Cytotoxic T Lymphocyte-Associated Antigen-4 Protects Against Angiotensin II-Induced Kidney Injury in Mice

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Background: Chronic inflammation caused by pathogenic immune response is crucial in the pathogenesis of kidney disease. In particular, T-cell-mediated adaptive immune responses evoke pathogenic immunoinflammatory responses and contribute to kidney injury (KI). Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), a potent negative regulator of T-cell immune responses, protects against immunoinflammatory diseases of the arteries such as atherosclerosis and abdominal aortic aneurysm. However, the role of this molecule in kidney disease remains underdetermined.

Methods and Results: To examine the effects of CTLA-4 overexpression on angiotensin II (AngII)-induced KI, we induced KI in CTLA-4 transgenic/apolipoprotein E-deficient (CTLA-4-Tg/Apoe−/−) mice or Apoe−/− mice fed a high-cholesterol diet by continuously infusing AngII. Overexpression of CTLA-4 ameliorated the development of AngII-induced KI and fibrosis. Moreover, CTLA-4-Tg/Apoe−/− mice had decreased expression of pro-inflammatory molecules in the kidney.

Conclusions: CTLA-4 overexpression has a protective effect on AngII-induced KI, and increasing CTLA-4 may be a novel therapeutic strategy to prevent the progression of kidney disease.

Key Words: Angiotensin II; Co-inhibitory molecule; Inflammation; Kidney injury; T cell

Kidney disease is an important cause of mortality in developed and developing countries and has become a significant health problem. Kidney disease typically results from diabetes, hypertension, autoimmune disease, or infection. However, interventions to regulate these diseases are not sufficient to slow the progression of kidney dysfunction. Although a large number of experimental and clinical studies have investigated the mechanisms underlying the process of kidney dysfunction, precise mechanisms have not been fully clarified. A thorough understanding of the mechanisms leading to kidney disease could contribute to the development of novel therapeutic strategies to prevent the progression of this disease.

Accumulating evidence suggests that the innate and adaptive immune system plays a critical role in the development and progression of kidney disease. In particular, chronic renal inflammation via T-cell-mediated immune responses has been shown to be involved in the pathogenesis of kidney disease. Angiotensin II (AngII) is one of the critical hypertensive stimuli, and its role in various diseases including atherosclerotic disease, hypertension, and kidney disease has been extensively investigated. AngII stimulation activates immune cells including macrophages, dendritic cells, and, in particular, T cells, and mediates several key events of the inflammatory process in the development of cardiovascular and kidney disease. Based on this, it can be speculated that regulation of T-cell immune responses may be a possible strategy to prevent AngII-induced kidney injury (KI).

Naïve T cells are activated by receiving 2 critical signals from antigen-presenting cells. T cells receive the first signal via the T-cell receptor by interacting with antigenic peptide/major histocompatibility complex ligand on the antigen-presenting cells. They also receive the second signal provided by co-stimulatory molecules on antigen-presenting cells to enhance or inhibit their activation depending on the type of co-stimulatory pathways. Co-stimulatory pathways play a crucial role in the regulation of pro-inflammatory effector T cells and anti-inflammatory regulatory T cells, and have a significant influence on atherosclerosis, abdominal aortic aneurysm, and hypertension. Regulatory T cells and activated effector T cells express co-inhibitory molecule cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), a homolog of CD28 that competitively binds to CD80 and CD86 on antigen-presenting cells, leading to blockade of the co-stimulatory CD80/CD86-CD28 pathway.
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Kidney Histology

Mice were anesthetized and the kidney was perfused with saline. The kidney was embedded in OCT compound (Tissue-Tek; Sakura Finetek, Tokyo, Japan), and 10-μm-thick cross-sections were prepared. Two sections in each mouse (7 mice per group) were stained with hematoxylin-eosin (HE) and the stained sections were digitally captured using an All-in-one Type Fluorescence Microscope (BZ-8000; Keyence, Osaka, Japan). Masson’s trichrome staining was performed to detect the fibrous area of the kidney. The stained sections were digitally captured using an All-in-one Type Fluorescence Microscope, and the percentage of the fibrotic area was calculated using ImageJ (National Institutes of Health, Bethesda, MD, USA). Two randomly chosen fields per section of the kidney were analyzed in each mouse and the average of the 2 sections was used for statistical analysis.

Methods

Animals and Experimental Design

We used apolipoprotein E-deficient (Apoe−/−) mice and CTLA-4-Tg/Apoe−/− mice on a C57BL/6 background and fed them a high-cholesterol diet containing 0.2% cholesterol and 21% fat (CLEA, Tokyo, Japan) and water ad libitum. We implanted ALZET mini-osmotic pumps (Model 2004; DURECT, Cupertino, CA, USA) in 12-week-old mice under anesthesia and continuously infused AngII (1,000 ng/kg/min, Sigma, St Louis, MO, USA) for 28 days as described previously. The mice were killed at 16 weeks of age under anesthesia to evaluate KI. The mice were housed in specific pathogen-free animal facilities. All animal experiments were approved by the Animal Care Committee of Kobe Pharmaceutical University (Permit Numbers: 2018-003, 2019-008) and conformed to the NIH guidelines.

