AT$_1$R blockade reduces IFN-γ production in lymphocytes in vivo and in vitro

**Jon A. Weidanz, Lynn M. Jacobson, Rebecca J. Muehrer, Ariang Djamali, Debra A. Hullett, Jenifer Sprague, Maurizio Chiriva-Internati, Vaughan Wittman, Thomas J. Thekkumkara, and Bryan N. Becker**

**Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, Texas; Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas; Division of Transplantation, Department of Surgery, University of Wisconsin-Madison, Madison, Wisconsin; and Section of Nephrology, Department of Medicine, University of Wisconsin-Madison, Madison, Wisconsin**

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**Background.** Type I angiotensin II (Ang II) receptor (AT$_1$R) signaling induces proinflammatory responses. Recent studies suggest that T lymphocytes express AT$_1$R; yet the effects of Ang II binding to AT$_1$R on T cells are poorly understood. We examined the effect of AT$_1$R blockade on release of the proinflammatory cytokine, interferon-gamma (IFN-γ) by human lymphocytes in vivo and in vitro.

**Methods.** We used an AT$_1$R blocker losartan in a randomized clinical trial in kidney transplant recipients over a 12-month period [AT$_1$R blocker (N = 11) and control (N = 10)]. Peripheral blood lymphocytes, isolated from both cohorts, were analyzed by enzyme-linked immunosorbent spot assays (ELISPOT) and real-time reverse transcription-polymerase chain reaction (RT-PCR) to enumerate IFN-γ producing T cells and IFN-γ mRNA levels. The effects of AT$_1$R blockade in vitro were assessed using human alloreactive T cells and an IFN-γ producing human cytotoxic T-lymphocyte line. Alloreactive T cells were treated with losartan or candesartan and enzyme-linked immunosorbent assay (ELISA) was used to measure IFN-γ protein release. The cytotoxic T-lymphocyte line also was AT$_1$R blocker–treated prior to determining IFN-γ producing cells by intracellular cytokine staining.

**Results.** The AT$_1$R blocker cohort had a significant decrease in IFN-γ producing peripheral blood lymphocytes (P ≤ 0.05 for each time point) and IFN-γ mRNA levels (P = 0.01 vs. control patients). Losartan also decreased IFN-γ production (P < 0.001) in purified alloreactive T cells in vitro as did candesartan. Moreover, Ang II amplified IFN-γ generation (P < 0.05) in alloreactive T cells while AT$_1$R blocker treatment inhibited Ang II’s effect (P < 0.04). AT$_1$R blocker treatment furthermore also inhibited IFN-γ production in the cytotoxic T-lymphocyte line.

**Conclusion.** AT$_1$R blockers may have a clinically relevant immunomodulatory role by blocking IFN-γ production in T cells.

There is evidence that angiotensin II (Ang II) can affect immune cell activity by amplifying chemokine expression in macrophages, regulating dendritic cell differentiation, and promoting lymphocyte proliferation [1, 2]. Ang II affects peripheral blood mononuclear cells (PBMCs) in a manner akin to that of cytokines, stimulating production of tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), monocyte chemotactic protein-1 (MCP-1), interleukin (IL)-1β, and tissue factor [3, 4]. Ang II binding to Ang II type 1 receptor (AT$_1$R) initiates signal transduction that can transactivate other signaling pathways, including the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway that can also result in cytokine production [5, 6]. This suggests that blocking the effects of Ang II on immune cells could blunt the inflammatory response that characterizes many chronic systemic disorders.

Classic immunosuppressive agents fail to block all of the mechanisms involved in immune cell activation and function. As such, there is great advantage in identifying other agents with anti-inflammatory and immunomodulatory properties that might be used either in combination with current immunosuppressive agents or alone. AT$_1$R blockers represent one such group of medications. Theoretically, they could modulate the effect of Ang II on lymphocytes and alter their inflammatory phenotype.

