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The Role of Type 1 Angiotensin Receptors on T Lymphocytes in Cardiovascular and Renal Diseases

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Abstract

The renin–angiotensin system plays a critical role in the pathogenesis of several cardiovascular diseases, largely through activation of type I angiotensin (AT₁) receptors by angiotensin II. Treatment with AT₁ receptor blockers (ARBs) is a proven successful intervention for hypertension and progressive heart and kidney disease. However, the divergent actions of AT₁ receptors on individual cell lineages in hypertension may present novel opportunities to optimize the therapeutic benefits of ARBs. For example, T lymphocytes make important contributions to the induction and progression of various cardiovascular diseases, but new experiments indicate that activation of AT₁ receptors on T cells paradoxically limits inflammation and target organ damage in hypertension. Future studies should illustrate how these discrepant functions of AT₁ receptors in target organs versus mononuclear cells can be exploited for the benefit of patients with recalcitrant hypertension and other cardiovascular diseases.

Keywords

Renin–angiotensin system; RAS; Angiotensin receptors; AT₁ receptor blockers; ARBs; T lymphocyte; Adaptive immunity; Disease pathogenesis; Hypertension; Atherosclerosis; Myocardial infarction; Heart failure; Kidney damage; Cardiac remodeling

Introduction

The primary effector molecule of the renin–angiotensin system (RAS), angiotensin II, makes critical contributions to the pathogenesis of hypertension, atherosclerosis, vascular and myocardial remodeling, and congestive heart failure [1, 2]. Angiotensin II (Ang II) propagates signals via two specific subtypes of receptors: type 1 (AT₁) and type 2 (AT₂) [3]. However, the classical effects of Ang II to mediate blood pressure elevation and cardiovascular injury are conferred predominantly through its activation of AT₁ receptors. Accordingly, specific AT₁ receptor blockers (ARBs) dramatically lower blood pressure and improve vascular and myocardial function in patients with cardiovascular diseases [4, 5]. Similarly, mice lacking the AT_{1A} receptor, the closest murine homologue to the single human AT₁ receptor, are protected in different experimental models of cardiovascular disease [6–8]. Studies examining the mechanisms underlying these effects have primarily focused on cells bearing AT₁ receptors in solid organs such as the heart, kidney, and vasculature [9, 10]. By contrast, the actions of AT₁ receptors expressed on infiltrating mononuclear cells have

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only recently received more intense scrutiny regarding their potential role in cardiovascular disease.

AT₁ receptors, a key target for cardiovascular protection

AT₁ receptors belong to a class of seven-transmembrane G-protein coupled receptors and are expressed ubiquitously throughout the body. Upon ligation with Ang II, AT₁ receptors trigger multiple intracellular signaling pathways involving inositol 1,4,5-triphosphate(IP₃), calcium, and adenylate cyclase [11]. Based on the cell lineage, stimulation of these signals elicits distinct local and systemic effects including vasoconstriction, cellular hypertrophy/proliferation, and hormone release. In turn, these effects ultimately contribute to the pathogenesis of a wide range of cardiovascular diseases such as hypertension, atherosclerosis, myocardial infarction, and congestive heart failure.

Hypertension

Activation of AT₁ receptors raises blood pressure by inducing vasoconstriction, sodium retention, and aldosterone generation. Accordingly, large human clinical trials have shown that treatment with AT₁ receptor antagonists lowers blood pressure effectively in the majority of hypertensive patients [12]. Moreover, recent studies have elucidated distinct and independent contributions of individual tissue pools of AT₁ receptors to hypertension. For example, we found previously that stimulation of AT₁ receptors exclusively on kidney cells is a primary driver of Ang II-dependent hypertension through effects on renal sodium handling [9].

