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The Gut-Immune-Kidney Axis: Influence of Dietary Protein in Salt-Sensitive Hypertension

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Abstract

Humans with salt-sensitive hypertension demonstrate increased morbidity, increased mortality, and renal end-organ damage when compared to normotensive subjects or those with salt-resistant hypertension. Substantial evidence from humans and animals has also demonstrated the role of dietary components other than salt to modulate hypertension. Evidence presented in this review provides support for the view that immunity and inflammation serve to amplify the development of salt-sensitive hypertension and leads to malignant disease accompanied by end-organ damage. Interestingly, salt-sensitive disease is modulated by changes in dietary protein intake, which also influences immune mechanisms. Together, the evidence presented in this review from animal and human studies indicates that changes in dietary protein source have profound effects on the gut microbiota, microbiota-derived metabolites, DNA methylation, gene expression, immune cell activation, the production of cytokines and other factors, and the development of salt-sensitive hypertension and related disease phenotypes.

Keywords

hypertension; kidney; immune cells; microbiota

Introduction

Cardiovascular diseases were listed as the most common cause of mortality in the US in 2019, accounting for over 870,000 deaths[1]. High blood pressure, or hypertension is a major risk factor for coronary heart disease, heart failure, and stroke; and has been reported to be the largest individual contributing factor to disease and mortality worldwide[1]. A particularly damaging form of hypertension is salt-sensitive hypertension, in which blood pressure changes in parallel with sodium intake[2]. Salt-sensitive hypertensives demonstrate increased end-organ damage with particularly high morbidity and mortality[3,4]. Of interest,

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Conflict of Interest

The authors declare that they have no conflict of interest with the work presented in this manuscript.

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None

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salt-sensitive hypertensive subjects demonstrate ventricular hypertrophy, renal damage, and stroke[1], but the cause of salt-sensitive hypertension remains unclear. Potential modifiers of salt-sensitive hypertension and associated end-organ damage are mechanisms related to the activation of inflammation and immunity. Multiple reviews have described studies performed over the past five decades that implicate the immune system in hypertension, vascular disease, and renal disease[5,6,7,8,9]. The present review will focus upon observations in experimental animals and humans demonstrating the role of environmental factors, particularly dietary protein, which modulates inflammation and immunity with resulting alterations in the development of hypertension and related end-organ damage.

Dietary Consumption of Protein and Other Macronutrients in Immunity and Hypertension

Epidemiological studies correlated the consumption of diets with elevated sodium chloride (salt), carbohydrate, saturated fat, cholesterol, and fiber with blood pressure[10,11,12,13]. The effects of elevated protein diets are somewhat controversial, with evidence supporting protein-induced decreases[12,14] and increases[15] in blood pressure. In humans with preexisting renal insufficiency, however, it is clear that high protein diets accelerate a decline in renal function[16,17], though it is unclear if diets with reduced protein improve outcomes related to chronic kidney disease[18]. The source of dietary protein is also important: vegetarians have lower blood pressure than omnivores[19] while vegetable protein intake inversely correlates with blood pressure[20]. The Optimal Macronutrient Intake Trial to Prevent Heart Disease (OmniHeart) and the interventional Dietary Approaches to Stop Hypertension (DASH) trials also demonstrated the blood pressure benefit of diets rich in plant protein[21,22]. Of interest, the DASH-Sodium trial assessed the impact of the reduction of sodium intake on a control diet and demonstrated that hypertensive individuals, African Americans, and those over 45 years of age demonstrated an enhanced blood pressure sensitivity to sodium intake[23]. Moreover, the generic “animal versus plant” distinction is an oversimplification with the concomitant contribution of other environmental factors including the consumption of nonprotein dietary components[24] as well as genetic influences[25].

Blood pressure is modulated by the amount of dietary fat[26,27], carbohydrate[27,28], and protein[29] in experimental hypertension. Investigations in our lab indicated that Dahl Salt Sensitive (SS) rats fed a purified, animal-based diet developed a greater degree of salt-sensitive hypertension compared to genetically-identical Dahl SS fed a grain-based diet[30,35]. Subsequent studies attributed the effect of the animal versus grain diet to the difference in the source of dietary protein (casein versus wheat gluten)[31] and revealed the contribution of the immune system[32,33,34]. Data in humans and animals provide compelling evidence that dietary protein is an important determinant of salt-sensitive hypertension and renal damage.