and negative regulation of T-cell function. In addition, effector T-cell responses are negatively regulated by receiving an inhibitory signal through the CD80/CD86–CTLA-4 pathway. We recently generated CTLA-4 transgenic (CTLA-4-Tg) mice on an atherosclerosis-prone background and demonstrated that overexpression of CTLA-4 protected against the development of immunoinflammatory diseases of the arteries such as atherosclerosis and abdominal aortic aneurysm.7 However, the effect of CTLA-4 overexpression on kidney disease remains unknown. In the present study, we used the CTLA-4-Tg mice that we have recently established, to investigate the role of CTLA-4 in the development of AngII-induced experimental kidney disease.
Real-Time Reverse Transcription–Polymerase Chain Reaction
Total RNA was extracted from the kidney after perfusion with RNA (Life Technologies) using TRizol reagent (Life Technologies). Quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed using a PrimeScript RT reagent Kit (Takara, Shiga, Japan), a SYBR Premix Ex Taq (Takara), and a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific) according to the manufacturer’s protocol. The following primers were used to amplify interleukin (IL)-1β, IL-6, monocyte chemotactic protein-1 (MCP-1), CD68 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): IL-1β, 5′-TGG GAC TGT TGG TCT A-3′ and 5′-CTC CCT ACT CTA GCC CTG CGT GAC TCT A-3′; MCP-1, 5′-GCA TCC AGG TGT TGG CTG CTC A-3′ and 5′-GCA AGT GCA TCA TGG CTC ATG GTG CCT A-3′; GAPDH, 5′-TGC GCC CAT AGT GCA TCA TGG CTC ATG GTG CCT A-3′ and 5′-GGT CTA TTG AAG TGG CTC ATG GTG CCT A-3′; IL-6, 5′-TCC AGG ATG AGG ACA TGA GCA C-3′ and 5′-CCA TTG TTC CCA AAG TGG TTC GAG T-3′; monocyte chemoattractant protein-1 (MCP-1), 5′-GCA TCC AGG TGT TGG CTG CTC A-3′ and 5′-GCA AGT GCA TCA TGG CTC ATG GTG CCT A-3′; and macrophage specific marker CD68, 5′-GAA CGT CAC ACA CCA GCA GGT TA-3′ and 5′-CCA CTT AAC AAG TCG GAG GCT TTA-3′. Amplification reactions were performed in duplicate and fluorescence curves were analyzed with included software. GAPDH was used as an endogenous control reference.

Statistical Analysis
Mann-Whitney U-test was used to detect significant differences between 2 groups. P<0.05 was considered statistically significant. For statistical analysis, GraphPad Prism version 7.0 (GraphPad Software) was used.

Results
Effects of CTLA-4 Overexpression
Amelioration of AngII-Induced KI
CTLA-4 overexpression did not change mouse body weight or plasma lipid profile.7 Although there was a marked increase in systolic blood pressure (SBP) in both Apoe−/− and CTLA-4-Tg/Apoe−/− mice following AngII infusion for 4 weeks, there was no difference in SBP between the 2 groups.7 We next evaluated the effect of AngII infusion on morphological changes in kidney structure in both Apoe−/− and CTLA-4-Tg/Apoe−/− mice by evaluating HE-stained cryosections. On histology of the kidney tissues, AngII-infused Apoe−/− mice had glomerular abnormalities such as decreased Bowman’s space and increased mesangial cells, whereas only minimal changes were observed in the kidney tissues of CTLA-4-Tg/Apoe−/− mice (Figure 1). Collectively, these data indicate that CTLA-4 plays a protective role in AngII-induced KI without lowering plasma lipid profile or blood pressure.

Inhibition of AngII-Induced Kidney Fibrosis
The extent of fibrosis in AngII-induced KI was analyzed using Masson’s trichrome staining. Collagen deposition in the kidney of AngII-infused CTLA-4-Tg/Apoe−/− mice was significantly decreased compared with that of AngII-infused Apoe−/− mice (Figure 2).

To clarify the mechanisms of AngII-induced KI fibrosis, we focused on immunoinflammatory responses in the injured kidney. Consistent with our previous findings of suppressed aortic inflammation in AngII-infused CTLA-4-Tg/Apoe−/− mice,7 on quantitative RT-PCR the mRNA expression of pro-inflammatory cytokines or chemokines (IL-1β, IL-6, MCP-1) and macrophage specific marker CD68 was markedly decreased in the kidney of AngII-infused CTLA-4-Tg/Apoe−/− mice compared with that of AngII-infused Apoe−/− mice (Figure 3). Taken together, these data indicate that CTLA-4 may prevent AngII-induced kidney fibrosis through the downregulation of renal immunoinflammatory responses.

