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Parental Dietary Protein Source and the Role of *CMKLR1* in Determining the Severity of Dahl SS Hypertension

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Abstract

Studies from our laboratory have revealed an important role for the maternal diet as well as the dietary protein source in the development of hypertension and renal injury in Dahl Salt-Sensitive (SS) rats. The current study sought to compare salt-induced hypertension, renal damage, and immune cell infiltration in the offspring of breeders fed either a casein- or gluten-based diet, with the hypothesis that offspring from gluten-fed breeders would fail to develop these salt-sensitive phenotypes. When fed identical diets postweaning, the F1 generation gluten offspring demonstrated lower mean arterial pressure (MAP, 149.1±3.1 vs 162.5±5.8 mmHg), albuminuria (166.2±34.6 vs 250.9±27.8 mg/day) and outer medullary protein casting (7.4±0.8% vs 13.1±1.3%) in response to high salt compared to the casein offspring (n=9–11). The gluten offspring also had fewer CD45+ leukocytes, CD11b/c+ monocytes/macrophages, CD3+ T-cells, and CD45R+ B-cells infiltrating the kidney. Analysis of the F2 generation gluten offspring also exhibited lower MAP and renal damage compared to rats born from casein breeders (n=7–9), with no difference in renal immune cell infiltration. *CMKLR1* (chemokine like receptor 1), receptor for the novel pro-hypertensive adipokine chemerin, was found via PCR array to be significantly upregulated (2.99-fold) in renal T-cells isolated from F2 offspring of casein-fed versus gluten-fed parents. Furthermore, *CMKLR1* inhibition via 2-(α-naphthoyl) ethyltrimethylammonium iodide (α-NETA) treatment significantly attenuated renal immune cell infiltration, hypertension, and renal damage in SS rats fed high salt. Together these data demonstrate the influence of the parental diet in determining the salt-induced hypertensive, renal damage, and inflammatory phenotype of the offspring.

Keywords

salt-sensitive hypertension; renal injury; dietary protein; T lymphocytes; CMKLR1; chemerin

Introduction

Diet is a risk factor for many diseases, especially hypertension and cardiovascular disease. In animal models of hypertension, the elevation of blood pressure has been shown to be

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Disclosures

None.

increased as a function of the fat[1–4], carbohydrate[2, 5–7], and protein[8] composition of the diet. Previous studies in our laboratory have shown that the source and amount of dietary protein can alter the severity of salt-induced hypertension and associated renal damage in Dahl salt-sensitive (SS) rats maintained on a highly purified, casein-based diet (AIN-76A, Dyets Inc)[9–12]. Interestingly, SS rats fed a grain-based diet also containing high salt (4.0% NaCl, HS) were protected from the salt-induced increases in mean arterial pressure (MAP) and albuminuria compared to SS rats fed the casein-based diet[9]. Both the casein and grain diets contain the same amount of sodium, suggesting sodium-independent components of the diet affect salt-sensitive hypertension. The major differences between the purified casein and the grain-based diets are the sources of carbohydrates (sucrose versus flour, respectively), protein (casein versus wheat gluten), and fat (corn oil versus soybean oil). Subsequent studies found that the source of protein in the diet was largely responsible for this phenotype in the grain-fed SS rats since substitution of casein in the purified diet with wheat gluten recapitulated the protective phenotype[10]. Furthermore, a high protein (casein) diet exacerbates SS hypertension, which is mediated primarily via immune mechanisms[11, 13].

Additional studies showed that the diet of the parents was just as important in determining the salt-sensitive phenotype in the offspring[9]. Maternal nutrition has been recognized as a major factor in determining fetal growth and more importantly, programming risk and susceptibility to future disease[14–16]. Embryo transfer experiments between purified diet-fed Dahl SS rats from the Medical College of Wisconsin and grain-fed Dahl SS rats from Charles River Laboratories demonstrated that, regardless of whether an embryo originated from parents on either the casein or grain diet, what predicted salt-sensitivity in the offspring was the diet of the surrogate mother[12]. This study demonstrated the profound effect of the maternal diet in determining the susceptibility or resistance of their offspring to hypertension later in life, independent of genetic predisposition.

Given the established importance of the source of dietary protein as well as the influence of the maternal diet in determining the degree of salt-sensitive hypertension, the current study seeks to investigate these two factors by creating a new breeding colony of Dahl SS rats maintained on a modified version of the purified AIN-76A diet containing wheat gluten. We hypothesize that specifically replacing the protein source of casein to wheat gluten in the parental diet (SS/gluten breeders) would protect the offspring from hypertension and renal damage, despite being placed on the purified casein-based chow after weaning. By placing all of the offspring on the same diet after weaning, we would be able to isolate potential phenotypic differences to one single component of the parental diet. The present experiments compared the effect of switching the parental generation to a “protective” diet just prior to conception versus lifelong maintenance of the parents on the same diet. Finally, since immune-dependent mechanisms have been shown to mediate both salt-dependent and protein-dependent elevations in blood pressure and renal damage in the SS rat[11, 13], experiments were also performed to examine the differences in immune cell infiltration and immune signaling in the kidney.

Methods

The authors declare that all supporting data are available within the article and its online supplementary files.

Animals, Diets, and Breeding Strategy.