Dietary Protein and Immune Mechanisms

An approach to modulate inflammatory disease is through dietary modification. A randomized interventional study compared the relationship between dietary protein intake and inflammation. The Metabolic Syndrome Reduction in Navarra (RESMENA) study revealed a direct relationship between dietary protein intake and inflammation. Interestingly,

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the relationship was specific for increases in animal/meat protein but not for increases in fish or vegetable protein intake[36]. Additional evidence links increased dietary protein from animal sources to an enhanced incidence of inflammatory bowel disease[37], while increased protein in the diet, particularly from red- and processed meats, increases potential relapse of ulcerative colitis[38]. While increased dietary protein enhances gut inflammation, less is known regarding the potential influence on other organ systems. The link between dietary protein and inflammation holds great interest since immune mechanisms are implicated in the pathology of salt-sensitive hypertension and associated renal end-organ damage.

Immunity in Salt-Sensitive Hypertension

Immune mechanisms have been demonstrated in multiple experimental models of hypertension[6,7,8,9], including the Dahl SS rat[6,7]. Much like salt-sensitive humans, the Dahl SS develops hypertension and end-organ damage when fed high salt[6,7]. Accompanying the hypertension, Dahl SS also develop renal histological damage and albuminuria[6,7]. The renal damage parallels the albuminuria observed in salt-sensitive hypertensive humans when compared to salt-resistant subjects[6,7].

The damaged kidneys of hypertensive Dahl SS rats after high salt feeding contain increased macrophages, T cells, and B cells when compared to the kidneys of Dahl SS fed low salt[6,7]. In contrast, infiltration of immune cells is not observed in the kidneys of age-matched, control rats that do not develop hypertension or renal damage when fed high salt. The infiltrating cells in Dahl SS and other rat models of hypertension surround glomeruli, vessels, and tubules in the kidney[6,7,39,40], indicating that they may play a role in vascular and tubular changes as well as the development of kidney damage. Supporting these observations, the degree of salt-sensitive hypertension and kidney damage in other rodent models of disease also correlates with immune cell infiltration in the kidney[5,6,8,9]. Moreover, the localization of immune cells in the renal interstitial spaces adjacent to damaged tubules, vessels, and glomeruli of the Dahl SS is similar to that in hypertensive subjects[5]. To examine the functional role of immune cells in hypertension, it was shown that treatment with immunosuppressive agents prevented infiltration of T cells in the kidney and attenuated salt-sensitive hypertension and renal damage in Dahl SS rats [6,7,32]. Protective effects of immunosuppressive treatment were observed in multiple other models of hypertension in animals[5,6,8,9].

Genetic editing strategies in Dahl SS rats targeted individual immune cell types to examine their importance in salt-sensitive hypertension[6,7]. Null mutation of *Rag1* significantly reduced T and B cells in the Dahl SS genetic background ($SS^{Rag1-/-}$) and significantly blunted salt-sensitive hypertension and renal damage compared to Dahl SS controls with intact lymphocytes[6,7]. Subsequent studies generated Dahl SS with a selective deletion of T cells by inducing a null mutation in *CD247* ($SS^{CD247-/-}$), a gene encoding the CD3 zeta chain[6,7]. Similar to the $SS^{Rag1-/-}$, the severity of hypertension and renal damage was attenuated in the $SS^{CD247-/-}$. To confirm the functional role of T cells in salt-sensitive hypertension, reconstitution of T cells by splenocyte transfer to $SS^{CD247-/-}$ restored high salt blood pressure, albuminuria, and renal histological damage to levels similar to that observed in wild type Dahl SS rats fed high salt. These observations are in agreement with

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reports demonstrating the critical role of T cells for the full development of angiotensin II-mediated hypertension in mice[42]. Of note, the role of lymphocytes in hypertension has not been universally observed in all laboratories[43]. Divergent effects of angiotensin II in Rag1−/− mice have been reported from different labs[43,44]. An explanation for the discrepancy noted in experimental results is unclear but may be due to the influence of environmental effects as described below. With some exceptions, the above-discussed data indicate that adaptive immune mechanisms, mediated by T cells, amplify salt-sensitive hypertension and kidney damage[6,7,32].

