure S4A). The reduction of the nonsustained ventricular arrhythmia was even more apparent when the percentage of inducible protocols among Ang II–infused IFN-γR KO (8%) and WT mice (62%) was compared (Figure 2A). Representative recordings are shown (Figure 2B). QTc interval was longer in Ang II–treated WT mice than in Ang II–treated IFN-γR KO mice (Figure S4B). Connexin 43 (Cx43) is the major gap junction protein in intercalated disks in the heart that has a crucial role in synchronized contraction. In untreated IFN-γR KO and WT mice, Cx43 expression was restricted to the intercalated disc regions (Figure 2C). On Ang II treatment in WT mice, Cx43 expression was no longer restricted to the intercalated disc regions. Instead, Ang II–treated IFN-γR KO mice showed an almost unchanged intercalated disk-restricted normal Cx43 gap junction protein localization similar to control mice (Figure 2C). In contrast, neutralizing IL-23R antibodies did not affect the inducibility of arrhythmias (Figure 2D and 2E), Cx43 expression was similarly altered in both groups after Ang II treatment (Figure 2F), and QTc intervals were also not different (data not shown). Altogether, these results indicate that blockade of the IFN-γ signaling pathway ameliorated cardiac damage and electric remodeling, whereas blocking the IL-23R signaling was not protective.

We next evaluated renal damage. Glomerular filtration rate was diminished in Ang II–treated mice (Figure 3A). The decrease in glomerular filtration rate in Ang II–treated IFN-γR–deficient mice was less pronounced than in Ang II–treated WT mice (Figure 3A). Neutrophil gelatinase-associated lipocalin, a marker of renal damage, was reduced in Ang II–treated IFN-γR KO.
Figure 1. Interferon-γ receptor (IFN-γR) deficiency and anti-interleukin-23 receptor (IL-23R) neutralizing antibody treatment, mean arterial blood pressure (MAP), and angiotensin II (Ang II)-induced cardiac damage are shown. A, Telemetric MAP was elevated during Ang II in both groups. B, Heart weight:body weight ratio was significantly attenuated in Ang II–treated IFN-γR knockout (KO) vs wild-type (WT) mice. C, Fibronectin and collagen I were reduced in the heart of IFN-γR KO mice after Ang II. D, Telemetric MAP was elevated during Ang II in isotype and anti-IL-23R–treated mice. Heart weight:body weight ratio (E) fibronectin and collagen I deposition (F) was similar after Ang II. G, Infiltrating CD4+, CD8+, and F4/80+ cells were reduced in IFN-γR KO+Ang II. H, Anti-IL-23R antibody treatment led to a very modest but significant reduction after Ang II compared with IgG isotype-treated mice. I, Expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expression on the endothelium was higher in both groups after Ang II, but it was substantially lower in IFN-γR KO+Ang II. J, ICAM-1 and VCAM-1 expression showed a very similar pattern in anti-IL-23R and IgG isotype-treated mice. *P<0.05 vs WT and IFN-γR KO, #P<0.05 vs WT+Ang II, §P<0.05 vs Ang II+IgG1, n≥5 per group.
KO mice versus Ang II–treated WT mice (Figure 3B). Ang II–treated IFN-γR–deficient mice also showed less cell infiltration compared with Ang II–treated WT mice (Figure 3C). Masson trichrome stains demonstrated less tubulointerstitial damage with less interstitial fibrosis (Figure 4A). In contrast, albuminuria, which reflects hypertensive glomerular damage, was increased in Ang II–infused WT, but even to a significantly higher degree in Ang II–treated IFN-γR mice (Figure 4B). Blockade of IL-23 signaling or IL-17A blockade did not ameliorate or induce albuminuria (Figure S5A and S5B).