T cells play a major role in mediating inflammatory disorders by contributing to or causing tissue damage through the release of interferon-γ (IFN-γ). Recently, Shao et al [7] demonstrated that continuous infusion of Ang II into rats significantly increased IFN-γ levels. This suggests that Ang II could be an important

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**Key words:** angiotensin II, kidney transplantation, angiotensin receptor blockers, chronic allograft nephropathy, lymphocyte proliferation; cytotoxic T-lymphocyte line; peripheral blood lymphocytes, IFN-γ.

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but heretofore, poorly examined hormonal factor driving IFN-γ production in lymphocytes.

There is a paucity of data assessing the effects of AT1R blockers on purified T cells or on T cells in vivo. We examined these possible effects in vivo and in vitro using peripheral blood lymphocytes from kidney transplant recipients, hypertensive patients, alloreactive T cells derived from blood donors, and cells from a cytotoxic T-lymphocyte line. We assessed IFN-γ mRNA levels and protein levels and proliferative responses in peripheral blood lymphocytes from patients randomized to AT1R blockers or no anti-Ang II treatment [no AT1R blocker or angiotensin-converting enzyme (ACE) inhibitor] as part of their medical therapy. We used a human cytotoxic T-lymphocyte line and human alloreactive T cells as in vitro models to confirm in vivo findings that AT1R blockade significantly reduced IFN-γ expression. As these are indirect observations of Ang II’s effect on peripheral blood lymphocytes, we also determined the direct effect of Ang II addition on IFN-γ production in alloreactive T cells. These data, in aggregate, indicate a role for Ang II in modulating T-cell IFN-γ production.

METHODS

Patient population

The University of Wisconsin Institutional Review Board approved the study, enrollment process, and protocol. Kidney transplant patients (study subjects) with mild allograft dysfunction (serum creatinine ≥0.3 mg/dL above baseline at 6 months after transplant) were randomized to treatment with either losartan (25 to 100 mg daily by mouth) as part of their medical regimen or with other types of antihypertensive agents (e.g., calcium channel blockers), excluding agents that serve as AT1R blocker or angiotensin-converting enzyme (ACE) inhibitor as part of their medical therapy. We used a human cytotoxic T-lymphocyte line and human alloreactive T cells as in vitro models to confirm in vivo findings that AT1R blockade significantly reduced IFN-γ expression. As these are indirect observations of Ang II’s effect on peripheral blood lymphocytes, we also determined the direct effect of Ang II addition on IFN-γ production in alloreactive T cells. These data, in aggregate, indicate a role for Ang II in modulating T-cell IFN-γ production.

Periphe ral blood lymphocytes

Whole blood was taken from individuals enrolled in the study, washed in saline solution and treated with ACK lysis buffer [150 mmol/L NH₄Cl, 10 mmol/L KHCO₃, and 0.1 mmol/L Na₂ ethylenediaminetetraacetic acid (EDTA), pH 7.3] to lyse red blood cells. Samples were then centrifuged and cells were resuspended at 1.5 × 10⁸ cells per mL in 10 mL complete RPMI media (RPMI-1640) (BioWhitaker, Walkersville, MD, USA), supplemented with fetal calf serum (FCS) (Sigma Aldrich, St. Louis, MO, USA), 2 mmol/L L-glutamine (BioWhitaker), 50 μmol/L 2-β-mercaptoethanol (Sigma Aldrich), 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate (Sigma Aldrich).

Proliferation assay

Purified peripheral blood lymphocytes were plated at 1 × 10⁶ cells per well in six-well plates followed by incubation for 3 days at 37°C with immobilized anti-CD3ε monoclonal antibody (Immunotech, Marseille, France) and antihuman CD28 monoclonal antibody (BD Pharmingen, San Diego, CA, USA) at 2.5 μg and 1 μg, respectively. Control wells were exposed to phosphate-buffered saline (PBS). T cells were assessed by flow cytometry after 72 hours of stimulation and found to contain >90% CD3+ cells. Other harvesting, control and stimulated cells were plated at 1 × 10⁶ cells per well in triplicate in a 96-well plate. Media containing 0.5 μCi [³H]-thymidine (Amersham Bioscience, Piscataway, NJ, USA) was added to each well, plates were then incubated overnight at 37°C and analyzed for [³H]-thymidine incorporation determined using a Packard TopCount NXT Scintillation plate reader. The proliferation index was calculated as previously described [9]. The Δ proliferation index was calculated by assessing the difference between the proliferation index at baseline and the proliferation index at various time points indicated.