Discussion
Kidney disease is one of the leading causes of morbidity and mortality worldwide. However, pharmacological treatment for several risk factors including diabetes or hypertension has little effect on the progression of this disease, and until now there has been no effective medical treatment for this disease. AngII is considered to be one of the main causes of kidney disease. AngII activates T cells and promotes T-cell-mediated vascular and renal dysfunction.8 Therefore, therapeutic interventions to regulate T-cell activation could be effective to prevent AngII-induced kidney damage. Here, we provide evidence that overexpression of the co-inhibitory molecule CTLA-4 inhibits renal inflammation and limits the damage and fibrosis in the kidney in an AngII-induced KI mouse model. The increasing of CTLA-4 is therefore suggested as a possible therapeutic strategy to prevent the progression of kidney disease.

Accumulating experimental and clinical evidence indicates that T-cell-mediated inflammation critically contributes to the pathogenesis of hypertension.8 An experimental study using genetic or antibody-mediated blockade approaches demonstrated that inhibition of the co-stimulatory CD80/CD86–CD28 pathway prevented the development of hypertension by regulating T-cell-mediated vascular inflammation.5 In the present study, however, we found no changes in blood pressure following blockade of the CD80/CD86–CD28 pathway by CTLA-4 overexpression in AngII-infused hypercholesterolemic mice, suggesting that...
the protective actions of CTLA-4 overexpression in KI may depend on direct anti-inflammatory effects.

With regard to the clinical implications of the present results, CTLA-4-Ig is a soluble fusion protein consisting of the extracellular CTLA-4 portion and mimics the inhibitory effect of CTLA-4 through a cell extrinsic pathway. CTLA-4-Ig has beneficial effects in the treatment of autoimmune diseases such as type 1 diabetes mellitus and rheumatoid arthritis, which are linked to the progression of kidney disease. Notably, previous experimental studies using CTLA-4-Ig showed that blockade of the CD80/CD86–CD28 pathway prevented renal allograft rejection and diabetic nephropathy. Furthermore, not only in experimental studies, but also in a case series of focal segmental glomerulosclerosis patients, treatment with CTLA-4-Ig induced partial or complete remission of proteinuria. Taken together with the present findings on the protective role of CTLA-4 in AngII-induced KI, it will be of great interest to test the hypothesis that therapeutic interventions to modulate the adaptive immune response by modulating CTLA-4 function would be effective for preventing the development of kidney disease. Although careful observation is needed for application in clinical settings in consideration of the detrimental side-effects such as general immunosuppression by the co-stimulatory blockade, the present data may provide a novel strategy for the treatment and prevention of kidney disease.

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Disclosures

The authors declare no conflicts of interest.

IRB Information

This study was approved by the Animal Care Committee of Kobe Pharmaceutical University (reference no., 2018-003, 2019-008).

References

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: Global dimension and perspectives. Lancet 2013; 382: 260–272.
2. Imig JD, Ryan MJ. Immune and inflammatory role in renal disease. Compr Physiol 2013; 3: 957–976.
3. Ley K, Gerdes N, Winkels H. ATVB Distinguished Scientist Award: How costimulatory and coinhibitory pathways shape atherosclerosis. Arterioscler Thromb Vasc Biol 2017; 37: 764–777.
4. Ait-Oufella H, Wang Y, Herbin O, Bourcier S, Potteaux S, Joffre J, et al. Natural regulatory T cells limit angiotensin II-induced aneurysm formation and rupture in mice. Arterioscler Thromb Vasc Biol 2013; 33: 2374–2379.
5. Vinh A, Chen W, Blinder Y, Weiss D, Taylor WR, Goronzy JJ, et al. Inhibition and genetic ablation of the B7/CD28 T-cell costimulation axis prevents experimental hypertension. Circulation 2010; 122: 2529–2537.
6. Matsumoto T, Sasaki N, Yamashita T, Emoto T, Kasahara K, Mizoguchi T, et al. Overexpression of cytotoxic T-lymphocyte-associated antigen-4 prevents atherosclerosis in mice. Arterioscler Thromb Vasc Biol 2016; 36: 1141–1151.
7. Amin HZ, Sasaki N, Yamashita T, Mizoguchi T, Hayashi T, Emoto T, et al. CTLA-4 protects against angiotensin II-induced abdominal aortic aneurysm formation in mice. Sci Rep 2019; 9: 8065.
8. McMaster WG, Kirabo A, Madhur MS, Harrison DG. Inflammation, immunity, and hypertensive end-organ damage. Circ Res 2015; 116: 1022–1033.
9. Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, et al; Type 1 Diabetes TrialNet Abatacept Study Group. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: A randomised, double-blind, placebo-controlled trial. Lancet 2011; 378: 412–419.
10. Kremer JM, Westhoven R, Leon M, Di Giorgio E, Alten R, Steinfeld S, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4-Ig. N Engl J Med 2003; 349: 1907–1915.
11. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, et al. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proc Natl Acad Sci USA 1997; 94: 8789–8794.
12. Fiorina P, Vergani A, Bassi R, Niewczas MA, Altintas MM, Pezzolesi MG, et al. Role of podocyte B7-1 in diabetic nephropathy. J Am Soc Nephrol 2014; 25: 1415–1429.
13. Yu CC, Fornoni A, Weins A, Hakroush S, Maiguel D, Sageshima J, et al. Abatacept in B7-1-positive proteinuric kidney disease. N Engl J Med 2013; 369: 2416–2423.