To address the cause of the increased glomerular damage in Ang II–treated IFN-γR KO mice, we performed a detailed podocyte analysis, because IFN-γR is highly expressed on these cells (Figure S6A and S6B). We first discovered a massive decrease in podocyte number indicated by Wilms tumor-1-positive cells (Figure 4C). Podocyte reduction was even greater in Ang II–infused WT, but even to a significantly higher degree in Ang II–treated IFN-γR KO mice (Figure 4F). Both sham-treated groups showed no accumulation of LC3B (data not shown).

**Discussion**

We found that IFN-γR deficiency, but not blockade of the IL-23 pathway, improved Ang II–induced cardiac hypertrophy and electric remodeling without affecting MAP. Similarly, tubulointerstitial renal damage and glomerular filtration rate were initially improved. Nonetheless, Ang II–induced glomerular damage was worsened in IFN-γR–deficient mice. Albuminuria was increased and podocyte number, as well as nephrin and synaptopodin expression, was reduced. Ang II–infused IFN-γR–deficient mice also showed impaired podocyte autophagy, which explains, in part, the accelerated proteinuria.

Mice with altered Th1 and Th17 signaling have been intensively studied in autoimmune mouse models of experimental autoimmune encephalomyelitis and collagen-induced arthritis. Moreover, genetic or pharmacological blockade of the Th17 pathway, including the inhibition of the IL-23R, improved Ang II–induced cardiac hypertrophy and electric remodeling without affecting MAP. Similarly, tubulointerstitial renal damage and glomerular filtration rate were initially improved. Nonetheless, Ang II–induced glomerular damage was worsened in IFN-γR–deficient mice. Albuminuria was increased and podocyte number, as well as nephrin and synaptopodin expression, was reduced. Ang II–infused IFN-γR–deficient mice also showed impaired podocyte autophagy, which explains, in part, the accelerated proteinuria.

Mice with altered Th1 and Th17 signaling have been intensively studied in autoimmune mouse models of experimental autoimmune encephalomyelitis and collagen-induced arthritis. Moreover, genetic or pharmacological blockade of the Th17 pathway, including the inhibition of the IL-23R, improved such models. Cua et al. showed that IL-23 injections into the central nervous system of p19 knockout mice abrogated resistance to experimental autoimmune encephalomyelitis, suggesting that IL-23 might also promote encephalitis after macrophage activation by the production of IL-1 and TNF-α. This finding suggests an important role for IL-23 in the regulation of myeloid cells other than its direct effect on Th cells in autoimmune diseases. We found that Ang II also plays a role in experimental autoimmune encephalomyelitis induction because Ang II blockade was protective in this model. Nonetheless, Th1 axis in many chronic infectious diseases is thought to be anti-inflammatory. However, adaptive immunity has not been extensively studied in cardiovascular disease models. Our data provide