Experiments were performed on age-matched, inbred, male Dahl SS rats (SS/JrHSDMcwi). We utilized Dahl SS breeders who had previously demonstrated successful pregnancies with healthy, viable pups. These proven Dahl SS breeders, originally maintained on a 0.4% NaCl purified casein-based AIN-76A diet (#113755, Dyets Inc), were switched to a modified wheat gluten-based AIN-76A diet (#103932, Dyets Inc). This custom-made diet contained an isocaloric protein substitution of wheat gluten for casein, while all other components, including fat (corn oil), carbohydrate (sucrose), vitamin, and mineral source and amount, remained the same. A diagram describing our breeding strategy can be found in Figure S1. A subset of the F1 generation from the gluten breeders was immediately studied and phenotyped, while another subset of the F1 generation was maintained on the gluten diet to serve as future breeders. Phenotyping of the F1 offspring investigated the effects of switching the diet of the parents just prior to conception, while the F2 offspring who were born of parents maintained on the gluten diet their entire lives provided insight into the effects of lifelong exposure of the parents to the modified diet. All of the wheat gluten offspring to be studied (SS/gluten) were weaned to the original 0.4% NaCl casein-based diet and compared to offspring from regular Dahl SS breeders (SS/casein), thus isolating the difference between groups solely to the protein source of the parental diet. All protocols were approved by the Medical College of Wisconsin IACUC.

A detailed description of all other methods can be found in the Online Supplement.

Results

F1 generation offspring of gluten-fed breeders developed a lesser extent of salt-induced hypertension, renal damage, and renal immune cell infiltration.

All rats studied in this protocol were fed the same diet after weaning, but the F1 generation rats were born from parents who had their diet switched to the modified wheat gluten chow just prior to conception (Figure S1). At baseline, no differences in MAP or albuminuria were observed in the offspring of either the casein or gluten breeders. However, in response to a three-week HS challenge, the offspring of the SS/gluten breeders demonstrated a significantly lower MAP compared to offspring from the SS/casein breeders (149.1±3.1 vs 162.5±5.8 mmHg, SS/gluten vs SS/casein at HS21, Figure 1A). This blunted hypertensive phenotype in the SS/gluten offspring corresponded with significantly lower albuminuria (166.2±34.6 vs 250.9±27.8 mg/day, Figure 1B) and proteinuria (257.8±43.6 vs 382.2±38.2 mg/day, data not shown). The SS/gluten offspring furthermore demonstrated improved renal histology, with fewer protein casts found in the outer medulla (7.4±0.8 vs 13.1±1.3%, Figure 1C) and a lower glomerular damage index score (2.19±0.02 vs 2.55±0.06, Figure 1D). Analysis of the immune cell profile in the circulation after three weeks of HS challenge revealed no differences between the two groups (data not shown). Upon analysis of the

immune cells infiltrating the kidneys, the blunted renal damage in the offspring of the SS/gluten breeders was accompanied with fewer total CD45+ leukocytes (43.5% reduction), CD11b/c+ monocytes and macrophages (44.8%), CD3+ T-cells (37.0%) and CD45R+ B-cells (46.6%) compared to offspring of the SS/casein breeders (Figure 2). By changing one component of the parental diet just prior to conception, these data altogether suggest that the offspring of the SS/gluten breeders failed to develop salt-sensitive hypertension, renal damage, and renal inflammation to the same extent as the SS/casein offspring, despite both groups of offspring being fed the exact same casein-based diet after weaning.

F2 generation offspring of gluten-fed breeders exhibited a blunted hypertensive and renal damage response, with no difference in renal immune cell infiltration.

The F2 generation offspring were born from SS/gluten breeders who were born and also bred on the modified wheat gluten chow their entire lives (Figure S1). Thus, phenotyping of the F2 SS/gluten offspring would examine the effects of a *lifelong parental* exposure to the wheat gluten diet. During the 0.4% NaCl period, there was no significant statistical difference in MAP or albuminuria between the SS/casein or SS/gluten offspring. In response to HS, the F2 offspring from the SS/gluten breeders demonstrated a blunted rise in MAP (141.5±1.5 vs 156.0±7.3 mmHg, SS/gluten vs SS/casein at HS21, Figure 3A), and to a greater extent than observed in the F1s. This perhaps is expected due to the longer exposure of the parents to the gluten diet. The F2 gluten offspring also had less salt-induced renal damage compared to the casein offspring, evidenced by less albuminuria (74.4±14.7 vs 192.4±48.6 mg/day, Figure 3B), fewer outer medullary protein casts (5.4±1.0 vs 9.2±1.6%, Figure 3C), and lower glomerular damage score (2.29±0.04 vs 2.49±0.06, Figure 3D). Interestingly, the F2 offspring from gluten breeders did not demonstrate a reduction in the number of CD45+ total leukocytes, CD11b/c+ monocytes and macrophages, CD3+ T-cells, or CD45R+ B-cells (Figure 4) infiltrating the kidney after HS challenge. This result was intriguing since the F2 gluten offspring demonstrated a blunted phenotype compared to the SS/casein offspring, despite the continued presence of immune cells in the kidney.

PCR array analysis of kidney T-cells identifies CMKLR1 as a potential target.