Atherosclerosis

The atheromatous plaque in the vessel wall contains macrophages that contribute to the pathogenesis of atherosclerosis by facilitating the accumulation of lipid in the fatty streak. Hyperlipidemia, in turn, upregulates AT₁ receptors whose activation augments vascular oxidative stress and accelerates atherosclerosis [13–15], particularly as oxidized lipid becomes a neo-antigen that targets adaptive immune responses to the vascular wall [16, 17]. Through these mechanisms, AT₁ receptor activation potentiates endothelial dysfunction, recruitment of mononuclear cells, fatty streak formation, and proliferation and migration of vascular smooth muscle cells. Inversely, pharmacologic blockade or genetic deletion of the AT₁ receptor dramatically attenuates the severity of atherosclerotic lesions [8, 18]. Thus, global inhibition of the renin–angiotensin system has become a frontline intervention to prevent cardiovascular morbidity in patients at risk [19].

Myocardial infarction

Together, hypertension and atherosclerotic disease of the coronary arteries (CAD) lead to the catastrophic outcome of myocardial infarction. By ameliorating hypertension and CAD, AT₁ receptor blockade reduces the likelihood of myocardial infarction. Once myocardial infarction occurs, AT₁ receptor activation triggers myocardial cell apoptosis and fibrosis, resulting in pathologic cardiac remodeling [20]. Accordingly, both pre-clinical and clinical studies confirmed that RAS inhibition reduces morbidity and mortality rates following myocardial infarction [21, 22, 7].

Congestive heart failure

Congestive heart failure (CHF) is the final common pathway of several progressive heart diseases, including those due to chronic hypertension and ischemic heart disease. By augmenting the severity of hypertension and CAD, AT₁ receptor activation speeds the progression of cardiac dysfunction. The efficacy of the AT₁ receptor blockade in preventing the onset of experimental heart failure is therefore not surprising [23, 24]. Consistent with

these findings, agents inhibiting AT₁ receptor activation have shown consistent efficacy in treating patients with CHF in large randomized clinical trials [25–27].

T lymphocytes, a new player in cardiovascular injury

During the progression of cardiovascular disease, T lymphocytes accumulate in target organs, particularly around the vasculature [28–30]. Recent studies illustrate that these infiltrating T lymphocytes and the inflammatory mediators they secrete may play a causal role in mediating cardiovascular damage.

Hypertension

The pathophysiology of essential hypertension is complex, with the vasculature, the kidney, and central nervous system all playing critical roles in this disorder. In addition, several earlier experiments indicated that T lymphocytes could participate in the development of hypertension. For example, adoptive transfer of lymph node cells from a rat subjected to renal infarction induced hypertension in the recipient [31], and thymectomy protected against blood pressure elevation in a genetically hypertensive rat model [32]. More recently, in a landmark study Guzik et al. demonstrated that hypertension induced by Ang II or DOCA salt was blunted in RAG1^{−/−} mice lacking functional lymphocytes, whereas the hypertensive response was restored by adoptive transfer of T but not B lymphocytes [29]. Conversely, blockade of lymphocyte proliferation can ameliorate damage to the heart and kidney during hypertension through blood-pressure-independent mechanisms [30, 33]. T lymphocytes appear to raise blood pressure by inducing vascular endothelial dysfunction and/or promoting sodium retention in the kidney [29, 34]. Although T cells may influence blood pressure and target organ damage through direct cytotoxic effects, cytokines produced by T cells may also contribute to Ang II-dependent hypertension [35, 36]. Moreover, the induction of oxidative stress by these perivascular T cells may “feed forward” to promote further inflammation in cardiovascular control organs. For example, activated T cells infiltrate the fat adjacent to blood vessels, secrete cytokines, and promote the generation of excessive oxidative stress both in the vascular compartment and the circumventricular organs (CVO) of the brain [37]. The CVO, in turn, signal to the paraventricular nucleus of the hypothalamus where the generation of reactive oxygen species induces systemic production of pro-hypertensive, pro-inflammatory cytokines via an NF- κ B-dependent pathway [38]. Therefore, T lymphocytes play a unique role in the pathogenesis of essential hypertension by integrating dysfunctional responses of the vasculature, the kidney, and the central nervous system.