**Figure 2.** Interferon-γ receptor (IFN-γR) deficiency and anti-interleukin-23 receptor (IL-23R) neutralizing antibody treatment on angiotensin II (Ang II)–induced electric remodeling are shown. A, Programmed electric stimulation in Ang II–infused IFN-γR KO knockout (KO) mice showed reduced reproducible nonsustained ventricular arrhythmia compared with Ang II wild-type (WT) mice. B, Representative ECG recordings. C, Representative connexin 43 (Cx43) (green) and N-cadherin (red) double immunostained heart sections show similar dyslocalization. #P<0.05 vs WT+Ang II, n=6 per group. KO mice versus Ang II–treated WT mice (Figure 3B). Ang II–treated IFN-γR–deficient mice also showed less cell infiltration compared with Ang II–treated WT mice (Figure 3C). Masson trichrome stains demonstrated less tubulointerstitial damage with less interstitial fibrosis (Figure 4A). In contrast, albuminuria, which reflects hypertensive glomerular damage, was increased in Ang II–infused WT, but even to a significantly higher degree in Ang II–treated IFN-γR mice (Figure 4B). Blockade of IL-23 signaling or IL-17A blockade did not ameliorate or induce albuminuria (Figure S5A and S5B).
evidence that blocking the IFN-γ pathway improves cardiac hypertrophy, cardiac fibrosis, and electric remodeling, whereas IL-23R inhibition was ineffective. Blockade of IFN-γ signaling, IL-23R, and IL-17A did not affect Ang II–induced MAP elevation. The latter finding is surprising because Madhur et al\(^23\) recently demonstrated a blunted blood pressure response in an IL-17A gene–deficient mouse model. The group also reported that IL-17A deficiency decreased inflammation in the aortas of IL-17A/apolipoprotein E double-gene–deficient mice without affecting the size of atherosclerotic plaque area or aneurysm formation.\(^24\) One reason for the MAP discrepancy could be related to study duration. Ours was a 14-day study, where we measured MAP up to day 11. Madhur et al\(^23\) first measured a difference in blood pressure in a more chronic phase, after day 21. Additionally, in contrast to the genetic approach, neutralizing antibody treatment can have potential confounding off-target and nonspecific effects. Moreover, it is known that IL-17F, which has the highest homology to IL-17A and is also produced by Th17 cells, can be upregulated in the absence of IL-17A.\(^24\) Because IL-17F uses the same receptor (IL-17RA) as IL-17A to signal (although with much lower affinity), IL-17F may compensate for the neutralized IL-17A and, therefore, may bias the efficacy of the anti-IL-17A antibody treatment in our setting. However, the antibody clones and doses were chosen to be in line with earlier studies, which showed efficacy in other disease models.\(^13,15,16,25\) Moreover, we also cannot fully exclude whether our current Ang II treatment design would have benefited from a higher dosing regime. To exclude this hypothesis for the anti-IL-23R study, we measured the concentration of the injected anti-IL-23R antibody at the end of our studies in each mouse and we found good concentrations (173 ± 47 µg/mL). Cayatte et al\(^15\) reported that 30 mg/kg of anti-IL-23R were fully inhibitory, which led to >100 µg/mL of IL-23R drug levels (personal communication with E.P.B.) We agree that our findings with neutralizing antibodies should be verified using genetic approaches. IFN-γR–deficient mice develop a normal immune system but do exhibit increased susceptibility to Listeria and Vaccinia despite normal cytotoxic and Th cell responses.\(^14\) Surprisingly, defective IFN-γ signaling by either IFN-γ deficiency or receptor deficiency resulted in increased susceptibility of experimental autoimmune encephalomyelitis and collagen-induced arthritis.\(^19\)

In contrast to the increased pathogenesis in various autoimmune models, we observed an improvement in cardiac hypertrophy and electric remodeling, a strong anti-inflammatory effect in the heart, and renal tubulointerstitial damage in the kidney. Interestingly, inhibition of IL-23R or IL-17A did not result in ameliorated cardiac hypertrophy and fibrosis. Whereas IL-23R inhibition reduced cardiac cell infiltration by only ±10%, IL-17A inhibition showed no reduction of cell infiltration.

There is an ongoing debate regarding the origin of cardiac and renal fibroblasts and their contribution to fibrosis.\(^26\) Furthermore, how Ang II modulates these processes is unknown. Our data suggest that the blockade of IFN-γR/IFN-γR plays a role in Ang II–induced renal and cardiac fibrosis at least in part attributable to a reduced adhesion molecule expression on the endothelium and consequently a lower infiltration of inflammatory cells. However, a reduced differentiation of resident fibroblasts may also play a role.

The IFN-γR deficiency had complex effects on the kidney. One of the major histological features of Ang II–induced nephropathy is CD4+ lymphocyte infiltration and mononuclear phagocyte infiltration. IFN-γ, which is predominantly secreted by Th1 cells, is a potent activator of mononuclear phagocytes and has been linked to disease progression in Ang II–induced kidney injury.\(^7\) We found that IFN-γ signaling deficiency ameliorated Ang II–induced renal damage and inflammation, although albuminuria was increased. Albuminuria has been