Enzyme-linked immunosorbent spot assays (ELISPOT) assay

The CD3/CD28-stimulated cells that remained after setting up the proliferation assay were used for ELISPOT
analysis. ELISPOT assays were conducted according to the manufacturer’s directions (Cellular Technology, Ltd., Cleveland, OH, USA). Cells were resuspended to \(3 \times 10^5\) per mL and serial two fold dilutions were made across 96-well plates coated with anti-IFN-\(\gamma\) or anti-IL-5 antibody. Plates were incubated for 2 days at 37°C, washed three times with PBS and three times with PBS/Tween (0.1%). Biotinylated secondary detection antibodies (OptiEIA IFN-\(\gamma\) and IL-5 obtained from the aforementioned kits) were diluted in 1% bovine serum albumin (BSA)/PBS before addition to the wells. The plates were incubated for 24 hours, washed four times with PBS/Tween, and streptavidin alkaline phosphatase (Dako #D0396) (Dako, Glostrup, Denmark) was added in the plates for 2 hours. The plates were washed with PBS, substrate was added, developed for 15 to 60 minutes, and then washed with distilled water to stop the reaction and allowed to air dry. Preselected wells were incubated with phytohemagglutinin as a positive control (10 \(\mu\)g/mL), similar to studies from Gebauer et al [10]. The final volume for all assay wells was 200 \(\mu\)L/well. Plates were then scanned and analyzed by Immunospot® Software (CTL Technologies).

**Real-time polymerase chain reaction (PCR) of peripheral blood lymphocyte samples**

Total RNA was extracted as described [11]. cDNA, obtained from sample RNA (Roche First Strand cDNA Synthesis Kit) (Roche, Indianapolis, IN, USA), was then used for PCR reactions. PCR reactions were prepared using 2× TaqMan™ Master Mix, cDNA template and RNAse-free water, and 20× Target assay mix. Nontemplate samples were run as controls. Samples were also assayed for 18S ribosomal RNA as an internal control. Assays contained 12.5 ng sample cDNA plus primers and probe at concentrations of 0.9 \(\mu\)mol/L and 0.25 \(\mu\)mol/L, respectively. Cycle conditions were 50°C for 2 minutes then 10 minutes at 95°C then 40 cycles consisting of 15 seconds at 95°C (denature) followed by 1 minute at 60°C for annealing and extension. All samples were assayed in quadruplicate. We have used this methodology successfully to compare relative mRNA levels across tissue samples in large-scale studies [11].

**Generation of human alloreactive T cells**

Blood was collected from volunteers using a protocol approved by the Institutional Review Board at Texas Tech University Health Sciences Center. Human alloreactive T cells were generated in mixed lymphocyte reactions using irradiated allogeneic stimulator cells. T-cell purity was determined to be greater than 90% based on flow cytometry and staining with anti-CD3e monoclonal antibody (UCHT1) (BD PharMingen).

**Effect of Ang II and AT1R blocker on IFN-\(\gamma\) production in human alloreactive T cells**

Alloreactive T cells (\(1 \times 10^6\) cells/well) were added to a 48-well plate and stimulated using either irradiated human leukocyte antigen (HLA) mismatched stimulator cells (\(2 \times 10^6\) cells/well) or on plates containing immobilized anti-CD3 antibody (UCHT1). Cells were cultured in the absence of any additions (positive control), in the presence of Ang II (Sigma, St. Louis, MO, USA) at 10 nmol/L and 0.1 nmol/L, in the presence of 10 \(\mu\)mol/L candesartan (CV-11974), or in the presence of 10 and 0.1 nmol/L Ang II concentrations plus 10 \(\mu\)mol/L candesartan. Candesartan was kindly provided by Astrazeneca (Wilmington, DE, USA). At 24 hours, culture supernatant was harvested and IFN-\(\gamma\) was detected by enzyme-linked immunosorbent assay (ELISA) according to manufacturer’s instructions (BD PharMingen).

