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Antony Vinh, Emory University
Wei Chen, Emory University
Yelena Blinder, Emory University
Daiana Weiss, Emory University
W Robert Taylor, Emory University
Jorg Goronzy, Stanford University
Cornelia Weyand, Stanford University
David G Harrison, Emory University
Tomasz J. Guzik, Jagiellonian University

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Inhibition and Genetic Ablation of the B7/CD28 T cell Costimulation Axis Prevents Experimental Hypertension

Antony Vinh, PhD, Wei Chen, PhD, Yelena Blinder, MD, Daiana Weiss, PhD, W. Robert Taylor, MD, PhD, Jorg Goronzy, MD, PhD, Cornelia Weyand, MD, PhD, David G. Harrison, MD, PhD, and Tomasz J. Guzik, MD, PhD

1Division of Cardiology, Department of Medicine, Emory University School of Medicine and the Atlanta Veteran Administration Hospital, Atlanta, GA 2Division of Rheumatology and Immunology, Department of Medicine, Stanford University School of Medicine 3Department of Medicine, Jagiellonian University School of Medicine, Cracow, Poland

Abstract

Background—The pathogenesis of hypertension remains poorly understood and treatment is often unsuccessful. Recent evidence suggests that the adaptive immune response plays an important role in this disease. Various hypertensive stimuli cause T cell activation and infiltration into target organs such as the vessel and the kidney, promoting vascular dysfunction and blood pressure elevation. Classically, T cell activation requires T cell receptor ligation and costimulation. The latter often involves interaction between B7 ligands (CD80 and CD86) on antigen presenting cells with the T cell co-receptor CD28. This study was therefore performed to examine the role of this pathway in hypertension.

Methods and Results—Angiotensin II-induced hypertension increased the presence of activated (CD86+) dendritic cells in secondary lymphatic tissues. Blockade of B7-dependent costimulation with CTLA4-Ig reduced both angiotensin II- and DOCA-salt induced hypertension. Activation of circulating T cells, T cell cytokine production and vascular T cell accumulation caused by these hypertensive stimuli was abrogated by CTLA4-Ig. Furthermore, in mice lacking B7 ligands, angiotensin II caused minimal blood pressure elevation and vascular inflammation, and these effects were restored by transplant with wild-type bone marrow.

Conclusion—T cell costimulation via B7 ligands is essential for development of experimental hypertension and inhibition of this process could have therapeutic benefit in the treatment of this disease.

Keywords
hypertension; immune system; inflammation; lymphocytes
INTRODUCTION

Despite extensive study, the cause of most cases of human hypertension remains unknown. Investigation for more than a century has focused on the kidney, the central nervous system and the vasculature as mediators of this common disease. Increasing evidence suggests that inflammation might contribute to hypertension. Various factors common to the hypertensive milieu, including reactive oxygen species, angiotensin II and altered physical forces promote activation of inflammatory cells, including macrophages and T cells, and their infiltration into the vasculature and kidney. Importantly, recent evidence suggests that T cells are essential for development of experimental hypertension. RAG-1−/− mice, which lack lymphocytes, have blunted hypertensive responses to angiotensin II infusion and DOCA-salt challenge, and adoptive transfer of T cells completely restores hypertension in these animals. Moreover, angiotensin II infusion increases circulating CD44high/CCR5+/CD69+ T cells. Such cells mimic the phenotype of activated, effector T cells; although the mechanism of how hypertension causes T cell activation remains undefined.

Classically, T cells require two signals for activation. The first involves interaction of the T cell receptor with an antigenic peptide presented in the context of major histocompatibility complex on antigen-presenting cells (APCs). The second, referred to as costimulation, involves the simultaneous interaction of receptors in proximity to the TCR with ligands on the APC. Among several potential costimulatory interactions, the binding of T cell CD28 with B7 ligands CD80 and CD86 on APCs is important, particularly for activation of naïve T cells. When TCR ligation occurs without costimulation of CD28, T cell activation is prevented and apoptosis is favored. Pharmacological approaches to inhibit B7-dependent costimulation have been employed to treat autoimmune diseases and prevent transplant rejection. Immune modulation using this approach might thus inhibit the inflammatory processes that underlie hypertension and vascular dysfunction. The current study was therefore performed to determine if interruption of costimulation, either pharmacologically or by genetic deletion of B7 ligands, would prevent T cell activation in response to hypertensive stimuli and have anti-hypertensive effects. Our findings suggest that T cell activation in the setting of elevated blood pressure requires costimulation and that strategies to prevent this could be useful in special cases of difficult to treat hypertension.