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### Figure 3

Interferon-γ receptor (IFN-γR) deficiency reduces hypertensive kidney injury. **A** Decrease of glomerular filtration rate (GFR) on angiotensin II (Ang II) treatment was less pronounced in IFN-γR KO vs wild-type (WT) mice. **B** Neutrophil gelatinase-associated lipocalin (NGAL) mRNA expression was reduced in Ang II–treated IFN-γR knockout (KO) vs WT mice. **C** Similar to the heart, infiltrating cells were reduced in Ang II–treated IFN-γR KO vs Ang II–treated WT mice. *

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reported previously in a murine glomerulonephritis IFN-γ gene–deficient model.27 Chang et al28 reported that IFN-γ induces autophagy in WT mouse embryonic fibroblasts but not in cells deficient in the autophagy gene, Atg5. Recently, Hartleben et al29 showed that podocyte-specific Atg5 deletion leads to an impaired podocyte autophagy. These 2 studies suggested that IFN-γR KO mice after Ang II could also exhibit impaired autophagy. We found that podocyte damage was higher in IFN-γR KO mice after Ang II, indicated by reduced podocyte number, nephrin, and synaptopodin expression. We found a profound LC3B accumulation only in podocytes of IFN-γR KO mice after Ang II, whereas Ang II infusion in WT mice resulted in a very mild onset of disturbed autophagy, as indicated by the lower percentage of affected glomeruli. Our data suggest that IFN-γ signaling blockade in Ang II–infused mice impaired podocyte integrity, decreased podocyte number, and disturbed podocyte autophagy. We believe that albuminuria resulted from podocyte injury in our model.

**Perspectives**

Our data underscore the complexity of immune mechanisms in the production of Ang II–induced target-organ damage. Shutting off various immune components does not necessarily result in a universal protective response. Our data suggest that some immune mechanisms, even in the face of Ang II, are necessary to preserve the integrity of certain cell types. Our findings could be relevant to clinical settings in which Ang II blockade has not invariably produced expected or desired responses.

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**Disclosures**

E.P. Bowman is an employee of Merck Sharp & Dohme and as such owns stocks and stock options.

**References**


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**Figure 4.** Lack of interferon-γ receptor (IFN-γR) leads to a more pronounced podocyte injury after angiotensin II (Ang II) infusion despite less tubulointerstitial damage. **A**, Kidney sections from Ang II–treated IFN-γR knockout (KO) mice showed reduced interstitial fibrosis compared with Ang II–treated wild-type (WT) mice. **B**, Urinary albumin excretion was 4 times higher in IFN-γR KO+Ang II compared with WT+Ang II. **C**, Podocyte number (Wilms tumor-1 [WT-1]-positive nuclei) was reduced in the Ang II–treated groups; however, this reduction was more pronounced in the IFN-γR KO group. **D**, Representative photomicrograph of nephrin staining of nephrin expression. **F**, Autophagosomal marker LC3B in podocytes indicates a disturbed autophagy in IFN-γR KO+Ang II mice compared with WT+Ang II mice. *P* < 0.05 vs WT and IFN-γR KO, #P < 0.05 vs WT+Ang II, n>5 per group.


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**What Is New?**

- The role of the different Th subsets in angiostatin II-induced end-organ damage and electric remodeling is not known.

**What Is Relevant?**

- Infiltrating immune cells and altered cytokine levels are characteristic for hypertensive target-organ damage.
Interferon-γ Signaling Inhibition Ameliorates Angiotensin II–Induced Cardiac Damage
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INTERFERON GAMMA (IFN-γ) SIGNALING INHIBITION AMELIORATES ANG II-INDUCED CARDIAC DAMAGE

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Detailed Methods

Measurement of glomerular filtration rate
Mice were anesthetized with isoflurane (1.5 % with 350 ml/min air flow) using an Univentor 400 anesthesia unit (AgnTho's AB, Lidingo, Sweden). Both jugular veins were of surgically prepared. One side was used to inject FITC-inulin and the other side was used to obtain blood samples after 3, 7, 10, 15, 35, 55, and 75 min after the FITC-inulin injection. During the procedure mice were kept warm (37°C) using a warming pad. FITC fluorescence intensity of buffered plasma was measured at 485 excitation and 535 emission using a TECAN SpectraFluor Plus microplate reader (Tecan Group Ltd., Maennedorf, Switzerland). GraphPad Prism 5.04 (GraphPad Software Inc., La Jolla, CA, USA) was used to fit plasma fluorescence to a two-phase exponential decay using nonlinear regression, and glomerular filtration rate (GFR) was calculated as using standard formulas.¹