With no difference observed in the renal infiltration of immune cells despite the F2 SS/gluten offspring being protected from salt-induced increases in blood pressure and renal injury, we sought to determine whether there was a functional difference in the T-cells infiltrating the kidneys. Utilizing a PCR array approach, 84 genes related to rat chemokines and their receptors were examined between T-cells isolated from both the blood and kidneys of SS/casein rats on 0.4% versus 4.0% NaCl, as well as between SS/casein and SS/gluten F2 offspring after HS challenge. Interestingly, only two out of the 84 genes were significantly differentially expressed between kidney T-cells from the SS/casein and SS/gluten offspring after HS (Table 1). One of the two genes was *CMKLR1*, which was significantly upregulated (4.68-fold) when comparing HS to 0.4% NaCl in the SS/casein offspring. However, *CMKLR1* was significantly downregulated (2.99-fold) in the SS/gluten compared to SS/casein offspring after HS challenge. This effect was also true in peripheral T-cells isolated from the circulation. *CMKLR1* was the only gene found to be significantly differentially expressed in each of the comparisons made between SS/casein and SS/gluten, as well as between 0.4% and 4.0% NaCl. *CMKLR1* (chemokine like receptor 1) is one of

the three receptors associated with the prohypertensive and proinflammatory adipokine, chemerin[17], and the benefits of either *CMKLR1* inhibition or chemerin neutralization as potential therapeutics is a new and exciting area of research[18–21]. Utilizing a commercially available ELISA (LifeSpan BioSciences, Inc.), chemerin levels were measured in the serum, urine, and renal tissue (cortex and outer medulla) of Dahl SS rats fed either 0.4% or 4.0% NaCl for 3 weeks (Figure S2). Interestingly, there was a slight but significant decrease in serum chemerin (41.1±1.2 vs 45.1±1.3 ng/mL, 4.0% vs 0.4% respectively) upon 4.0% NaCl high salt challenge, but an increase in urinary chemerin excretion (13.5±2.2 vs 5.3±1.8 pg chemerin/μg total protein). In the renal tissue, there was significantly greater expression of chemerin in the cortex versus medulla, regardless of chow salt content, and a surprising reduction in chemerin expression in response to 4.0% NaCl in the medulla (0.95±0.07 vs 1.65±0.22 pg chemerin/μg total protein). However, the interplay between chemerin levels and the extent of receptor expression as a driving force for disease progression remains an important subject for future exploration.

CMKLR1 inhibition by α-NETA attenuates Dahl SS hypertension and renal damage.

In order to determine whether this difference in *CMKLR1* gene expression played a physiological role, we administered 2-(α-naphthoyl) ethyltrimethylammonium iodide (α-NETA), a small molecule inhibitor of *CMKLR1*, to see if it would have any effect in attenuating Dahl SS hypertension and renal injury. α-NETA, originally identified as an inhibitor of choline acetyltransferase (IC_{50}=9 μM)[22], has more recently been determined to be a more potent inhibitor of chemerin/*CMKLR1* signaling (IC_{50}=375 nM)[18]. We began α-NETA treatment one week prior to the switch to HS, and even during the 0.4% NaCl period, a significant reduction in blood pressure was already observed in the α-NETA group (108.7±2.1 vs 122.0±1.7 mmHg, α-NETA vs Vehicle at LS-5, Figure 5A). Throughout the course of HS for 21 days, daily α-NETA treatment clearly prevented the salt-induced rise in MAP compared to vehicle-treated rats (138.3±2.1 vs 158.1±5.3 mmHg at HS21). This protection from hypertension was accompanied with less renal injury, evident by attenuated albuminuria (88.2±22.6 vs 237.2±44.5 mg/day, Figure 5B), fewer outer medullary casts (5.0±0.9 vs 15.1±1.8%, Figure 5C), and a lower glomerular damage score (2.20±0.04 vs 2.83±0.14, Figure 5D) by the end of the 3 weeks HS challenge. While there was no difference in the peripheral immune cell populations between the two groups (data not shown), there were significantly fewer CD45+ total leukocytes (49.2% reduction) infiltrating the kidneys of α-NETA-treated SS rats compared to vehicle (Figure 5E). This reduction was primarily due to significantly fewer CD11b/c+ monocytes and macrophages (59.9%). Interestingly, for the lymphocyte populations, α-NETA treatment had no effect on the number of total CD3+ T-cells and there was a non-significant trend towards a reduction in CD45R+ B-cells. However, upon closer look at the T-cell subsets, there was a 37.6% reduction in CD3+CD4+ helper T-cells and a 49.2% increase in CD3+CD8+ cytotoxic T-cells. Altogether, these α-NETA studies have clearly provided evidence for an important role for *CMKLR1* in the development of Dahl SS hypertension, renal damage, and renal immune cell infiltration.

Discussion

The current study has demonstrated that switching the parental dietary protein source of casein to gluten just prior to conception resulted in F1 offspring that developed a blunted hypertensive, renal damage, and immune cell infiltration response to high salt. In contrast, the F2 offspring whose parents had lifelong exposure to the gluten diet developed a lesser extent of hypertension and renal injury, but interestingly not the renal immune cell infiltration. We have provided evidence that although there is no difference in the number of infiltrating renal immune cells, there may be a functional difference in the infiltrating T cells, which may instigate different signaling pathways upon infiltration into the kidney.

Different protein sources have been associated with different degrees of cardiovascular disease risk. There is a significant amount of observational evidence highlighting the influence of animal versus plant protein consumption on cardiovascular disease[23]. Vegetarians have been shown to have lower blood pressure than omnivores[24], and since then, a number of studies since then have indicated an inverse relationship between plant protein intake and blood pressure[25–28]. The Dietary Approaches to Stop Hypertension (DASH) trial[29] and the Optimal Macronutrient Intake Trial to Prevent Heart Disease (OmniHeart)[30] were two large-scale interventional studies seeking to evaluate the effect of implementing healthy dietary patterns in adults, and the overall results from both trials confirmed the health benefit associated with greater plant protein consumption. These observations parallel the protection observed in the Dahl SS rats fed the grain-based diet compared to those fed the purified, casein-based diet[9].