**Antigen-specific stimulation of the anti-p53 cytotoxic T lymphocytes**

An IFN-\(\gamma\) producing human cytotoxic T-lymphocyte line [12] was kindly provided by Dr. Albert De Leo (University of Pittsburgh, PA, USA). This cytotoxic T-lymphocyte line was used for characterizing the effects of AT1R blockade on IFN-\(\gamma\) expression. The cytotoxic T-lymphocyte line was maintained using irradiated HLA-A2 matched feeder cells pulsed with the p53 peptide (aa 264–272 LLGRNSFEV) and supplemented with 10 U/mL of human recombinant IL-2 (rhIL-2). Cells were cultured in the absence of any additions (positive control) or presence of 10 \(\mu\)mol/L candesartan. Celles were grown in six-well plates at 10^6 cells per well and stimulated using an irradiated HLA-A2 positive lymphoblastoid cell line at 1 \(\times 10^4\)/well pulsed with 10 \(\mu\)g/well of p53 peptide.

**Intracellular staining**

Cells from the human cytotoxic T-lymphocyte line (2 \(\times 10^5\)/well) were added to a 96-well plate and stimulated with irradiated lymphoblastoid cell line as described above in the absence (positive control) or presence of 10 \(\mu\)mol/L candesartan. At 96 hours, cells were harvested and intracellular cytokine staining was performed per protocol. Intracellular staining of human cytotoxic T-lymphocyte controls (untreated/unstimulated and untreated/stimulated) and experimental cells (candesartan-treated/stimulated) was performed using IL-4 [anti-IL-4-fluorescein isothiocyanate (FITC)-conjugated, mouse IgG3,\(\kappa\)] (#34019.111) (R&D Systems, Minneapolis, MN, USA) and IFN-\(\gamma\) (anti-IFN-\(\gamma\)-phycoerythrin-conjugated, mouse IgG2a,\(\kappa\)] (#25718.11) (R&D Systems) specific antibodies per the protocol provided by BD PharMingen.

**Statistical methodology**

Differences between groups were assessed using Student t test and analysis of variance (ANOVA). Statistical
analyses were performed using SigmaStat for Windows version 3.0 (SPSS Inc., Chicago, IL, USA). Data are reported as mean ± SD.

RESULTS
Study population
Twenty-one kidney transplant recipients were randomly divided into two groups. One group received losartan as part of their high blood pressure regimen; the other did not. Neither group received ACE inhibitors. Kidney function, as assessed by mean serum creatinine and eGFR [8], was comparable between the two groups as were the number of acute rejection episodes (data not shown). Patients in both cohorts were given the same immunosuppressive agents that included MMF and calcineurin inhibitors during the time course of the study.

Blood pressure and eGFR values were similar in both groups during the study time course (data not shown). Proteinuria declined in the AT1R blocker cohort (spot urine protein/creatinine ratio study entry 1.9 ± 0.8 g/L; 12-month spot urinary protein/urinary creatinine 1.1 ± 0.45 g/L) (P = 0.016). There was a slight (nonsignificant) reduction in the urinary protein/urinary creatinine ratio in the control cohort [study entry 2.1 ± 1.1 g/L; not significant (NS) vs. losartan study entry; 12 months 1.66 ± 0.93 g/L].