Classically, the two major subsets of T lymphocytes are CD4⁺ T cells (T helper cells) and CD8⁺ T cells (cytotoxic T cells). Of these two subsets, CD8⁺ T cells appear to play the dominant role in raising blood pressure in response to Ang II [39]. T helper cells have been further divided into several subsets, such as Th1, Th2, Th17, and T regulatory cells based on their functions and the cytokines they produce. Proinflammatory Th1 cells produce IFN- γ and stimulate the production of TNF- α . Almost a decade ago, Shao et al. reported that an imbalance of Th1/Th2 cells in the spleen correlates with the extent of target organ damage induced by Ang II [35]. More recently, we found that mice unable to mount a Th1 immune response have attenuated hypertensive kidney injury but no alteration in the chronic hypertensive response to Ang II, confirming a critical role of Th1 lymphocytes in Ang II-induced target organ damage [40]. Similarly, Th17 cells, through the production of IL-17A, contribute to the induction of Ang-II-dependent hypertension and vascular dysfunction [41]. By contrast, T regulatory cells, previously characterized as potent suppressors of T-helper lymphocytes, prevent blood pressure elevation due to various stimuli and cardiac injury during Ang-II-induced hypertension [42–45]. Thus, depending on the specific T cell subset,

T lymphocytes can have divergent effects on blood pressure elevation and target organ damage.

Atherosclerosis

T lymphocytes are present in human atherosclerotic lesions throughout the course of disease evolution [46]. In the apoE^{-/-} mouse model of atherosclerosis, lymphocyte deficiency dramatically attenuates lesion severity, whereas adoptive transfer of T cells reactive to modified low-density lipoprotein (LDL) aggravates vascular injury [47]. These data suggest that adaptive immune responses to the oxidized LDL neo-antigen mediate atheromatous plaque formation.

T lymphocytes within the atherosclerotic plaque consist primarily of CD4⁺ T cells with a minority of CD8⁺ T cells present [48]. The preponderance of evidence implicates CD4⁺ T helper cells in the pathogenesis of atherosclerosis. For example, mice deficient of signature Th1 cytokines such as IFN- γ , IL-12, and IL-18 have lower lesion burdens, whereas injection of these molecules exaggerates atherosclerosis [49–51]. Furthermore, targeted deletion of the Th1 transcription factor, T-bet, ameliorates atheromatous lesions in LDLr^{-/-} mice [52]. Collectively, these data demonstrate that Th1 immunity is a key contributor to atherogenesis.

Data regarding the role of Th17 cells in the pathogenesis of atherosclerosis are challenging to interpret. Elevated levels of IL-17A, a hallmark cytokine of Th17 cells, are detectable within murine and human atherosclerotic plaques [53]. Measures to diminish IL-17A by antibodies or decoy receptors attenuate atherosclerosis, pointing to a proatherogenic role for this cytokine [54, 55]. By contrast, IL-17A-deficient mice have shown increased susceptibility OR resistance to atherosclerosis in selected models [56–59]. Thus, the actions of Th17 cells in atherogenesis may depend on the timing of disease and/or tissues subjected to injury.

As in hypertension, regulatory T cells are protective in models of atherosclerosis. Prototypical Treg cytokines TGF- β 1 and IL-10 exhibit anti-atherogenic functions in several models [60, 61]. Adoptive transfer of T regulatory cells mitigates the severity of atherosclerosis, while depletion of Tregs by anti-CD25 results in augmented atheromatous plaque size in apoE-deficient mice [59, 62]. Thus, based on the evidence to date, Treg cells play a uniformly salutary role in cardiovascular disease.

The precise function of CD8⁺ T cells found in atherosclerotic lesions will require further clarification [63]. Mice lacking the capacity to fully activate CD8⁺ T cells due to MHC-I deficiency have enlarged atherosclerotic lesions compared to controls, suggesting a protective role of CD8⁺ T cells in atherogenesis [64]. Adoptive transfer studies further indicate that the beneficial actions of CD8⁺ T cells in atherogenesis accrue from a CD8⁺CD25⁺ regulatory T cell population [65]. By contrast, activation of a CD8 co-stimulatory molecule, CD137, with an agonistic antibody accelerates the progression and increases the instability of atherosclerotic lesions [66]. The apparent discrepancy in this study versus the aforementioned experiments in which CD8⁺ T cells provided protection from atherogenesis may relate to off-target effects of the CD137 antibody as CD137 is expressed on other immune cell lineages including dendritic cells. Nevertheless, in contradistinction to the roles of CD4⁺ versus CD8⁺ T cells in raising blood pressure, CD4⁺ T lymphocytes seem to play the major pathogenetic role in the progression of atherosclerosis.