Furthermore, other reports have demonstrated the important contribution of maternal nutrition during the gestation and lactation periods to offspring immunity and their susceptibility to chronic disease in adult life. Multiple studies have provided evidence that maternal high salt or high fat intake during pregnancy and/or lactation can lead to the development of hypertension and metabolic syndrome in the offspring[31–33]. While most studies surrounding maternal dietary protein consumption focus on protein restriction and maternal undernutrition, the predisposing effects in the offspring are quite evident. Maternal protein restriction during pregnancy in stroke-prone spontaneously hypertensive rats led to an increase in salt sensitivity and stroke incidence in the offspring[34], which has been attributed to increased aldosterone[35]. However, offspring that had a normal prenatal environment but were reared by low protein diet-fed mothers led to hypertension in the offspring's adult life, indicating the importance of the maternal diet specifically during the lactation period[36]. Interestingly, a soya protein-based diet, compared with a casein diet, when consumed during gestation alone or throughout gestation and lactation increased the presence of characteristics of metabolic syndrome in the offspring[37], further demonstrating that the source as well as amount of dietary protein in the maternal diet can have predisposing effects in the offspring. In the current study, the protective effects on hypertension and renal damage observed in the SS/gluten offspring compared to SS/casein are due to changes made to the parental diet. Future studies in our animal model will require parsing out the specific influence of the maternal versus paternal diet, as well as the contribution of the maternal gestational diet versus the diet during lactation.

Additionally, nutrition and dietary factors have a tremendous impact on immune function and the host's ability to separate and protect itself from its environment[38–40], and the role of the immune system in the development and amplification of Dahl SS hypertension is well-established[41]. Recent studies in our laboratory utilizing the SS$^{Rag1^{-/-}}$ rat, an SS rat lacking functional T and B lymphocytes, demonstrate a clear role for immune mechanisms in the exacerbation of high dietary protein-induced hypertension and renal damage[13]. While decades worth of evidence has revealed the extensive effects of nutrition on all aspects of immunity[42–44], more recent studies have demonstrated postprandial induction of both physiological and pathological inflammatory processes, offering a potential mechanism whereby changes in dietary intake can alter functional phenotypes[45–47]. Moreover, it is unknown if inflammation precedes hypertension or vice versa, which is a limitation of the current study. A recent study utilizing a servo-control technique demonstrated increased renal perfusion pressure driving the infiltration of immune cells into SS rat kidneys[48]. However, it is undeterminable from the present study whether the effects of a parental gluten diet induces epigenetic changes in the offspring related to inflammation or kidney function and again, will be the major focus of future investigations.

While the precise mechanisms linking changes in dietary protein to the amplification of salt-sensitive hypertension, end-organ damage, and inflammation are not entirely clear, one specific dietary-induced immunological alteration observed in the current study was the upregulation of *CMKLR1* in the T cells in both the circulation and kidney of high salt-fed SS/casein offspring compared to SS/gluten. *CMKLR1* (chemokine like receptor 1), also known as ChemR23 (chemerinR), is a previously orphan G protein-coupled receptor expressed on immature dendritic cells and macrophages, whose natural ligand is chemerin[49]. Although chemerin is a chemoattractant protein best known for its role in adaptive and innate immunity, more recent studies have revealed its expression in adipose tissue[50] as well as in the tunica media and endothelial cell layer of rat aorta[19]. This novel adipokine has been shown to serve as an endogenous vasoconstrictor, significantly increase ROS production in smooth muscle cells to promote proliferation and migration[51], and increase mRNA expression of proinflammatory mediators (IL-6, MCP-1, and VCAM-1) in vascular cells to facilitate monocyte-to-endothelial cell attachment[20]. Furthermore, inhibition of *CMKLR1* prevented chemerin-induced vasoconstriction and increases in blood pressure[52]. In humans, circulating levels of chemerin are significantly higher in hypertensive individuals, positively correlate with increased blood pressure[53, 54], and is higher in the plasma of hypertensive non-dippers compared to both hypertensive dippers and normotensive individuals[55]. This is particularly interesting, as inhibition of *CMKLR1* via novel small molecule antagonist α-NETA resulted in a 2.26 hour phase shift in diurinal rhythm versus vehicle treated SS rats (Figure S3). Additionally, there exists a strong inverse association between serum chemerin levels and renal function, where diseased kidneys have an impaired ability to eliminate chemerin[56]. The current study is the first to demonstrate that immune cells infiltrating the target organ differentially express *CMKLR1* in response to dietary modulations, and has determined a deleterious role for *CMKLR1* expressed on T-cells specifically in the Dahl SS rat. Global and chronic blockade of *CMKLR1*-mediated chemerin signaling with α-NETA resulted in a remarkable attenuation of hypertension and renal damage in Dahl SS rats and clearly prevented the homing of immune cells into the kidney after high salt challenge.

However, α-NETA treatment demonstrated a strong antihypertensive effect even during the 0.4% NaCl control period, where there are presumably few immune cells, thus these studies are not able to distinguish the off-target effects related to the blockade of *CMKLR1* in non-immune cells. These immune-independent anti-hypertensive effects of *CMKLR1* inhibition, potentially related to mechanisms involving vasoconstriction, adipogenesis, and metabolism, remain to be the subject of investigation in future studies.

Perspectives

Together, these data demonstrate the influence that the parental diet can have in predisposing the offspring to salt-sensitive hypertension and renal damage later in adult life. Altering a single component of the parental diet, the protein source, improved the blood pressure and renal damage phenotypes in the offspring of gluten- versus casein-fed parents. This dietary change furthermore led to differences in immune cell function via *CMKLR1*, where inhibition of this novel target demonstrated its deleterious role in the development of Dahl SS hypertension and renal disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

1. What Is New –

Parents fed a protective wheat gluten-based diet, whether placed on the diet just prior to conception or throughout life, led to offspring who developed a lesser extent of Dahl SS hypertension and renal damage.