ELISPOT analyses for peripheral blood lymphocyte generation of IFN-γ
Anti-Ang II therapy may be renoprotective in the setting of chronic allograft nephropathy (CAN) [12]. Given that alloimmune factors contribute to CAN, it is logical to consider that therapies in that setting that are successful may have salutary effects on immune responses. We therefore examined the ability for AT1R blockers to inhibit T-cell–mediated IFN-γ production. This cytokine, produced primarily by T cells and natural killer (NK) cells, has effector functions that include activation of macrophages and endothelial cells. Peripheral blood lymphocytes isolated from patients were assayed for IFN-γ expression as described. Baseline values were not significantly different between treatment groups (spots per 1 × 10⁶ cells at study entry AT1R blocker 3654 ± 1471; control 3803 ± 1255; NS) The AT1R blocker cohort demonstrated a significant reduction in IFN-γ producing cells [(spots per 1 × 10⁶ cells) 3 months AT1R blocker 4127 ± 1289; control 6231 ± 1834 (P = 0.01); 6 months AT1R blocker 3899 ± 1508; control 5124 ± 2253 (P = 0.05); 9 months AT1R blocker 3845 ± 1493; control 6889 ± 1863 (P < 0.001); 12 months AT1R blocker 4276 ± 1456; control 6471 ± 1621 (P < 0.05)] (Fig. 1).

We also examined whether AT1R blocker treatment resulted in a shift from a Th1 to a Th2 phenotype by assaying for IL-4 and IL-5 expression. There was no demonstrable effect on IL-4 or IL-5 production suggesting AT1R blockers specifically inhibited IFN-γ production. The effect of AT1R blocker therapy was not restricted to kidney transplant patients alone. To determine whether the affect of losartan on T cells was specific for renal transplant recipients, we isolated peripheral blood lymphocytes from hypertensive patients (N = 5) without renal disease (serum creatinine 1.2 ± 0.3 mg/dL) or any other form of solid organ transplantation. Each individual (three women and one man; mean age 47 ± 7 years) received losartan 50 mg daily as treatment for hypertension during the time period noted. Mean systolic blood pressure was 138 ± 9 mm Hg on treatment and mean diastolic blood pressure was 82 ± 4 mm Hg. Samples were obtained from these individuals at baseline and then after 3 months of therapy. When we examined this set of nontransplant recipients with hypertension also treated with losartan, ELISPOT values at 3 months of treatment were not significantly different from the transplant AT1R-treated patients (spots per 1 × 10⁶ cells 4716 ± 1003 (P < 0.05 vs. control) (NS vs. AT1R blocker transplant).

Real-time PCR for IFN-γ mRNA levels
We assayed IFN-γ mRNA levels using real-time PCR in peripheral blood lymphocyte samples from patients treated with AT1R blockers versus control patients. AT1R blocker treatment was associated with a significant reduction in IFN-γ mRNA levels from peripheral blood lymphocytes. Figure 2 shows IFN-γ mRNA levels from real-time PCR results on peripheral blood lymphocytes isolated from AT1R blocker–treated versus control groups at early (3 months) and late (12 months) time points. The AT1R blocker–treated cohort had a significant reduction in peripheral blood lymphocyte
control.

Human alloreactive effector T cells were incubated with and without losartan (0.1 to 10 µmol/L concentration) and stimulated with immobilized anti-CD3 monoclonal antibody. Losartan treatment led to a significant dose-dependent reduction in IFN-γ production by human alloreactive T cells (Fig. 4). This suggested a losartan-specific effect on effector T-cell function.

**In vitro effect of Ang II blockade on IFN-γ production in a cytotoxic T-lymphocyte line**

To further define whether the reduction in IFN-γ production in T cells observed with losartan was mediated
through specific blockade of AT$_1$R, we expanded our studies with AT$_1$R blocker treatment to include candesartan CV-11974. We used an IFN-γ producing cytophilic T-lymphocyte line in stable culture to assess the direct effects of candesartan on IFN-γ generation. The cytotoxic T-lymphocyte line was NK and T-cell free as determined by staining for CD56 (>1% of cells stained positive) and there was no evidence of NK cell-specific cytotoxicity (data not shown). IFN-γ production was evaluated by intracellular cytokine staining and flow cytometry.