Mechanisms of Immune Activation in Salt-Sensitive Hypertension

The mechanisms activating immunity and inflammation in the kidney in salt-sensitive hypertension and the processes whereby immune cells mediate changes in function are the subject of active investigation. Observations in Dahl SS rats deficient in T cells[6,7] demonstrate an initial, immune-independent phase of salt-sensitive hypertension and kidney damage. We speculate that this initial phase, occurring in response to elevated salt intake, is mediated by numerous mechanisms including aberrant regulation of hormonal, neural, paracrine, or autocrine mechanisms which increase renal vascular resistance or enhance renal tubular sodium reabsorption[6]. The increase in blood pressure leads to elevated renal perfusion pressure that is transmitted to the kidney, an effect that is pronounced when renal vascular autoregulatory mechanisms are impaired as observed in salt-sensitive hypertension[45].

We hypothesize (Figure 1)[6,7], based upon data from Dahl SS and angiotensin II-hypertensive rats[51,52,53], that an initial increase in blood pressure is transmitted to the renal vasculature and mediates the infiltration of immune cells into the kidney. The mechanism is unclear, but it is possible that the elevated perfusion pressure results in tissue damage and triggers the migration of innate and adaptive immune cells into the kidney. The infiltrating immune cells release cytokines, free radicals and/or other molecules that amplify the development of hypertension by increasing epithelial sodium reabsorption, constricting the vasculature, and directly mediating further tissue damage[6,7]. As previously described, other initiating mechanisms may include increased sympathetic nerve activity, antigens and neoantigens and environmental modifiers of immune cell activation[6,7,33,46,47,48,49,50].

Modulation of Immune Cell Infiltration and Activation Alters Salt-Sensitive Hypertension

The profound influence of T cells and other immune cells to amplify salt-sensitive hypertension and renal damage indicates that mechanisms that modulate the activation or infiltration of these cells into the kidney could alter the progression of disease. Recent data indicate that the source of dietary protein may alter immune activation and salt-sensitive blood pressure. Dahl SS fed diets identical in NaCl but with distinct sources of protein develop dramatic differences in salt-sensitive hypertension and renal damage[30,31,34,50,54]. Figure 2 summarizes experimental results demonstrating that Dahl SS fed an animal-based diet (SS/Animal) develop more severe salt-sensitive hypertension and renal damage than Dahl SS fed a grain-based diet (SS/Grain). Of note, the altered severity of salt-sensitive

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disease is associated with differential infiltration of immune cells, (particularly T cells) into the kidney. Moreover, in separate studies, pharmacologic or genetic approaches to suppress the immune system led to a blunting of the effects of an animal-based diet with elevated protein content to amplify salt-sensitive hypertension and renal damage[32,34]. The remarkable effects of dietary protein consumption on blood pressure led us to hypothesize that changes in the gut microbiota, potentially linked by altered immune activation, can enhance salt-sensitive hypertension.

Role of Diet and the Gut Microbiota in Hypertension

Human and animal data indicate that the gut microbiota can link diet with hypertension. Both hypertensive humans and animals exhibit gut dysbiosis and decreases in microbial diversity compared to normotensive controls[55,56]. Studies in animal models of hypertension indicate that pathogenic factors from the gut microbiota participate in disease development. Normotensive Wistar Kyoto rats developed hypertension after transfer of cecal contents from spontaneously hypertensive stroke-prone rats[57]. Similarly, transplantation of feces from hypertensive humans results in elevated blood pressure in germ-free mice[58]. Interestingly, the transfer of cecal contents from Dahl SS to Dahl Salt-Resistant (SR) rats did not affect blood pressure, but transfer from Dahl SR to Dahl SS led to an elevation of blood pressure[56]. Related to end-organ damage in hypertension, the microbiome is also implicated in chronic kidney disease[59]. Collectively, these studies support the role of the gut microbiota as a determinant of hypertension and end-organ damage.