Changes in gene expression of *CMKLR1* may explain a functional difference in infiltrating T-cells, and *in vivo* inhibition of *CMKLR1* via α-NETA inhibits Dahl SS hypertension and renal injury.

2. What Is Relevant –

The Dahl SS rat exhibits similar traits observed in humans with salt-sensitive hypertension. These studies demonstrate how either dietary modifications or targeting of the immune system can ameliorate the severity of this salt-induced disease.

3. Summary –

By altering a single component of the parental dietary protein source, these data reveal the influence that the parental diet can have in predisposing the offspring to salt-sensitive hypertension, renal damage, and inflammation later in adult life.

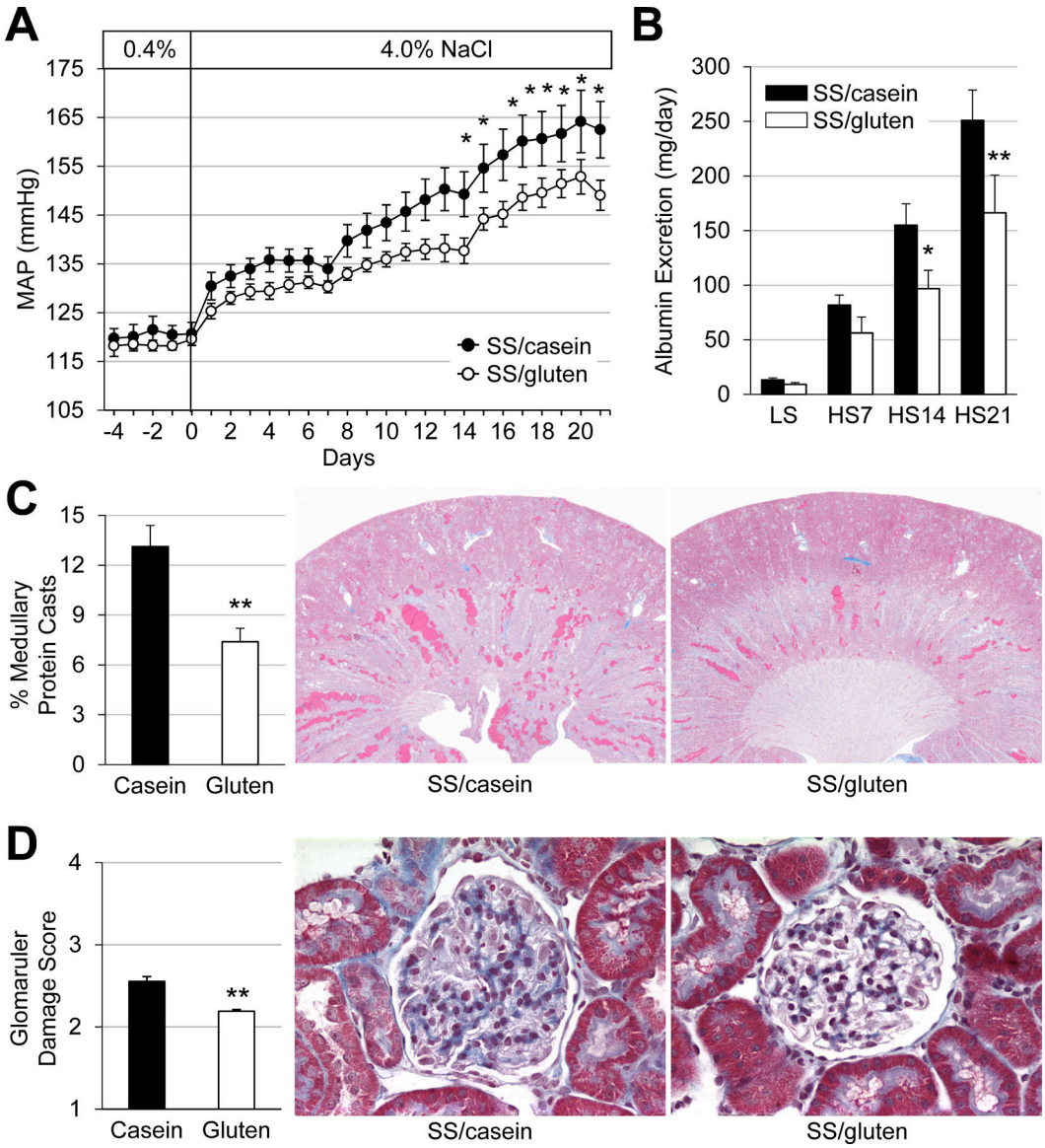

Figure 1.

The F1 SS/gluten offspring were born from Dahl SS breeders switched to the modified wheat gluten chow just prior to conception. (A) Mean arterial blood pressure, (B) urinary albumin excretion, (C) medullary protein cast formation, and (D) glomerular damage were all lower after 3 weeks of 4.0% high salt challenge in the F1 SS/gluten offspring compared to SS/casein offspring. n=9–14, *p<0.05 and **p<0.01 versus SS/casein via Two Way RM ANOVA (A-B) or t-test (C-D).