MATERIALS AND METHODS

Animal Models

C57Bl/6J mice and mice with genetic deletion of both CD80 and CD86 (B7−/− mice) on a C57Bl/6J background were obtained from Jackson Laboratories (Bar Harbor, ME) and were fed regular chow. Diet and water were provided ad libitum. The Emory University Animal Care and Use Committee approved the protocol for animal use. Mice at 12 weeks of age were selected at random from their cages for surgical and treatment interventions. In one series of experiments, we employed a 2 × 2 design to compare the effect of co-administration of CTLA4-Ig on the hypertensive response to angiotensin II. Angiotensin II was administered subcutaneously at a rate of 490 ng/kg/min for 14 days using osmotic minipumps as previously described. Sham-operated animals underwent an identical surgical procedure, except the diluent without angiotensin II was infused. The investigator was blinded to which mice received sham or angiotensin II infusions. The fusion protein CTLA4-Ig (250 µg), which inhibits T cell costimulation, was administered intraperitoneally (IP) every three days beginning 3 days prior to minipump implantation. As a control, either saline or an isotype control antibody was administered to mice not receiving CTLA4-Ig.

In additional experiments we employed a 2 × 2 design to determine if CTLA4-Ig could prevent a separate form of hypertension caused by administration of deoxycorticosterone
acetate and salt (DOCA-salt hypertension). For creation of DOCA-salt hypertension, mice underwent uninephrectomy, subcutaneous placement of a DOCA pellet and then received 0.9% NaCl in the drinking water. The sham surgery involved a surgical incision and placement of a placebo pellet. Animals were treated with either CTLA4-Ig or its placebo as described above, beginning 3 days prior to surgery and every three days thereafter.

In two separate experiments, we sought to determine if CTLA4-Ig would reverse established hypertension. To accomplish this, we performed two additional experiments using 2 × 2 designs in which CTLA4-Ig treatment was initiated two weeks following either angiotensin II infusion (Sham/AngII + Vehicle/CTLA4-Ig) or the DOCA-salt challenge (Sham/DOCA + Vehicle/CTLA4-Ig). CTLA4-Ig was administered every 3 days during the 2- or 4-week treatment regimens respectively.

Bone Marrow Engraftment

In a separate experiment, bone marrow was obtained from 8-week-old WT donor mice by flushing femurs and tibiae with RPMI 1640 medium. Nucleated cells were counted, and bone marrow was resuspended at a concentration of 2 × 10^7/ml. Eight week old recipient B7−/− mice were irradiated with 1100 rads and then were immediately reconstituted with either 2 × 10^6 WT bone marrow cells via retro-orbital injection. After 6 weeks of engraftment, the recipient mice were randomized to receive either sham or angiotensin II osmotic minipump surgery. Following 14-days of infusion, spleen-derived DCs from recipient mice were analyzed by flow cytometry for CD80+ or CD86+ expression to confirm successful engraftment.

Blood pressure measurements

Blood pressure was measured using the tail-cuff method at days 0, 7 and 14 (and 21 for DOCA-salt model) using the MC4000 Multichannel System for mice (Hatteras, Inc). In studies examining the effect of CTLA4-Ig on angiotensin II-induced hypertension only, we also measured intra-arterial blood pressure in freely moving mice using radiotelemetry. For reversal studies blood pressures were measured weekly for 4–6 weeks during the course of angiotensin II or DOCA salt challenge.

Flow Cytometry

At the end of each treatment period, mice were euthanized and blood and tissue were harvested for immediate flow cytometric analysis. Flow cytometry was employed to characterize circulating T cell activation phenotypes and vascular T cell infiltration. Dendritic cell (DC) maturation was examined in spleens and lymph nodes of sham and angiotensin II treated mice. Please refer to online data supplement for a detailed description of tissue digestion and flow cytometry.