Minimal nonspecific staining of the cytotoxic T-cell lymphocyte line was observed with isotype antibody controls. Eighty-seven percent of the gated T-cell population present in the cytotoxic T-lymphocyte line produced IFN-γ (Fig. 5A) after peptide stimulation. Unstimulated cells showed little production of either cytokine (Fig. 5A). Candesartan (10 μmol/L) significantly decreased the number of IFN-γ producing cells (> 90% reduction) to a level seen in the unstimulated T-cell population (N = 3) (Fig. 5A) without any significant change in IL-4 production. Candesartan also had no effect on cell viability as assessed by trypan blue exclusion after any period of incubation or in a 3-[4, 5] diphenyltetrazolium bromide (MTT) viability assay read after 96 hours (data not shown).

**AT$_1$R blockade of human alloreactive effector T cells leads to reduction in IFN-γ production**

We then conducted experiments with candesartan in human alloreactive effector T cells. These cells (55% CD4+ and 45% CD8+), when stimulated with irradiated HLA mismatched stimulator cells for 96 hours in the presence of 10 μmol/L candesartan elaborated less IFN-γ secretion compared to untreated cells (control) (N = 3) (P < 0.001) (Fig. 5B). These findings corroborated both the in vivo results and the decrease in IFN-γ production in the cytotoxic T-lymphocyte line following AT$_1$R blockade.

**Ang II induction of IFN-γ production in human alloreactive effector T cells**

Stimulation of alloreactive effector T cells with irradiated HLA mismatched stimulator cells for 24 hours in the presence of Ang II (0.1 nmol/L) led to a significant increase in IFN-γ production compared to untreated cells (control) (N = 3) (P < 0.05) (Fig. 5C). To determine whether Ang II induction of IFN-γ was mediated via AT$_1$R, effector cell cultures were co-cultured with both Ang II and candesartan. Candesartan significantly reduced the Ang II–mediated increase in IFN-γ production in the supernatant at 24 hours (N = 3) (P < 0.04 for 10 nmol/L Ang II vs. 10 nmol/L Ang II + candesartan) (N = 3) (P < 0.02 for 0.1 nmol/L Ang II vs. 0.1 nmol/L Ang II + candesartan). This suggested that the Ang II effect on IFN-γ production occurred via AT$_1$R on effector T cells (Fig. 5C).

**DISCUSSION**

In vitro studies have suggested that Ang II exerts effects on lymphocytes [13, 14]. We examined a clinical population for in vivo evidence of an AT$_1$R blocker’s ability to decrease T-cell production of IFN-γ. We evaluated both short-term (3 months) and long-term (12 months) effects of AT$_1$R blocker treatment on IFN-γ production in peripheral blood lymphocytes and found a significant reduction in the number of IFN-γ producing cells in the AT$_1$R blocker–treated cohort compared to control subjects. Our findings include a limited analysis of peripheral blood lymphocytes derived from hypertensive individuals prior to and 3 months after treatment with losartan that parallel our observations for a reduction of peripheral blood lymphocyte IFN-γ production and proliferation after AT$_1$R blockade in kidney transplant recipients. We then conducted in vitro tests to assess AT$_1$R blocker effects on T-cell IFN-γ generation to confirm our in vivo observations. We used a human cytototoxic T-lymphocyte line and physiologically relevant human alloreactive effector T cells for these studies and demonstrated Ang II–mediated induction of IFN-γ. AT$_1$R blockers significantly inhibited Ang II–mediated IFN-γ production in these cells, suggesting that AT$_1$R mediated this effect. The similar results in vivo and in vitro show that AT$_1$R blockers in general exert an immunomodulatory function by altering IFN-γ levels in T cells.

Our kidney transplant recipients represent an intriguing population in which to investigate the effects of AT$_1$R blockers. Transplantation, by its very nature, often evokes lymphocyte and, even more specifically, T-cell–mediated responses. Therefore, it is interesting that T-cell–mediated injury in kidney tissue can be linked to renin-angiotensin system activity independent of any hemodynamic effects [13]. It follows logically that AT$_1$R blockade could have beneficial effects under conditions of inappropriate T-cell activation. Nataraj et al [14] found that Ang II, acting through AT$_1$R, stimulated lymphocyte proliferation via activation of calcineurin phosphatase and suggested that AT$_1$R blockers could be used in conjunction with calcineurin inhibitors to achieve an additive immunomodulatory effect. The current findings expand upon these observations by demonstrating an additive immunomodulatory response in vivo in the presence of AT$_1$R blockade, exemplified by a reduction in IFN-γ generation.