The gut microbiota and diet are closely linked; diet shapes the gut microbiota and the bacterial metabolites which drive the pathophysiological effects of the diet. Observational evidence illustrated the benefit of plant protein consumption on cardiovascular disease[19,24]. An inverse relationship between plant protein intake and blood pressure has been observed in humans[20], and dietary interventional trials have illustrated the beneficial effects associated with greater plant protein consumption for blood pressure control[21,22]. Plant-based diets are associated with a distinct microbiota[60] and are linked to an improved Firmicutes-to-Bacteroidetes ratio, unique bacterial speciation, and greater fecal short-chain fatty acids[61]. While diet drives microbial composition, gut bacteria play critical roles in digestion, proteolysis and the fermentation of dietary proteins essential for amino acid balance, utilization, and bioavailability[62,63,64]. The gut microbiota and diet thus play important roles in host health.

Alterations in the Microbiome Mediate Enhanced Salt-Sensitive Hypertension and Renal Damage in Dahl SS Fed Animal-Based Diets

Significant differences were observed in salt-sensitive hypertension between Dahl SS rats fed an animal- compared to a grain-based diet[30,31,33,35]. A comparison of the fecal microbiota demonstrated remarkable differences between the two Dahl SS colonies (Figure 3A)[54]. Significant differences were also noted in the microbiota between the colonies of rats while maintained on a low or high salt diet, consistent with the concept that the microbiota participates in hypertensive disease. To explore the role of changes in the microbiota in disease development, a fecal material transplant (FMT) from the Dahl SS/

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animal to the Dahl SS/grain. The FMT from rats fed the pro-hypertensive diet amplified the elevation in systolic arterial blood pressure (Figure 3B) and albuminuria (Figure 3C) in the recipient rats. Of great interest, the rats receiving the FMT also demonstrated a specific increase in T cells infiltrating the kidney (Figure 3D). Since T cells amplify salt-sensitive hypertension and renal damage in the Dahl SS[6,7,41], altered immune mechanisms may link dietary-induced alterations in the microbiota with salt-sensitive disease. These compelling experiments demonstrate that the microbiota is important in the amplification of salt-sensitive hypertension and related end-organ inflammation and damage in salt-sensitive hypertension, but the mechanisms are unclear.

Though gut dysbiosis in hypertensive humans and experimental animals is associated with increased intestinal inflammation and activation of antigen presenting cells[65], the mechanisms of microbiota-mediated inflammatory effects are undefined. Possible mediators include alterations in short-chain fatty acid (SCFA) production[67], altered levels of beta-hydroxybuyrate[66], trimethylamine N-oxide (TMAO)[68,69], changes in LPS[58], or other gut-derived metabolites These and other mechanisms remain to be explored, but evidence indicates that SCFA can modulate blood pressure[67] while plasma L-carnitine and TMAO levels predict an increased risk for cardiovascular disease and adverse cardiac events[68,70,71].

Epigenetic Effects

The mechanisms transducing changes in diet to alter function are unclear, but the microbiota and microbial metabolites may directly or indirectly affect physiological processes. Evidence indicates that epigenetics play a critical role in the development of hypertension and renal disease in humans and animal models of disease[73,74]. In particular, changes in gut microbial composition have been linked to changes in DNA methylation in metabolic syndrome[75], inflammatory bowel disease[76], and colorectal cancer[77]. Since immune cells, T cells in particular, infiltrate the kidney in salt-sensitive hypertension and amplify the development of renal damage and elevation in arterial blood pressure[6,7,41], the T cell is a potential target of environmental factors to modulate function by epigenetic mechanism in hypertension. Furthermore, epigenetic modifications have been shown to play a principal role in the regulation of the immune system through the development, activation and function of different individual immune cell types[78,79]. DNA methylation in the T cell may therefore affect gene expression and modify the cell’s capacity to differentiate and function[80].

To examine the mechanisms whereby changes in the diet and microbiota alter T cell activation, a DNA methylation and gene expression analysis was performed on T cells of Dahl SS fed an animal- or plant-based diet[33,50]. In response to high salt, the methylome of T cells isolated from the kidney of Dahl SS/animal exhibited a significant increase in hypermethylated regions compared to T cells isolated from the kidneys of SS/grain (Figure 4A-B)[50]. Moreover, a predominant negative correlation was observed between gene expression and DNA methylation, indicating that DNA methylation modulates gene expression in salt-sensitive hypertension[50]. Finally, the inhibition of DNA methyltransferase blunted salt-induced hypertension and renal damage in the SS rats fed the pro-hypertensive diet, providing a functional role for DNA methylation. These studies demonstrated the

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potential of diet-induced changes in the microbiota and DNA methylation to modulate the severity of salt-sensitive hypertension.