Cytokine detection

Spleen or lymph nodes were digested as described above and were purified using autoMACS and a pan T cell isolation kit (Miltenyi Biotech). Cell purity was confirmed to be > 95%. Splenic and lymph node T cells were plated at a density of 2×10^5 per well in 96-well plates coated with anti-CD3 antibody (BD PharMingen) and cultured for 48 hours. Cytometric Bead Array (BD PharMingen) was used to determine levels of TNF-α, IFN-γ, IL-2, IL-4, and IL-5 that had been released into the media.

Quantification of vascular superoxide production

In a separate set of mice (n=5–6), aortic superoxide production was determined by monitoring conversion of dihydroethidium to 2-hydroxyethidium as previously described 10.
Statistical analyses

All data are expressed as mean ± SEM. For blood pressure measurements over time, repeated measures ANOVA with a Scheffe’s post-hoc test was performed using PASW Statistics 18.0 for Mac. For measurements obtained at one point in time, comparisons of animals or treatments were made using two-way ANOVA using Graphpad Prism 5.0. When significance was indicated, specific comparisons were made based on pre-designated hypotheses using Bonferroni post-hoc two-tailed analysis. To assure that the overall risk of a type 1 error for multiple comparisons did not exceed 0.05, a Bonferroni correction was applied by dividing 0.05 by the number of comparisons. The reported level of significance indicated in the manuscript for these comparisons represents values after the Bonferroni correction. When two comparisons were made, a two-tailed t-test was employed.

RESULTS

Pharmacological inhibition of costimulation reduces angiotensin II-induced hypertension

Using both non-invasive tail-cuff measurements (Figure 1A) and invasive monitoring of blood pressure by radiotelemetry (Figure 1B and 1C), we found that co-treatment with the fusion protein CTLA4-Ig, which blocks CD28 interactions with B7 ligands, significantly attenuated the hypertension induced by angiotensin II (Figure 1A–C, and Supplementary table 1). Radiotelemetry demonstrated that systolic pressure averaged 168 ± 1.5 mmHg in mice co-treated with isotype control antibodies and 139 ± 2.8 mmHg in CTLA4-Ig-treated mice during the last week of angiotensin II infusion (p < 0.001). Likewise diastolic pressure was reduced from 139 ± 1.3 to 116 ± 1.6 (p < 0.001) by CTLA4-Ig co-treatment (Figure 1C).

A hallmark of hypertension is an increase in vascular superoxide production, which in turn alters vascular tone and promotes vascular disease. Angiotensin II-induced hypertension was associated with a 3-fold increase in aortic superoxide production, and this was not observed in aortas obtained from mice treated with CTLA4-Ig (Figure 1D).

Inhibition of T cell activation and vascular inflammation by CTLA4-Ig in angiotensin II-induced hypertension

As described previously, chronic angiotensin II infusion caused a modest, but significant increase in the percentage of T cells expressing the early activation marker CD69 (Figure 2A and 2B). The percentage of circulating T cells expressing CD44high (Figure 2C) and the surface marker CCR5 (Figure 2D), which plays a critical role in tissue homing, were also increased by angiotensin II. These responses were eliminated by co-treatment with CTLA4-Ig (Figure 2).

Previously, we have showed that chronic angiotensin II infusion increases infiltration of total leukocytes and CD3+ cells into the aorta, and that the predominant site of infiltration is the perivascular adipose tissue. In the present study, we confirmed these findings. Analysis of aortic homogenates revealed that angiotensin II infusion increased total leukocytes by 4-fold (Figure 3A). CTLA4-Ig co-treatment attenuated aortic accumulation of total leukocytes (Figure 3A), largely due to a decrease in CD3+ cells (Figure 3C). Consistent with our previous study, angiotensin II increased aortic infiltration of CD4+, CD8+ and double negative T cell subsets, and this was reduced by CTLA4-Ig treatment (Supplemental Figure 1). The increase in aortic macrophages caused by angiotensin II was not significantly altered by CTLA4-Ig co-treatment (Supplemental Figure 2A). CTLA4-Ig had no effect on the aortic content of B cells, DCs or natural killer cells (Supplemental Figure 2B). Angiotensin II-induced hypertension was associated with a modest increase in TNF-α and IFN-γ by T cells isolated from the spleen, but a marked increase in production of these cytokines in cells...
isolated from lymph nodes (Figure 3D and 3E). Importantly, CTLA4-Ig prevented these increases in T cell cytokine production caused by angiotensin II. T cell production of IL-4 and IL-5 were below the limit of detection in all treatment groups (data not shown).