In vivo programmed electrical stimulation (EPS)
Programmed electrical stimulation was performed using a digital electrophysiology lab (EP Tracer, CardioTek, Maastricht, Netherlands) to test for physiological parameters such as refractory periods and the inducibility of ventricular and atrial arrhythmias. During the stimulation, mice were anesthetized with isoflurane (2 % with 360 ml/min air flow) using the Univentor 400 anesthesia unit (AgnTho's AB, Lidingo, Sweden). Body temperature was kept constant at 37°C using a 872/3HF homeothermic blanket control unit (Hugo Sachs Elektronik - Harvard Apparatus GmbH, March-Hugstetten, Germany). After surgical preparation of the right jugular vein, a 2 French octapolar electrophysiology catheter (CIB’ER mouse catheter; NuMed, Hopkinton, NY, USA) was placed in the right heart, including atrium and ventricle. Programmed stimulation was performed by using a standardized protocol that included trains of 10 basal stimuli (S1) followed by up to 3 extra stimuli (S2-S4), delivered with a coupling interval decreasing in steps of 5 ms until ventricular or atrial refractory was reached. The stimulation procedures were repeated at three different basal cycle lengths (100, 90 and 80 ms). Occurrence and duration of inducible arrhythmias were documented. Only stimulation protocols with reproducible arrhythmias longer than five consecutive beats in ventricle were considered positive.

Electrocardiography (ECG)
A standard limb lead surface ECG was performed under slight isoflurane anesthesia by using skin electrodes. Calculation of standard ECG time-interval parameters were performed by two independent operators based on averaged ECG lead II waveforms. Correction for heart rate and definition of time points was performed as describe before.²

Echocardiography
Detailed protocol was published elsewhere.³ Briefly, anesthetized mice were analyzed with a 30-MHz transducer (RMV 707b, VisualSonics). Two-dimensionally targeted M-mode echocardiographic images were recorded from the parasternal short-axis view at the level of the papillary muscles as published. Intraventricular septal (IVS) and left ventricular posterior wall thickness (LVPW) were estimated at end diastole. Total left ventricular wall thickness was calculated by adding LVPW and IVS. An experienced reader blinded to treatment preformed all measurements.
RNA isolation and quantitative real-time PCR

Total RNA was isolated from snap-frozen kidney tissues by using the RNeasy RNA isolation kit (Qiagen, Hamburg, Germany) according to the manufacturer's instruction. Isolated RNA concentration was measured and RNA quality was tested by NanoDrop-1000 spectrophotometer (PeqLab, Erlangen, Germany). For the synthesis of cDNA, equivalent amounts of RNA (2 μg) were used and processed by a high capacity cDNA reverse transcription kit (Life Technologies GmbH, Darmstadt, Germany).

Quantitative analysis of target mRNA expression was performed with real-time PCR using the relative standard curve method. TaqMan or SYBR green analysis was conducted according to the manufacturer's instructions, using an Applied Biosystems 7500 Sequence Detector (Life Technologies Corporation, Carlsbad, CA, USA). The expression level of the target genes was normalized by beta actin (βACT). Primers were synthesized by Biotez (Berlin, Germany) and the sequences are as follows:

mβACT forward: 5’-GTGAAAAGATGACCCAGATCA-3’,
mβACT probe: 5’-FAM-TGAGACCTTCAACACCCAGCCATG-TAMRA-3’,
mβACT reverse: 5’-CACAGCTGGATGCGTACGT-3’,

mNGAL forward: 5’-TGATCCCTGCCCATCTCT-3’,
mNGAL probe: 5’-FAM-TCACTGTCCCCTGCAGCCAGA-TAMRA-3’,
mNGAL reverse: 5’-GGAACCTGATCGCCTCCGGA-3’,

mHPRT forward: 5’-GCTTTCCCTGGTAAGCAGTCA-3’,
mHPRT reverse: 5’-ACACTTGAGGCAGCTTTTTCAC-3’,