Cardiac remodeling

The contributions of innate immune cells including neutrophils and monocytes/macrophages to cardiac injury and remodeling have received more attention than a possible role for T cells in this setting [67–70]. Nevertheless, T lymphocytes may well participate in the pathogenesis of left ventricular remodeling following myocardial infarction. T lymphocytes infiltrate the penumbra surrounding the infarct zone, perhaps analogous to their accumulation around diseased blood vessels in hypertensive disease [71]. Belonging largely to the Th1 subset, these T cells regulate the wound healing process by directly interacting with fibroblasts and indirectly stimulating collagen production via secreted cytokines [72]. By contrast, more recent incisive studies illustrated that both conventional and regulatory CD4⁺ T cells become activated after acute myocardial infarction (AMI) and facilitate wound healing of the myocardium. Accordingly, genetic deletion of CD4, restriction of CD4⁺ T cell responses, or deficiency of the MHC II molecule that activates CD4⁺ T cells consistently led to increased mortality [73]. Similarly, adoptive transfer of T regulatory cells attenuates adverse ventricular remodeling after myocardial infarction, consistent with the aforementioned findings that T regulatory cells improve hypertensive cardiac remodeling induced by Ang II [74, 75, 45]. Here again, given the apparent contradictions in available data, the actions of CD4⁺ T cells in directing left ventricular remodeling after AMI may depend on the timing of CD4⁺ cell infiltration and/or the mechanisms through which these T cells are activated.

AT₁ receptors on T lymphocytes: a paradoxical target in cardiovascular diseases

AT₁ receptors are prominently expressed on cells of the innate and adaptive immune systems. For example, Nataraj and colleagues used autoradiography to demonstrate that Ang II can bind specifically to spleen cells through AT₁ receptors [76]. Moreover, mRNA expression of AT_{1A} receptors is detected in splenocyte lineages including T lymphocytes, B lymphocytes, and macrophages [76] and in bone-marrow-derived cell lineages [77]. More precisely, the major T cell subsets isolated from mouse spleen including CD4⁺ T cells, CD8⁺ T cells, double-negative T cells, NK T cells, and T regulatory cells each express AT₁ receptors as do circulating T cell subsets in humans [40, 78]. Hoch *et al* also detected expression of AT_{1B} receptors and AT₂ receptors on CD4⁺ and CD8⁺ T cells [79]. Finally, expression levels of the AT₁ receptor are increased upon activation of T cells in keeping with a relevant functional role for T lymphocytes in the setting of RAS activation [80].

As the classical effects of Ang II to drive vascular cell proliferation are mediated through its binding to AT₁ receptors, investigators suspected that Ang II might activate T lymphocytes through AT₁ receptor stimulation. In vitro experiments supported this notion. In primary cultured lymphocytes, activation of AT₁ receptors permits full proliferative responses to T cell receptor activation and allogeneic stimulation [76]. However, Jurewicz *et al*. indicated that angiotensin receptors other than AT₁ receptors also contribute to Ang II-induced activation of T cells isolated from healthy human volunteers [78], and work from Dr. Harrison's group further implicated AT₂ receptors in the regulation of T cell function [79]. In these latter experiments, endogenously produced Ang II influenced T cell activation, oxidative stress, and cytokine production through a putative intracellular RAS.

As in vitro assessments of T cell function do not uniformly match T cell behavior in disease models in vivo [81], exploring the actions of AT₁ receptors on T cells in vivo has presented an experimental challenge with obvious clinical relevance. One major obstacle towards this end has been to separate the actions of AT₁ receptors specifically on T cells from systemic

activation of AT₁ receptors. Accordingly, investigators have sought to generate experimental animals lacking AT₁ receptors on immune cell populations.