Evidence for maturation of APCs in response to chronic angiotensin II infusion and role for B7 ligands in hypertension

Following antigen uptake, DCs and other antigen processing cells undergo a maturation process characterized by increased surface expression of MHC II and the B7 ligand CD86. Flow cytometry was therefore employed to detect CD11c+ cells circulating in blood and in single cell isolates of the aorta, spleen and lymph nodes (Figure 4A). The percentage of these cells expressing CD80 and MHC II was unchanged by chronic angiotensin II infusion in all of these compartments (Figure 4C). In contrast, angiotensin II significantly increased the percent of CD11c cells expressing CD86 in the spleen and lymph nodes (Figure 4B).

These findings show that DC expression of CD86 is selectively increased by hypertension in secondary lymphoid organs, in keeping with a classical pathway for T cell activation and are compatible with the anti-hypertensive effects of CTLA4-Ig shown above. To further examine the role of B7 ligands in hypertension, we performed additional studies in mice deficient in CD80 and CD86 (B7−/− mice). The hypertensive response to angiotensin II was markedly reduced in these mice (Figure 5A). Moreover, the increase in circulating CD69+ and CD44high T cells caused by angiotensin II did not occur in B7−/− mice (Figure 5B). Finally, the absence of B7 ligands protected against the vascular infiltration of CD3+ positive cells during angiotensin II infusion (Figure 5C and 5D).

B7 molecules are not only expressed on DCs, but also on non-hemopoietic cells such as endothelial cells. To directly examine the role of B7 on bone marrow derived cells, we performed bone marrow transplant experiments. B7−/− mice were lethally irradiated and then given bone marrow from wild type C57Bl/6J mice. Six weeks later, these animals were treated with chronic infusions of either angiotensin II or vehicle for the ensuing 2 weeks. To confirm successful engraftment of WT bone marrow, we employed flow cytometry to analyze B7 ligand surface expression on spleen-derived DCs. Recipient B7−/− mice displayed an expression profile very similar to WT mice indicating restoration of B7 ligand expression on DCs (Figure 6B). Engrafting WT bone marrow into B7−/− mice completely restored angiotensin II-induced hypertension (Figure 6A) and aortic infiltration of leukocytes and T cells (Figures 6C and 6D).

CTLA4-Ig prevents DOCA-salt induced hypertension

It is conceivable that costimulation might affect only angiotensin II-induced hypertension and not be effective in other forms of hypertension. We therefore performed additional studies in mice with DOCA-salt hypertension. This model of hypertension is associated with suppression of the renin/angiotensin system and is sodium and volume dependent, but similar to angiotensin II-induced hypertension is associated with inflammation and oxidative tissue damage. We have previously shown that T cells are also critical in this model. As is the case of angiotensin II, CTLA4-Ig reduced the hypertensive response to the DOCA-salt challenge (Figure 7A). DOCA-salt hypertension also promoted T cell activation, as evidenced by an increase in circulating CD69+ and CD44high cells. Co-treatment with CTLA4-Ig prevented these changes in T cell activation markers (Figure 7B). The aortic infiltration of total leukocytes and T cells was significantly increased by DOCA-salt hypertension, and this was prevented by CTLA4-Ig co-treatment (Figures 7C, 7D and 7E).
Reversal of hypertension by CTLA4-Ig

We performed additional experiments to determine if CTLA4-Ig could reverse established angiotensin II and DOCA-salt induced hypertension. Two weeks of angiotensin II infusion or DOCA-salt challenge raised blood pressures to 175 ± 2.4 mmHg and 153 ± 4.1 mmHg respectively. As we have reported in the current and previous study, 2 weeks of angiotensin II infusion significantly increases activation of circulating T cell and infiltration of these cells. In the present study, we also found that 2 weeks of DOCA-salt induced hypertension had similar effects (Supplement Figure 3). At this time point we began treatment with CTLA4-Ig (250 µg/mouse) delivered I.P. every 3 days as described above for 2 or 4 weeks. Treatment with CTLA4-Ig significantly lowered blood pressure in mice made hypertensive by either angiotensin II (Figure 8A) or DOCA-salt (Figure 8B). Of note, in DOCA-salt hypertensive mice, 4 weeks of CTLA4-Ig treatment lowered blood pressure to values observed in mice that had received sham surgery. These data indicate that blockade of costimulation not only prevents hypertension but also lowers established hypertension.