Transcriptomic Effects

Recent studies demonstrated that high salt intake or alterations in other dietary factors can alter immune cell activation. In particular, elevated extracellular sodium concentration induced T helper 17 (Th17) cell polarization from naïve T cells of mouse or human origin[46,47]. High-salt feeding was shown to alter the gut microbiome and modulate the induction of Th17 cells in mice and humans[48]. Since Il-17 released from Th17 cells is pro-hypertensive, elevated sodium concentrations may have a feed-forward effect on blood pressure. Interestingly, evidence indicates that large amounts of sodium can be stored in the skin[81]; while elevated skin sodium content has been reported to activate macrophages[49].

We examined gene expression in T cells of Dahl SS fed an animal- or plant-based diet[33]. A transcriptomic analysis of T cells isolated from the kidney demonstrated a shift in expression from genes related to inflammation in the Dahl SS/Animal to genes related to metabolism in SS/Grain (Figure 4C-F)[33]. An IPA analysis indicated significant upregulation in pro-inflammatory pathways in T cells isolated from the kidneys of Dahl SS/Animal. In contrast, downregulated gene expression in metabolic pathways was observed in the T cells of the Dahl SS/Grain when fed high salt. These studies therefore demonstrate the potential influence of diet-induced changes in the microbiota, DNA methylation, and gene expression to modulate immune cell function and ultimately the severity of salt-sensitive hypertension and associated renal end-organ damage.

To place these animal studies into a translational context, diets rich in vegetable and plant protein are beneficial for blood pressure[20,21,22]. A potential mechanism is the direct relationship between dietary protein intake and inflammation which is specific for increases in animal/meat protein but not for increases in fish or vegetable protein intake[36]. Similarly, changes in sodium intake have been associated with changes in inflammation. A sustained reduction in sodium intake in normal individuals associated with decreased total numbers of peripheral monocytes[82]. The levels of pathogenic IL-6 and IL-23 also decreased with reduced salt intake, whereas protective IL-10 increased[82]. These results in animals and humans indicate that changes in dietary protein source, as well as alterations in sodium intake, have profound effects on the microbiota, DNA methylation, gene expression, the activation of immune cells, and the development of salt-sensitive hypertension and related disease phenotypes.

Perspectives and Conclusions

Figure 5 illustrates a hypothesis whereby changes in protein source can alter salt-sensitive hypertension. Observations in rats and humans indicate that changes in the dietary protein source can modulate salt-sensitive hypertension. Many mechanisms may mediate these effects, but we demonstrated that ingestion of a pro-hypertensive diet containing animal-based protein shifts the gut microbiota. Further experiments demonstrated that microbiota transfer from animals fed the pro-hypertensive diet can confer an elevation of arterial

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pressure and increased end-organ damage to animals fed a plant-based diet. We speculate that the altered microbial community leads to the production and release of metabolites that act upon host physiology. The metabolites may directly affect function by specific interactions or indirectly alter function via effects on gene transcription. Specifically, we observed profound changes in DNA methylation and gene expression in T cells. Since T cells amplify salt-sensitive hypertension in the Dahl SS, metabolites which alter T cell function and number could profoundly affect the salt-sensitive disease phenotype. Rats fed the pro-hypertensive, animal-based diet, in comparison to rats fed the grain-based diet, have increased DNA methylation and upregulation of pro-inflammatory genes in the T cells that infiltrate the kidney. By contrast, T cells isolated from the kidneys of rats fed the grain-based diet demonstrate a downregulation of genes in metabolic pathways when comparing the low and high salt conditions. Finally, we observed increased infiltration of T cells and other immune cells into the kidney of Dahl SS fed the animal-based diets that develop the greatest degree of salt-sensitive hypertension and renal end-organ damage. These results provide a potential explanation for the deleterious effects of some diets to amplify salt-sensitive disease.