Laboratories have pursued several approaches to isolate the actions of AT₁ receptors on immune cell lineages. One approach has been to generate bone-marrow chimeras by transplanting bone marrow cells from AT₁ receptor-deficient donors into lethally irradiated wild-type recipients. Following bone marrow engraftment, AT₁ receptor-deficient bone marrow chimeras (BMKO) and their controls (BMWT) can then be subjected to models of cardiovascular disease. For example, we chronically infused BMKO and control mice with Ang II and found that AT₁ receptor deficiency solely on bone-marrow-derived cells leads to a greater degree of blood pressure elevation and more severe damage in the kidney. Kidneys from the Ang II-infused BMKO mice also contain more robust perivascular T cell aggregations. These data revealed an unexpected protective effect of AT₁ receptors on immune cells in the setting of hypertension [77].

In models of atherosclerosis, AT₁ receptors on immune cells have shown variable effects. In a model of low-level RAS activation combined with an atherogenic diet, mice lacking AT₁ receptors on bone marrow cells manifested accelerated atherogenesis [82]. In contrast to these findings, the AT₁ receptor did not influence atherosclerotic lesion formation in the LDL-receptor- or apoE-deficient models [83, 84]. The conflicting results in these various models of atherosclerosis may depend on the mechanisms of RAS activation and/or disease induction, but also on the degree of AT₁ receptor deletion in the wide variety of immune cell lineages derived from bone marrow progenitors, including macrophages, dendritic cells, and lymphocytes. Nevertheless, these studies of hypertension and atherosclerosis raised the possibility that AT₁ receptors on immune cells may have important functions to regulate the progression of cardiovascular diseases.

A second approach to explore the precise role of AT₁ receptors on T lymphocytes in hypertension has been through adoptive transfer of AT₁-receptor-deficient T cells into Rag 1^{-/-} mice that lack an adaptive immune system [29]. In this model, immune reconstitution with T cells lacking AT₁ receptors leads to a blunted hypertensive response and reduced oxidative stress in Ang-II-dependent hypertension. This approach avoids confounding effects of the lethal irradiation used in the bone marrow transfer models, but may still be prone to any aberrant immune cell trafficking or functions that occur following adoptive transfer of exogenous lymphocyte populations into an adult immune deficient animal [85, 86]. Nonetheless, this incisive study introduced the notion that AT₁ receptors specifically on T lymphocytes could modulate the pathogenesis of hypertension.

A third approach to study the actions of AT₁ receptors on T cells while avoiding potential confounding effects of lethal irradiation and/or adoptive transfer is through precise genetic deletion of the AT₁ receptor in T lymphocytes via a Cre/loxP strategy. The emergence of modern genetic engineering techniques such as the Cre/loxP system has afforded the opportunity to manipulate genes in target cell lineages driven by specific promoters. By using this strategy, we generated mice lacking the AT_{1A} receptor solely on T lymphocytes ("T cell KO") and their littermate controls ("T cell WT") [40] (Figure 1). This genetic approach is prone to a different set of confounding effects including the possible impact on immune system development of AT₁-receptor-deficient T cell precursors during gestation. However, in a cursory assessment of immune cell development in these animals, we found that thymocyte proportions were similar in the T cell WT and KO groups, suggesting that thymic maturation is intact in our T cell KO animals.

To study the role of the AT₁ receptor on T lymphocytes in hypertension, we challenged both T cell WT and KO mice with chronic Ang II infusion for 4 weeks, and their intra-arterial

blood pressures were measured via radiotelemetry. In this study, blood pressures remained similar in the two groups both at baseline and during Ang II, indicating that AT₁ receptors on T cells do not regulate normal blood pressure homeostasis or the chronic hypertensive response to Ang II.