DISCUSSION

In previous studies, we found that T cells play an important role in the genesis of hypertension. These studies showed that hypertensive stimuli are associated with T cell activation and the entry of effector-like T cells into the perivascular adipose tissue. We hypothesized that these cells release cytokines that affect function of the adjacent vessel, stimulating superoxide production and altering vasomotor tone. In the present study, we show that prevention of T cell costimulation by treatment with CTLA4-Ig or by genetic deletion of B7 ligands prevents T cell activation and vascular inflammation caused by hypertension. This was associated with a reduction in the hypertensive response and vascular superoxide production. In keeping with an anti-inflammatory effect, CTLA4-Ig prevented the increase in TNF-α and IFN-γ production by T cells isolated from the spleen and lymph nodes of hypertensive mice. We further showed that the effect of costimulation blockade was not limited to angiotensin II-induced hypertension, but also occurred in mice with DOCA-salt hypertension. Furthermore, we demonstrated that blockade of costimulation could also reverse established hypertension. The current study therefore provides unequivocal evidence that inhibition of the adaptive immune response can ameliorate high blood pressure and that modulation of costimulation might be effective in the treatment of otherwise refractory or malignant hypertension.

A previously unanswered question is how hypertensive stimuli like angiotensin II and high salt activate T cells. It is possible that hypertensive stimuli could mobilize T cells activated by other non-specific stimuli. Another possibility is that the major effect of angiotensin II is to increase adhesion and chemoattractant molecules like ICAM-1 and RANTES that attract T cells previously activated by other, unrelated stimuli. These “passive” mechanisms are possible because stimuli such as angiotensin II increase vascular expression of adhesion molecules and chemokines, largely via redox-mediated mechanisms. Further, even when mice are kept in clean conditions, modest T cell activation by exogenous antigens is unavoidable. Given that costimulation occurs simultaneously with T cell receptor activation, our current findings suggest that T cells are activated via a classical mechanism in the setting of hypertension. Our findings are therefore consistent with a scenario in which hypertensive stimuli prompt formation of neoantigens that are presented to T cells via APCs leading to T cell activation. The increase in circulating cells bearing markers such as CD69, CD44high and CCR5 in response to angiotensin II is compatible with this hypothesis. Moreover, our finding that angiotensin II increases the level of CD86+CD11c+ cells in secondary lymphoid organs is compatible with maturation of DCs, which occurs upon antigen presentation. The nature of neoantigens involved in hypertension remains undefined, however this situation is analogous to the setting of atherosclerosis, where
molecules such as altered oxidized lipoproteins, heat shock proteins, bacterial and viral proteins have been implicated as neoantigens. Importantly, blockade of costimulation was effective not only in angiotensin II-induced hypertension, but also in DOCA-salt hypertension. This form of hypertension is associated with suppressed circulating renin levels, and our findings therefore indicate that costimulation contributes to hypertension that is independent of angiotensin II. These data are compatible with our previous findings that RAG-1−/− mice are resistant to DOCA-salt hypertension, and that high salt promotes T cell recruitment into the kidney.

Agents that prevent costimulation, such as CTLA4-Ig, have been developed to treat autoimmune diseases such as rheumatoid arthritis and transplant rejection. There is some debate that these agents might also affect the immune response by outside-in signaling and modulation of APC function. As an example, it has recently been shown that CTLA4-Ig promotes formation and activation of T regulatory cells. These actions have been in part attributed to activation of 2,3-dioxygenase (IDO) in DCs. IDO competent cells in turn are potent suppressors of T cell function and can inhibit activation of these cells in an inflammatory environment. It is therefore possible that CTLA4-Ig exerts its anti-hypertensive effect via promotion of T regulatory cell function. In preliminary studies, however, we have not found an increase in circulating CD4+CD25+FoxP3+ cells in CTLA4-Ig-treated hypertensive mice (data not shown), indicating that an increase in T regulatory cells is probably not responsible for the effects of CTLA4-Ig in this setting. Moreover, these effects of CTLA4-Ig to increase immunoregulatory T cells are dependent on B7 signaling. Our finding that B7−/− mice are also resistant to the hypertension and vascular inflammation caused by angiotensin II indicates that APC signaling via by B7 is unlikely the only mechanism by which CTLA4-Ig reduces blood pressure, but does emphasize the role of B7-mediated costimulation in mediating hypertension. Importantly, engrafting WT bone marrow into B7−/− mice restored angiotensin II-induced hypertension and vascular inflammation, further supporting a role for the B7/CD28 axis in hypertension.