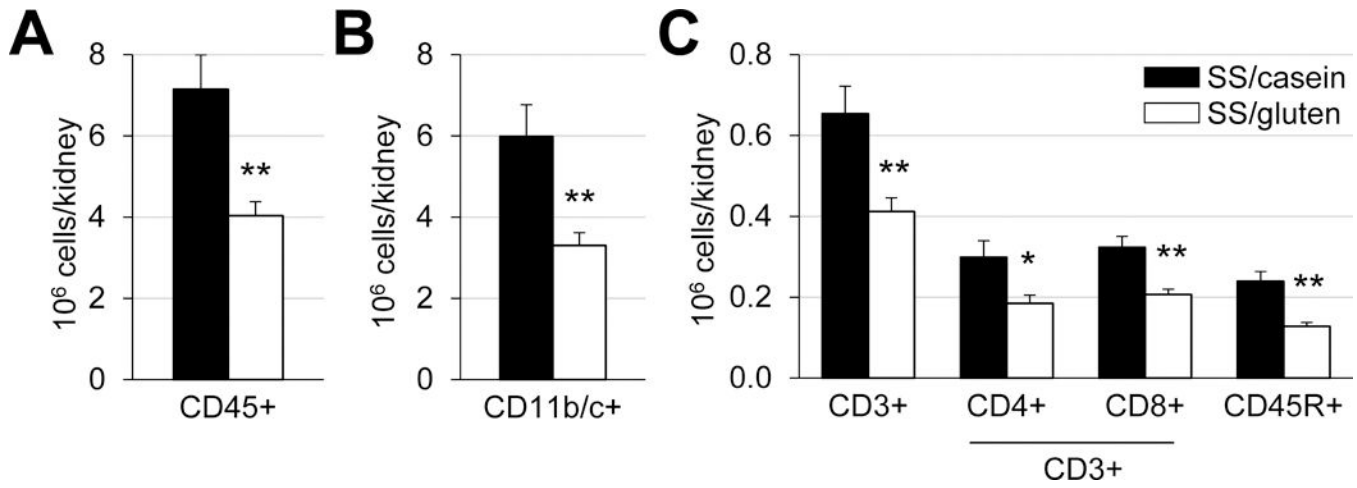

Figure 2.

Examination of the renal infiltrating immune cell profile of CD45+ leukocytes (A), CD11b/c + monocyte/macrophages (B), and CD3+ T-cells, CD3+CD4+ helper T-cells, CD3+CD8+ cytotoxic T-cells, and CD45R+ B-cells (C) showed a reduction in F1 SS/gluten offspring after 3 weeks of 4.0% high salt challenge compared to SS/casein offspring. n=14, *p<0.05 and **p<0.01 versus SS/casein via One Way ANOVA.

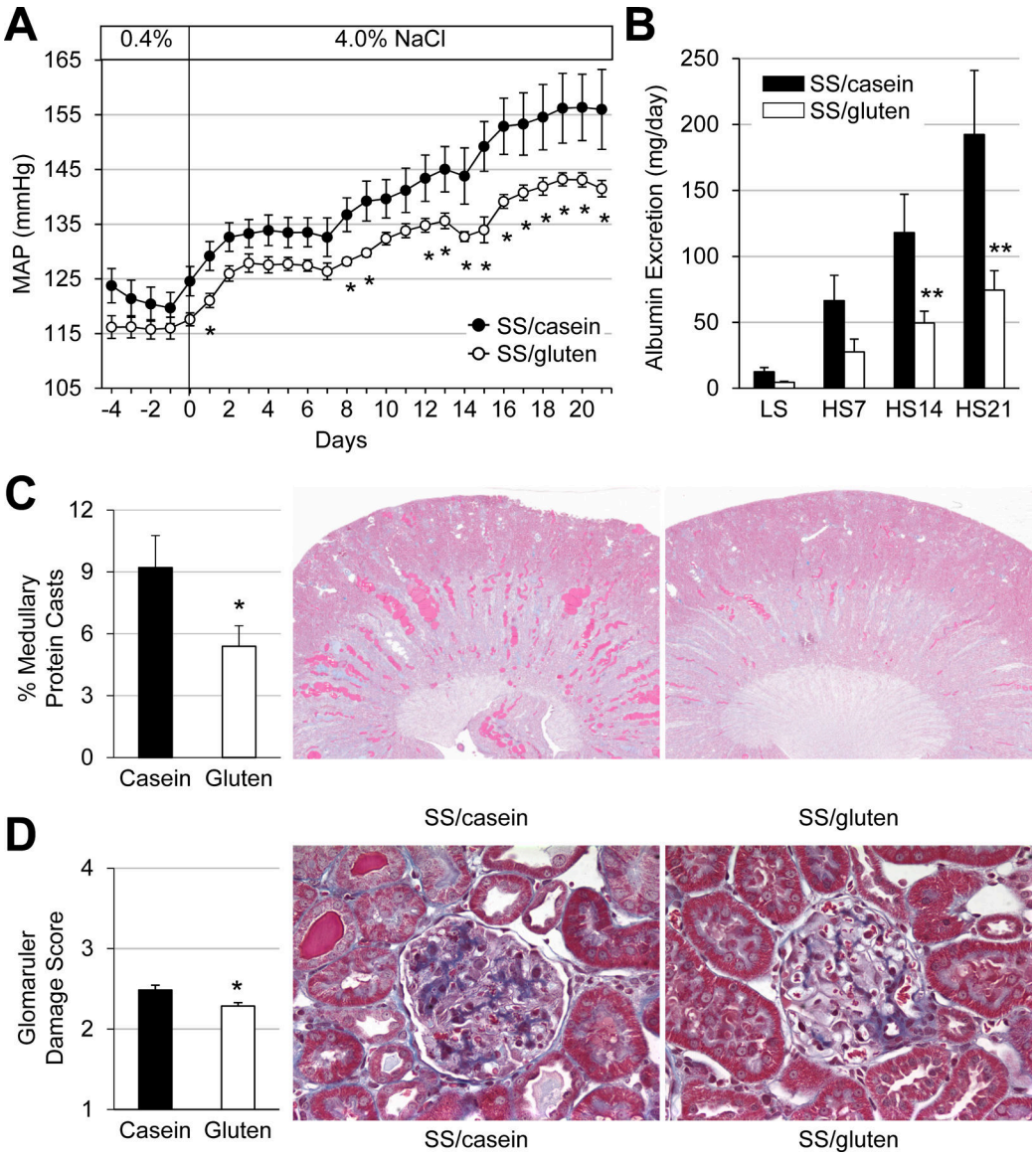

Figure 3.