Though much work has advanced this field, many questions remain. Fundamentally, the bacterial species influenced by changes in diet, the metabolites or other cellular products produced by those species, and the rapidity of reversibility of diet-induced effects on the microbiota and microbiota-derived metabolites are unknown. Similarly, the potential approaches to manipulate these parameters to treat salt-sensitive hypertension will require intense effort. This review has focused upon the potential role of T cells in the kidney in salt-sensitive hypertension, but effects could be mediated in multiple cell types in different tissues to mediate the biological effects of altered dietary protein sources on hypertension. Moreover, the mechanisms transducing infiltration, activation, and action of innate and adaptive immune mechanisms in hypertension and end-organ damage are unclear. Despite these challenges, this field provides great potential for the development of improved therapy for this life-threatening disease.

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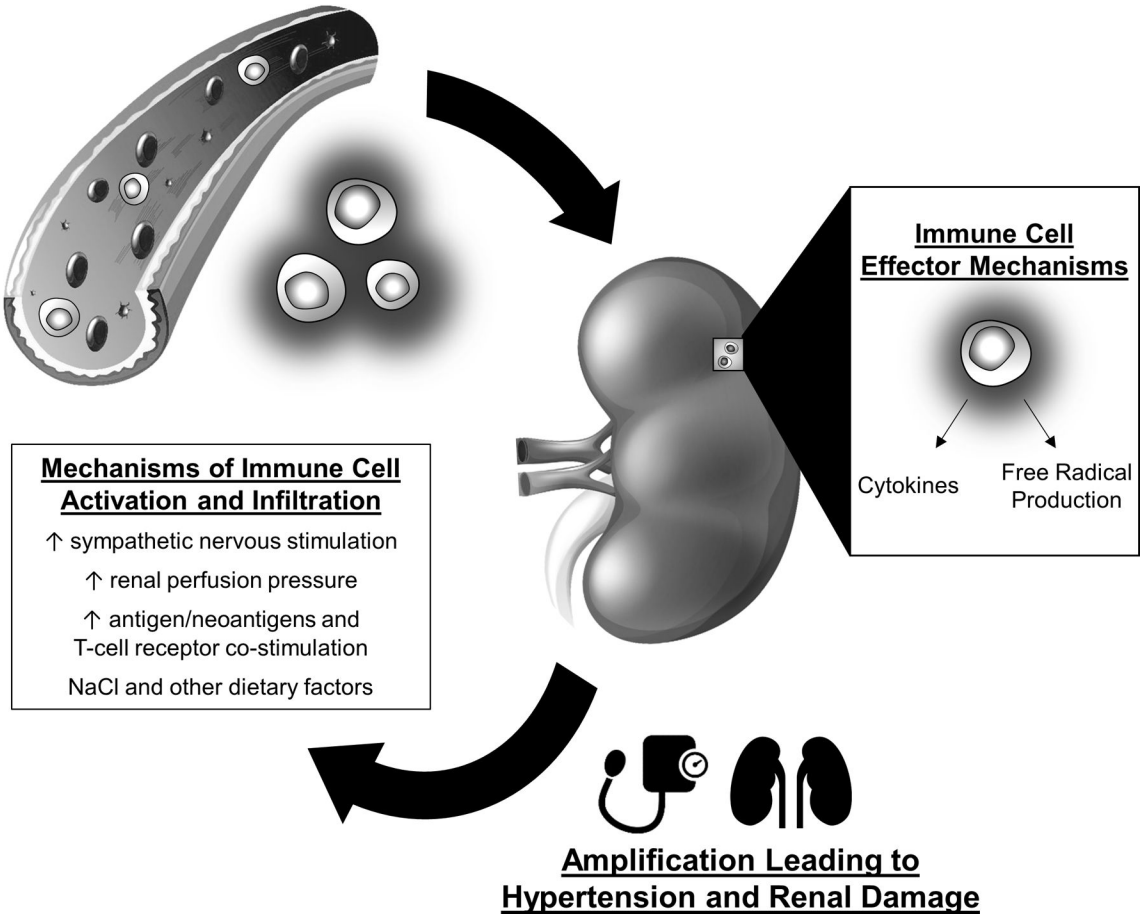

FIGURE 1:

Hypothesized role of immune mechanisms in the amplification of salt-sensitive hypertension. Elevations in sympathetic nerve stimulation, elevated renal perfusion pressure, increased antigens or neoantigens, and environmental factors activate immune cells in hypertension. The immune cells infiltrate target organs, including the kidney, and release free radicals, cytokines, and other molecules that mediate tissue damage and alter physiological function. As a result, the effector actions lead to the amplification of hypertension and tissue damage.