We also determined whether activation of AT₁ receptors on T cells influences the degree of hypertensive kidney damage. In our hypertension model, AT₁ receptor deficiency on T cells exaggerated urinary albumin excretion, expression of the kidney injury marker NGAL, glomerular podocyte loss, and renal T cell infiltration. These findings suggest that the augmented proteinuria and renal T cell accumulation seen in the BMKO animals from our earlier bone marrow transfer experiment were due to the absence of AT₁ receptors specifically on T cells rather than on another bone marrow-derived cell lineage [77]. Further analysis revealed that activation of AT₁ receptors on T cells constrains their propensity to propagate the Th1 pro-inflammatory response, which mediates glomerular damage in the kidney during hypertension [40].

Collectively, our experiments indicate that AT₁ receptors on T lymphocytes protect the kidney in hypertension despite a robust literature characterizing the prohypertensive and/or pro-inflammatory effects of activating AT₁ receptors in the kidney and the vasculature [9, 13]. As mentioned above, the consideration of several parameters in our experiments may facilitate the interpretation of our results in the context of previous studies. First, the genetic background and immune competency of the mouse strains employed and the Ang II dose differed in our experiments from those used by other groups. It is possible that the high dose of Ang II used in our studies induced apoptosis among T cells specific for a relevant neo-antigen in our model [87]. Second, life-long deletion of AT₁ receptors from T cells, as occurs with our genetic approach, may lead to compensatory alterations in other genes and related signals. Relevant to this concern, we recorded profound changes in gene expression profiles in T lymphocytes isolated from *T cell WT* and *KO* kidneys in the setting of hypertension [40]. Third, our approach deleted the AT₁ receptor from both CD4⁺ and CD8⁺ T lymphocytes. This strategy induced a pro-inflammatory CD4⁺ Th1 cell subset that accelerated hypertensive kidney damage. However, the infiltration of CD8⁺ T cells into the kidney during Ang II infusion was not influenced by our genetic strategy. Thus, our study has little to say about the functions of CD8⁺ T cells in the pathogenesis of hypertension, and further studies in this area will be critical to truly understand the role of adaptive immunity in hypertension.

Perspective and Conclusion

Treatment with AT₁ receptor blockers (ARBs) effectively lowers blood pressure and ameliorates the complications of hypertension, including ischemic heart disease, congestive heart failure, and progressive kidney damage. However, ARB therapy does not fully prevent or reverse these cardiovascular diseases. Understanding the mechanisms through which AT₁ receptor activation regulates disease pathogenesis is therefore critical for the identification of relevant signaling pathways and the design of potent, targeted therapeutics. To this end, we have identified a paradoxical protective effect of AT₁ receptor activation on T lymphocytes to slow the progression of glomerular damage in the kidney during hypertension. These findings hold promise for the design of novel therapeutics to globally suppress the renin–angiotensin system while sparing or even stimulating AT₁ receptors on T cells. Such a “selective” modulation of the renin–angiotensin system may yield even greater benefits in the treatment of cardiovascular diseases than the current strategy of global angiotensin receptor blockade.

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A

Control

CD4-Cre⁺
mT/mG

Thymus

X40

Spleen

X40

Kidney

X20

Figure 1. Verification of T cell-specific deletion of the type 1a angiotensin (AT_{1A}) receptor in *T cell knockout (KO)* mice

A, Representative histology of thymus, spleen, and kidney in *CD4-Cre⁺ mT/mG* and control (*CD4 Cre⁻ mT/mG*) mice. In these *mT/mG* mice, green fluorescence indicates the presence of *CD4 Cre* expression whereas red fluorescence indicates the absence of *CD4 Cre* expression. Blue fluorescence in kidney is a nuclear DAPI stain. **B**, Splenocytes were harvested from *T cell WT* and *T cell KO* littermates and sorted into 3 subpopulations. *Agtr1a* mRNA expression was quantitated in these purified immune cell populations and in kidney and heart from *T cell WT* and *T cell KO* groups (n = 6). This method revealed a 90% deletion of the AT_{1A} receptor from both CD4⁺ and CD8⁺ T lymphocytes in the *T cell KO* animals. * *P* < 0.00001 vs. *T cell WT*. (Adapted from Zhang et al. [40])