F2 SS/gluten offspring had parents who were born, maintained, and bred on the modified wheat gluten diet. Compared to the SS/casein offspring, the F2 offspring from gluten breeders demonstrated lower mean arterial pressure (A) and significantly improved renal injury indicated by reduced albuminuria (B), medullary protein cast formation (C), and glomerular damage (D) after 3 weeks of 4.0% high salt. n=7–10, *p<0.05 and **p<0.01 versus SS/casein via Two Way RM ANOVA (A-B) or t-test (C-D).

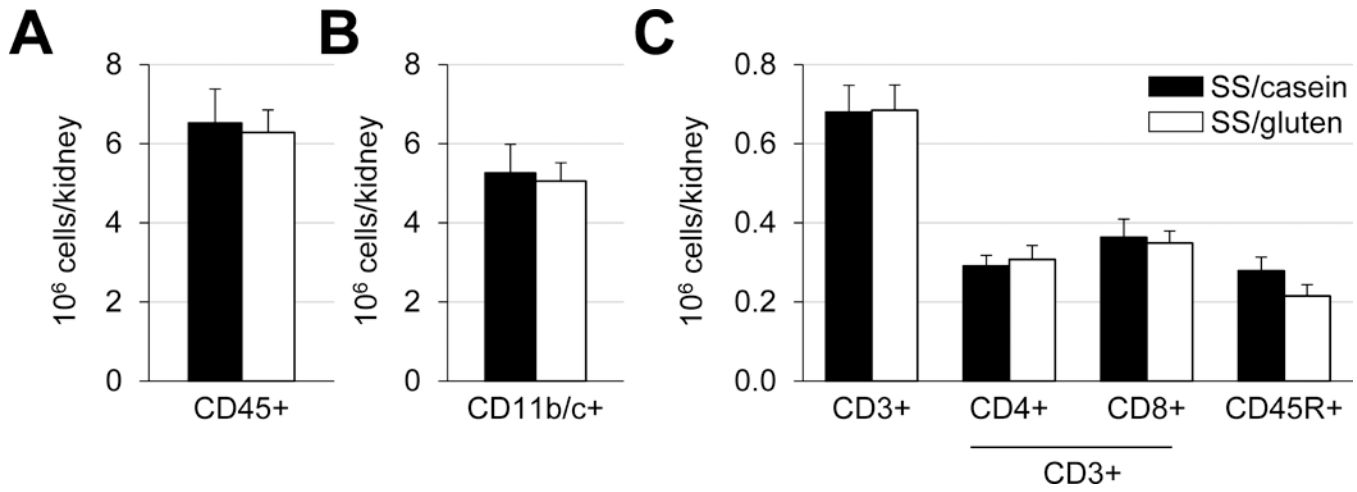

Figure 4.

Investigation of the renal infiltrating immune cell profile of CD45+ leukocytes (A), CD11b/c + monocyte/macrophages (B), and CD3+ T-cells, CD3+CD4+ helper T-cells, CD3+CD8+ cytotoxic T-cells, and CD45R+ B-cells (C) showed no difference between the F2 SS/gluten and SS/casein offspring after 3 weeks of 4.0% high salt challenge. n=7–10.