In keeping with our current studies, the T cell suppressing agent mycophenolate mofetil (MMF) reduces blood pressure in experimental animals. Moreover, MMF and the anti-inflammatory agent dexamethasone prevent renal damage in hypertensive transgenic rats expressing human renin and angiotensinogen. The levels of DCs in the kidney were elevated in these animals, and were reduced by dexamethasone. These findings support a role for T cell activation via interaction with DCs in the setting of hypertension. MMF has also been shown to lower blood pressure in humans with psoriasis and rheumatoid arthritis. This agent interferes with purine metabolism and therefore could have non-specific effects on other rapidly dividing cells, however when taken together with our present study, supports a role for T cell activation in hypertension.

It is of interest that the number of vascular macrophages was not affected by CTLA4-Ig. Despite this, this agent had anti-hypertensive effects and lowered vascular superoxide levels. This result is similar to the previously reported effect of T cell inhibition with an antibody against the Very Late Antigen-4 (anti-VLA4) on autoimmune encephalitis in rats. VLA4 inhibition suppressed disease and prevented T cell infiltration into the central nervous system, but did not alter macrophage accumulation. Studies such as these should not be interpreted as indicating that macrophages have no role, but that T cells and macrophages engage in substantial cross-talk to promote inflammation. Our present data would indicate that the ingress of macrophages in vessels in the absence of T cell accumulation has minimal effect on blood pressure and vascular superoxide production.

These findings might have clinical relevance. While numerous agents are available for treatment of hypertension, a sizable proportion of the hypertensive population is either...
refractory to treatment or requires multiple agents to achieve blood pressure control. Moreover, patients with accelerated or malignant hypertension are often difficult to treat, and suffer target organ damage that could be mediated by underlying inflammation. Co-stimulation blockade might provide an alternate form of therapy targeted toward reducing inflammatory-mediated tissue damage. Thus, while co-stimulation blockade might not routinely be used to treat all individuals with hypertension, this therapy might be employed in more difficult to control cases, particularly for brief periods during initiation of treatment.

In summary, these studies indicate that the hypertension caused by various stimuli such as angiotensin II, mineralocorticoids and high salt require T cell costimulation and that costimulation is essential for the vascular inflammation that occurs in response to these common stressors. Future studies are needed to define the triggering mechanisms that activate T cell and antigen-presenting cells in hypertension. Moreover, treatment with agents that suppress co-stimulation might have a role in certain cases of either difficult to treat or malignant hypertension.