18s forward: 5’-ACATCCAAGGAAGGCAGCAG-3’,
18s probe: 5’-FAM-CGCGCAAATTACCCACTCCCGAC-TAMRA-3’,
18s reverse: 5’-TTTTCGTCACTACCTCCGCG-3’,

mBNP forward: 5’-GCCAGTCTCCAGAGCAATTCA-3’,
mBNP reverse: 5’-GGGCCATTTTCCTCCGACT-3’,

mMCIP1 forward: 5’-CGTGGCTGGAAACAGTAGA-3’,
mMCIP1 sonde: 5’-FAM-ATGCCACCCCCGTCATAAATTACGATCT-TAMRA-3’,
mMCIP1 reverse: 5’-CCCAGCTGGAGATGGGATA-3’.

Histology and immunohistochemistry

For morphologic evaluation of the kidneys, 2-µm paraffin sections were stained with trichrome Masson-Goldner standard procedure. Immunohistochemistry was performed on 5-µm cryosections of hearts and kidneys using the following primary antibodies: anti-CD4, anti-CD8 (all BD PharMingen, San Diego, CA, USA) anti-F4/80 (AbD Serotec, Oxford, UK), anti-fibronectin (Abcam, Cambridge, UK), connexin-43 (Sigma-Aldrich Chemie GmbH, Munich, Germany), anti-LC3B (Cell Signaling, Danvers, MA, USA), anti-WT1 (SantaCruz, Santa Cruz, CA, USA), anti-VCAM-1, anti-ICAM-1 (AbD Serotec, Oxford, UK), anti-nephrin (PROGEN, Heidelberg Germany), anti-synaptopodin (PROGEN Biotechnik, Heidelberg, Germany), type I collagen (Southern Biotech, Birmingham, AL, USA) and N-cadherin (Invitrogen, Paisley, UK). For indirect immunofluorescence, nonspecific binding sites were blocked with 10% normal donkey serum (Jackson ImmunoResearch, West Grove, USA) for 30 min. Then sections were incubated with the primary antibody for 1 h at room temperature or overnight at 4°C. All incubations were performed in a humid chamber. For
fluorescence visualization of bound primary antibodies, sections were further incubated with Cy3-conjugated secondary antibodies (Jackson ImmunoResearch) for 1 h in a humid chamber at room temperature. For fluorescence visualization of N-cadherin biotin conjugated secondary antibody and streptavidin (Alexa Fluor 568) and in case of Cx43 secondary antibody conjugated with Alexa Fluor 488 (all Invitrogen, Paisley, UK) was used and was incubated as mentioned before. Specimens were analyzed using a Zeiss Axioplan-2 imaging microscope with the computer program AxioVision 4.8 (Zeiss, Jena, Germany). The investigator had no knowledge of the treatment group assignment. Analyses of infiltrating cells were done by counting CD4, CD8 or F4/80 positive cells in heart- and kidney-tissue sections. Data are expressed as mean number of whole (heart) or 20 areas (kidney) randomly chosen, non-overlapping fields per section. For WT-1 semi-quantitative analysis WT-1 positive podocyte nuclei were counted in 50 glomeruli of each kidney. Semi-quantitative analysis of nephrin was done by using the following scoring system: 0, no; 1, weak; 2, moderate; and 3, strong expression. Analysis of fibronectin and collagen I expression was done by semi-quantitative scoring as follows: 0, no; 1, very mild; 2, mild; 3, moderate, 4, intense; 5, very intense.