These data also extend the observations by Fernandez-Castelo et al [15]. They demonstrated a direct association between Ang II and IFN-γ production in lymphocytes in vitro. We used similar concentrations of Ang II in our studies. However, we did not identify the biphasic effect
that they reported. This is likely due to differences in T-cell purity, disparities in CD4+ and CD8+ T-cell ratios and an altered activation state of the cells in our studies. There is also a striking corollary set of data recently described by Shao et al [7], comparable to our findings. They showed that rat splenic T cells manifested a 30% increase in IFN-γ secretion following Ang II treatment. This compares favorably to increase in IFN-γ secretion caused by Ang II in human T cells.
we observed in response to Ang II. While these Ang II effects may appear modest in vitro, the same group noted potent effects of Ang II on IFN-γ production in vivo, supporting a physiologic role for Ang II. Moreover, AT₁R blocker treatment in vivo reduced Ang II–induced T-cell IFN-γ production in the animals.

We focused primarily on responses in human cells. Even in the in vitro studies, we attempted to be highly specific in examining IFN-γ generation from both a cytotoxic T-lymphocyte line and alloreactive effector T cells in an effort to substantiate the findings from the peripheral blood lymphocyte studies. Using both cell sets also afforded us the opportunity to determine if losartan had direct effects on T cells. Moreover, we could assess whether candesartan’s effects on T-cell IFN-γ production was AT₁R-specific. The fact that AT₁R blockers specifically decreased IFN-γ production in vitro in the absence of shift in Th2-type cytokine production suggests that AT₁R are immunomodulatory receptors.

Losartan and candesartan vary slightly in terms of their affinity for AT₁R and their duration of antagonism [16]. Candesartan, unlike losartan, does not appear to interact with the intracellular form of the angiotensin receptor or the thromboxane receptor [17, 18]. Thus, it may be more specific for in vitro testing. The significant effect of candesartan treatment on IFN-γ production in the cytotoxic T-lymphocyte studies and the strong effects of both AT₁R blockers on alloreactive effector T cells suggest that the primary effect of losartan on the peripheral blood lymphocytes was due to AT₁R antagonism. Intriguingly, our observations using two different AT₁R blockers and the finding that olmesartan reduced IFN-γ generation in T cells in treated rats [7] collectively support a general mechanism of action for AT₁R blockers to down-modulate T-cell production of IFN-γ.

CONCLUSION

These studies suggest that AT₁R blocker treatment alters IFN-γ generation in peripheral blood lymphocytes both in vitro and in vivo. Several caveats to our study are noteworthy in the context of this conclusion. We acknowledge there is no clinical evidence of immunosuppression with AT₁R blockers in people with hypertension or receiving immunosuppression. We also recognize that, despite the in vivo findings, the in vitro concentrations employed may not represent what occurs clinically. Further, we examined a small number of patients in each cohort of the clinical investigation. Although serial sampling, the lack of significant demographic differences between the cohorts, and the ability to use subjects as their own controls afforded us a degree of confidence in the resulting data, we recognize the possibility of α error. Yet, other studies of a similar nature and size have used the same methodologies. In addition, ex vivo manipulation of cells could alter their inherent properties. Nonetheless, the measured intraindividual responses still suggest a specific AT₁R blocker effect that crossed cohorts. The results are descriptive as befits a translational clinical trial in humans, and for that reason, it is important to recognize that the in vitro investigations help to corroborate the in vivo studies. While newer mechanisms involving Ang II and cytokines continue to be described [19], it is important to recognize that defining such mechanisms in vivo is difficult. Nevertheless, these data suggest that AT₁R blockers may have immunomodulatory functions as a component of their receptor antagonism.

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Reprint requests to Bryan Neil Becker, M.D., UW Nephrology, 3034 Fish Hatchery Road, Suite B, Madison, WI 53713–3125.
E-mail: bnb@medicine.wisc.edu

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