**Short Commentary**

Recent evidence indicates that inflammation, and in particular the adaptive immune response, contributes to hypertension. Hypertensive stimuli such as angiotensin II and mineralocorticoids promote T cell activation and infiltration into vessels and the kidney. Prior studies have also shown that mice lacking T cells are resistant to hypertensive stimuli. In this study, additional evidence supporting a role of T cells in the genesis of hypertension is provided. Inhibition of T cell costimulation using the agent CTLA4-Ig, or by genetic deletion of the costimulatory molecules CD80 and CD86, markedly lowered the hypertensive response to angiotensin II or DOCA-salt challenge in mice. Moreover, treatment with CTLA4-Ig had blood pressure lowering effects in experimental animals with established hypertension. These experiments support an immune mechanism for hypertension and point to new treatment strategies for difficult to treat cases of hypertension. In particular, CTLA4-Ig might prove beneficial in patients with malignant hypertension, which is often associated with end-organ damage that might be immune-mediated.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Inhibition of CD80/CD86 costimulation by CTLA4-Ig in angiotensin II (Ang II)-dependent hypertension. C57Bl/6J mice received either angiotensin II (490 ng/min/kg) or buffer (sham) for 14 days via osmotic minipump. Animals were also co-treated with either CTLA4-Ig (250 µg) or control i.p. injections 3 days prior to the onset of angiotensin II and every 3 days thereafter. (A) Non-invasive blood pressure measurements obtained via the tail cuff method at days 0 (pre) and 14 (post) were compared using two-way ANOVA (n = 5 in each group). (B) Example traces of telemetric systolic blood pressure recordings obtained in freely moving control C57Bl/6J and CTLA4-Ig administered mice 3 days prior to angiotensin II pump implantation (baseline) and during the last 3 days of angiotensin II infusion. (C) Average blood pressure values obtained by telemetry at baseline and during angiotensin II infusion (n=6 for vehicle; n=8 for CTLA4-Ig). Comparisons were made using repeated measures ANOVA with Scheffe’s post-hoc test. (D) Aortic superoxide levels measured by monitoring the oxidation of dihydroethidium to 2-hydroxyethidium using high pressure liquid chromatography following 14 days of either buffer or angiotensin II infusion (n=5 for sham; n=5 for Ang II+vehicle n=6 for Ang II+CTLA4-Ig).
Figure 2.
CTLA4-Ig inhibits T cell activation in angiotensin II dependent hypertension.
Representative histograms (A) and mean data (B) are shown comparing the effects of vehicle vs. CTLA4-Ig treatment on CD4+ T cell surface expression of the early activation antigen CD69 during angiotensin II-induced hypertension. (C and D) Effect of CTLA4-Ig on the percentage of circulating CD4+/CD44^hi and CD44/CCR5^hi cells in angiotensin II-induced hypertension (n=6 for sham+vehicle; n=9 for Ang II+vehicle; n=6 for sham+CTLA4-Ig; n=10 for Ang II+CTLA4-Ig). Comparisons were made using two-way ANOVA and statistical values reflect the Bonferroni correction.
Figure 3.
CTLA4-Ig inhibits vascular leukocyte and T cell infiltration in angiotensin II dependent hypertension. (A) Absolute numbers of total leukocytes (CD45+ cells) in the aorta of sham- and angiotensin II-infused mice treated with vehicle or CTLA4-Ig (n=6 for sham+vehicle; n=6 for Ang II+vehicle; n=6 for sham+CTLA4-Ig; n=8 for Ang II+CTLA4-Ig). (B) Flow cytometry showing T cells (CD45+CD3+) in aortic samples from sham- and angiotensin II-infused mice treated with vehicle or CTLA4-Ig. (C) Mean values for total aortic T cells (CD3+ cells) in all treatment groups (n=6 for sham+vehicle; n=6 for Ang II+vehicle; n=6 for sham+CTLA4-Ig; n=8 for Ang II+CTLA4-Ig). Panels D and E show production of inflammatory cytokines TNF-α and IFN-γ by T cells isolated from (D) spleen and (E) lymph nodes. Cells were stimulated for 48 hours on an anti-CD3 plate (n=5 for sham+vehicle; n=5 for Ang II+vehicle; n=5 for sham+CTLA4-Ig; n=6 for Ang II+CTLA4-Ig). Comparisons were made using two-way ANOVA and statistical values reflect the Bonferonni correction.
Figure 4.