**Detailed statistical analysis**
Statistical analysis was performed using GraphPad Prism 5.04 (GraphPad Software Inc., La Jolla, CA) and SPSS 13.0 (SPSS Inc., Chicago, IL) software. Normality of the data was tested by Kolmogorov-Smirnov test. Normally distributed data were analyzed by unpaired t-test in case of 2 groups and by one-way ANOVA using Tukey’s post-hoc test in multiple groups. Non-normally distributed data were analyzed with the Kruskall-Wallis test and the Mann-Whitney U test. Differences of MAP between the groups during time were analyzed by two-way ANOVA for repeated measurements. Data are presented as mean±SEM. p<0.05 were considered to be statistically significant.

**References**


Figure S1. Effect of IFN-γ signalling blockade on Ang II-induced cardiac damage. A. Total left ventricular (LV) wall thickness determined by echocardiography was significantly elevated in Ang II-treated WT mice compared to the other groups. B. mRNA level of cardiac hypertrophy marker brain natriuretic peptide (BNP) and modulatory calcineurin-interacting protein 1 (MCIP1) in the study groups. Two weeks of Ang II infusion elevated BNP expression in left ventricle of WT and IFN-γR KO hearts similarly, while MCIP1 was only induced significantly in WT hearts. C. Following 2 weeks of Ang II infusion IFN-γR KO mice developed significantly less collagen I and fibronectin deposition compared to WT control. $P<0.05$ versus WT and IFN-γR KO and IFN-γR KO+Ang II, *$P<0.05$ versus WT and IFN-γR KO, #P<0.05 versus WT+Ang II, n≥5/group.
Figure S2. Effect of treatment with IL-23R neutralizing antibody on Ang II-induced cardiac damage. A. Total left ventricular (LV) wall thickness determined by echocardiography was not altered by IL-23R antibody treatment. B. Treatment with IL-23R neutralizing antibody showed no effect on BNP, but increased MCIP1 mRNA level. C. Following 2 weeks of Ang II infusion treatment with IL-23R neutralizing antibody showed no difference compared to IgG isotype treated control. §P<0.05 versus Ang II+IgG1, n≥5/group.
Figure S3. Effect of treatment with IL-17A neutralizing antibody on mean arterial pressure (MAP), and Ang II-induced cardiac damage. A. Telemetric MAP was elevated during Ang II in both groups. B. Heart weight-to-body weight ratio did not show differences after two weeks of Ang II infusion. C. Total left ventricular (LV) wall thickness determined by echocardiography was not altered by IL-17A neutralizing antibody treatment. D. Treatment with IL-17A neutralizing antibody had no effect on BNP or MCIP1 mRNA expression. E. Number of infiltrating CD4+ , CD8+ , and F4/80+ cells was similar after two weeks of Ang II infusion. n≥4/group.
**Figure S4.** Electrophysiological integrity of the heart is better preserved in the absence of IFN-γR after Ang II infusion. A. Reproducible non-sustained ventricular arrhythmias were drastically less inducible in Ang II-treated IFN-γR KO mice (25%) than in Ang II-treated isotype controls (86%). B. Length of the QTc interval was significantly shorter in IFN-γR deficiency mice following Ang II infusion compared to Ang II-treated WT. *P<0.05 versus WT+Ang II, n≥6/group.
Figure S5. Blockade of IL-23 signaling by IL-23R neutralizing antibody (A) or IL-17 blockade with IL-17A neutralizing antibody (B) did not affect albuminuria after 2 weeks of Ang II infusion.
Figure S6. mRNA expression of the IFN-γ receptors in primary podocytes, isolated glomeruli and non-glomerular fraction of C57BL/6 mice. IFN-γR1 (A) and more significantly IFN-γR2 (B) is preferentially expressed in podocytes compared to other renal compartments.
Figure S7

Figure S7. Expression of synaptopodin, a molecule associated with actin microfilaments present in the podocyte foot processes. A. Synaptopodin expression was decreased after Ang II, compared to untreated mice. This decrease was greater in glomeruli from Ang II-treated IFN-γR KO mice compared to Ang II-treated WT mice.