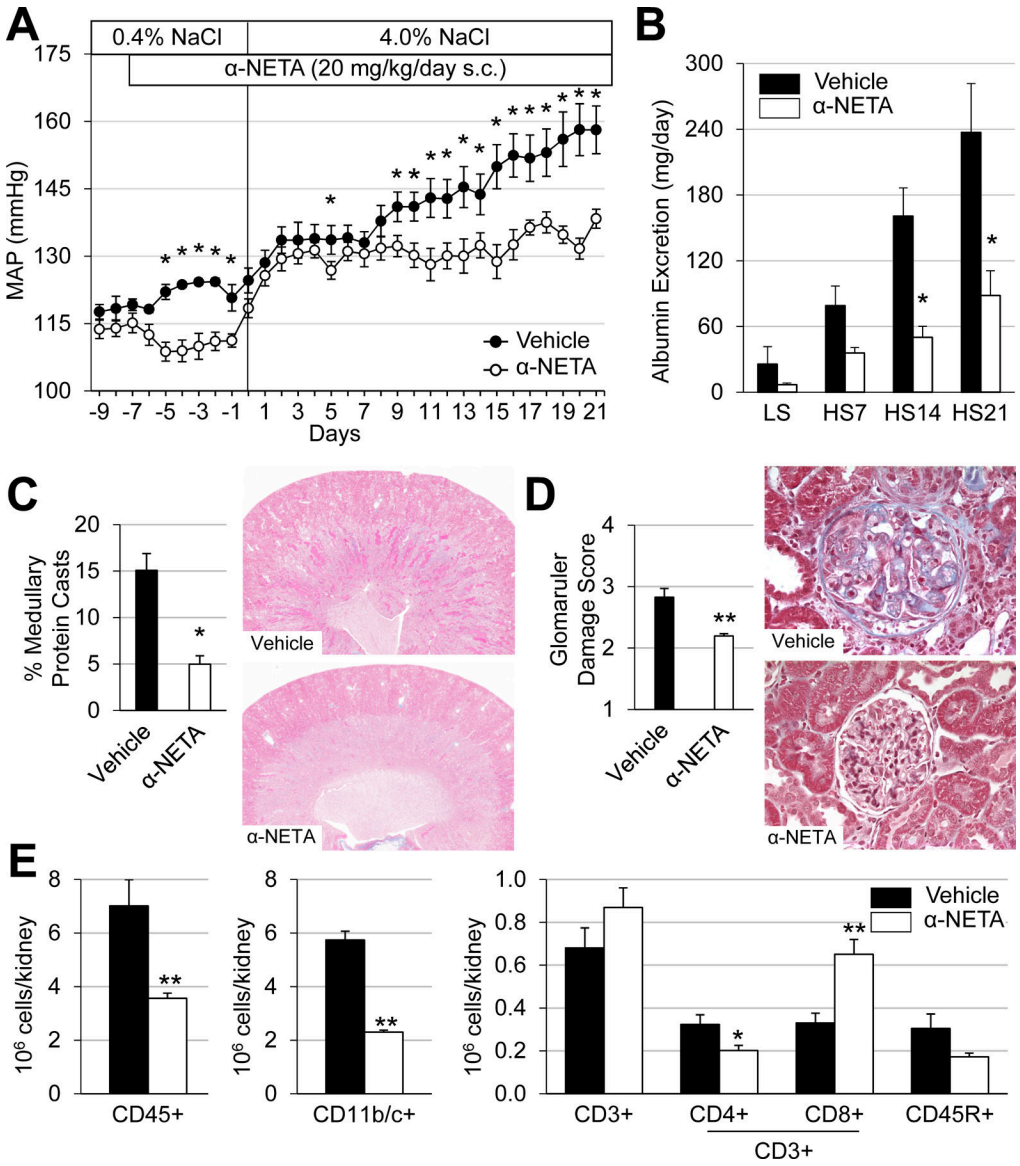

Figure 5.

Daily administration of small molecule CMKLR1 inhibitor, α-NETA (20 mg/kg s.c.), prevented Dahl SS rats from developing salt-induced hypertension (A) and renal damage, demonstrated by improved albuminuria (B), medullary protein cast formation (C), and glomerular morphology (D). This was accompanied with fewer immune cells infiltrating the kidneys of α-NETA-treated SS rats after 3 weeks of high salt (E). CD45: leukocytes, CD11b/c+: monocyte/macrophages, CD3+: T-cells, CD4+: helper T-cells, CD8+: cytotoxic T-cells, CD45R+: B-cells. *p<0.05 and **p<0.01 versus Vehicle via Two Way RM ANOVA (A-B), t-test (C-D), or One Way ANOVA (E).

Table 1.

Significantly differentially expressed genes determined by PCR array analysis of the T-cells isolated from both the blood and kidney of SS/casein and SS/gluten F2 offspring after 3 weeks of high salt. n=6/group.

BLOOD T-CELLS			**KIDNEY T-CELLS**		
SS/casein HS vs. SS/casein LS			SS/casein HS vs. SS/casein LS		
Gene	Fold Regulation	P-Value	Gene	Fold Regulation	P-Value
Cmklr1	***5.291454***	***0.030687***	*Xcl1*	*441.489*	*0.00641*
Ccr1	*3.977782*	*0.001551*	*Ccr1*	*4.90958*	*0.02379*
Pf4	*3.718699*	*0.02804*	***Cmklr1***	***4.68809***	***9.1E-05***
Ccl5	*2.827114*	*0.010781*	*Ccl19*	*4.26714*	*0.03782*
Ifng	*2.770214*	*0.002243*	*Ccl7*	*4.20654*	*0.01883*
Itgam	*2.215908*	*0.001178*	*Il1b*	*3.6972*	*0.00884*
Ppbp	*2.139734*	*0.01732*	*Cxcl2*	*3.37441*	*0.04407*
			Ccl6	*3.31062*	*0.0246*
Ccl3	*−5.89423*	*0.003349*	*Cx3cr1*	*2.92601*	*0.00032*
Hif1a	*−4.40651*	*0.000068*	*Tymp*	*2.73556*	*0.00238*
Mapk14	*−4.3566*	*0.002733*	*Cx3cl1*	*2.7276*	*0.01434*
Cmtm3	*−2.7864*	*0.000779*	*Ccl3*	*2.5959*	*0.01274*
Cmtm2a	*−2.61662*	*0.035572*	*Ccl5*	*2.29841*	*0.00323*
			Ccr5	*2.07882*	*0.00443*
SS/gluten HS vs. SS/casein HS			*Hif1a*	*−5.5622*	*0.00045*
Gene	Fold Regulation	P-Value	*Kdr*	*−5.0137*	*0.0145*
Xcl1	*3.54338*	*0.0486*	*Ccr2*	*−4.6568*	*0.02889*
Cmtm2a	*2.08198*	*0.02508*	*Cmtm3*	*−2.2108*	*0.01652*
Cmklr1	***−3.2608***	***0.02412***	SS/gluten HS vs. SS/casein HS		
Il1b	*−3.1265*	*0.01548*	Gene	Fold Regulation	P-Value
Ifng	*−2.2321*	*0.00074*	*Cmtm3*	*2.759998*	*0.0398*
			Cmklr1	***−2.988662***	***0.011801***