Angiotensin II increases CD86 expression on CD11c+ cells in secondary lymphoid organs. (A) Examples of staining of lymph node derived CD11c+ cells. Fluorescence minus one (FMO) controls were used to unequivocally identify CD45+, CD11c+, CD80+, CD86+ and MHC II+ DCs. CD11c+ positive cells were gated within CD45+ gate. Subsequently CD80+, CD86+ and MHC II+ cells were identified. (B) Bar graph showing frequencies of CD11c+CD86+ cells from blood aorta, spleen and lymph nodes (n=10; 10; 4; 16 for both sham and Ang II respectively). (C) Percentages of CD11c+CD80+ and CD11c+MHC II+ cells in lymph nodes from vehicle and angiotensin II-treated WT mice (n=6 for both groups).
Figure 5.
Role of CD80/CD86 costimulation on development of angiotensin II-dependent hypertension. Angiotensin II (490ng/min/kg) was infused for 14 days in either wild-type C57Bl/6J (WT) or B7−/− mice. (A) Repeated measures ANOVA with Scheffe’s post hoc test was used to compare non-invasive blood pressure measurements obtained via tail cuff (n=7 for WT-sham; n=6 for WT-Ang II; n=11 for B7−/−-sham; n=12 for B7−/−-Ang II). (B) Average percentage of circulating CD4+/CD69+ and CD4+/CD44high cells as determined by flow cytometry in blood from vehicle- or angiotensin II-treated WT or B7−/− mice (n=7 for WT-sham; n=6 for WT-Ang II; n=11 for B7−/−-sham; n=11 for B7−/−-Ang II). (C) Representative contour plots acquired using flow cytometry and (D) average aortic CD45+CD3+ cells in sham and angiotensin II-treated wild-type or B7−/− mice (n=7 for WT-sham; n=6 for WT-Ang II; n=9 for B7−/−-sham; n=9 for B7−/−-Ang II). Comparisons of flow cytometry data were made using two-way ANOVA and statistical values reflect the Bonferonni correction for multiple comparisons.
Figure 6.
Engrafting WT bone marrow into irradiated B7−/− mice restores angiotensin II-induced hypertension and vascular infiltration. (A) Repeated measures ANOVA with Scheffe’s post hoc test was used to compare non-invasive blood pressure measurements obtained via tail cuff (n=4 for sham; n=6 for Ang II; n=4 for sham+BMT; n=6 for Ang II+BMT). (B) Histograms showing CD80 and CD86 expression on DCs derived from WT and B7−/− mice and B7−/− mice engrafted with WT bone marrow. (C) Representative flow cytometry gated for aortic CD45+ and CD3+ cells from vehicle and angiotensin II-treated B7−/− mice engrafted with WT bone marrow. (D) Numbers of CD45+ and CD3+ cells in aortas of sham and angiotensin II-treated B7−/− mice engrafted with WT bone marrow (n=6 for sham; n=6 for Ang II). Statistical values shown in (D) were obtained using two-tailed t-test.
Figure 7.
CTLA4-Ig attenuates DOCA-salt hypertension and its related vascular inflammation. DOCA-salt hypertension was induced in C57Bl/6J mice by uninephrectomy, subcutaneous implantation of a pellet containing deoxycorticosterone acetate (DOCA) and addition of 0.9% NaCl to the drinking water. Vehicle or CTLA4-Ig (250µg) was administered i.p. 3 days prior to 27 uninephrectomy and every 3 days thereafter. (A) Repeated measures ANOVA with Scheffe’s post hoc test was used to compare non-invasive blood pressure measurements obtained via the tail cuff method (n=10 for sham+vehicle; n=10 for sham +CTLA4-Ig; n=12 for DOCA+vehicle; n=13 for DOCA+CTLA4-Ig). (B) Average percentage of circulating CD4+ lymphocytes expressing CD69+ and CD44high as determined by flow cytometry (n=10 for sham+vehicle; n=8 for sham+CTLA4-Ig; n=11 for DOCA +vehicle; n=9 for DOCA+CTLA4-Ig). (C) Absolute numbers of total leukocytes (CD45+ cells) in aortas of sham- and DOCA-salt mice treated with vehicle or CTLA4-Ig (n=10 for sham+vehicle; n=10 for sham+CTLA4-Ig; n=11 for DOCA+vehicle; n=11 for DOCA +CTLA4-Ig). (D) Representative flow cytometric and (E) average aortic T cells accumulation (CD3+/CD45+) in control mice and mice with DOCA-salt hypertension treated with either vehicle or CTLA4-Ig (n=10 for sham+vehicle group; n=10 for sham+CTLA4-Ig; n=11 for DOCA+vehicle; n=11 for DOCA+CTLA4-Ig). Comparisons of flow cytometry data were made using two-way ANOVA and statistical values reflect the Bonferroni correction for multiple comparisons.
Figure 8.
Treatment with CTLA4-Ig reverses angiotensin II and DOCA-salt induced hypertension. (A) Two weeks post-sham or angiotensin II treatment (4 week pump), mice were randomly assigned to receive either vehicle or CTLA4-Ig for the remaining 2 weeks of angiotensin II infusion (n=5 for all groups). (B) Similarly, after two weeks of sham/DOCA-salt challenge mice were randomly assigned to receive either vehicle or CTLA4-Ig for 4 weeks (n=5 for all groups). Non-invasive blood pressure measurements were obtained weekly obtained via tail cuff. Repeated measures ANOVA with Scheffe’s post hoc test was used to compare blood pressure responses during the vehicle/CTLA4-Ig treatment phase only.