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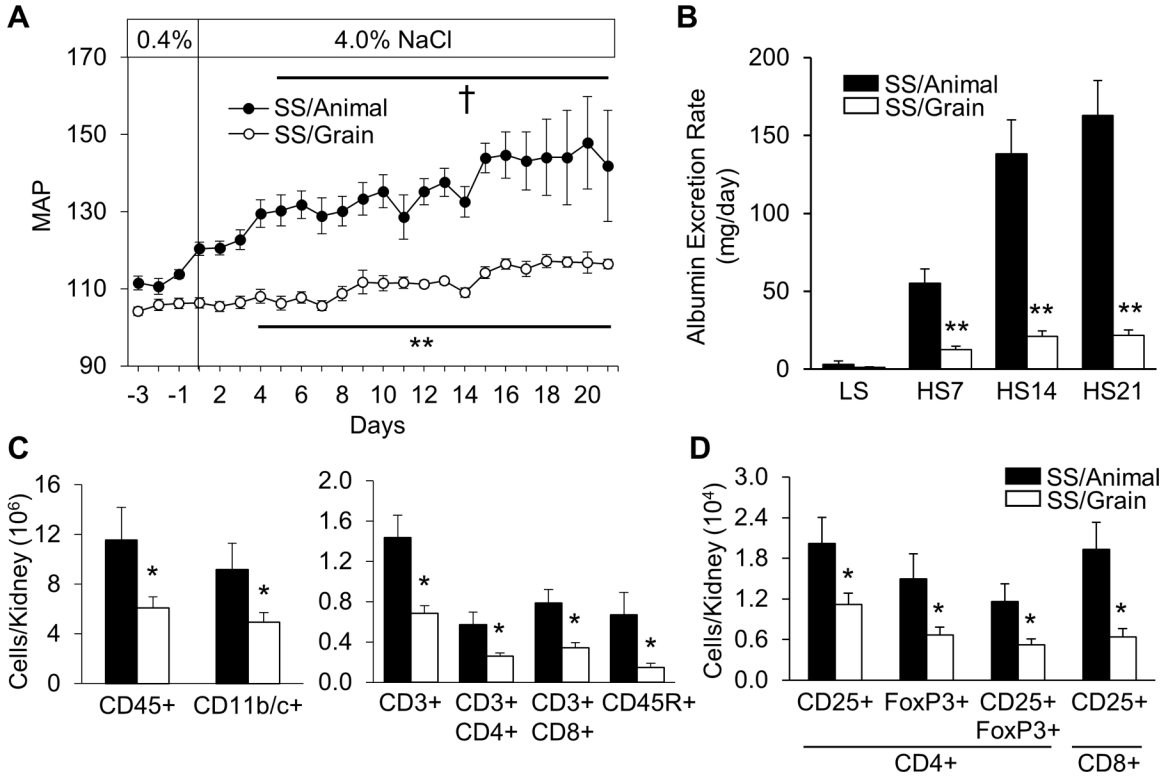

FIGURE 2:

Grain-fed Dahl SS (SS/Grain) demonstrate attenuated salt-induced hypertension (A), renal damage/albuminuria (B), and renal immune cell infiltration (C and D) compared to casein-fed Dahl SS (SS/Animal). Mean arterial pressure: MAP (mmHg). Low salt: LS, 0.4% NaCl; High salt: HS, 4.0% NaCl. CD45: leukocytes, CD11b/c+: monocyte/macrophages, CD3+: T-cells, CD4+: helper T-cells, CD8+: cytotoxic T-cells, CD45R+: B-cells, CD3+CD4+CD25+FoxP3+: T-regulatory cells, CD3+CD8+CD25+: activated cytotoxic T-cells (n=5–7/group, †P<0.05 vs LS Day 0, *P<0.05 and **P<0.001 vs SS/Animal). Reproduced from Abais-Battad *et al* [33].

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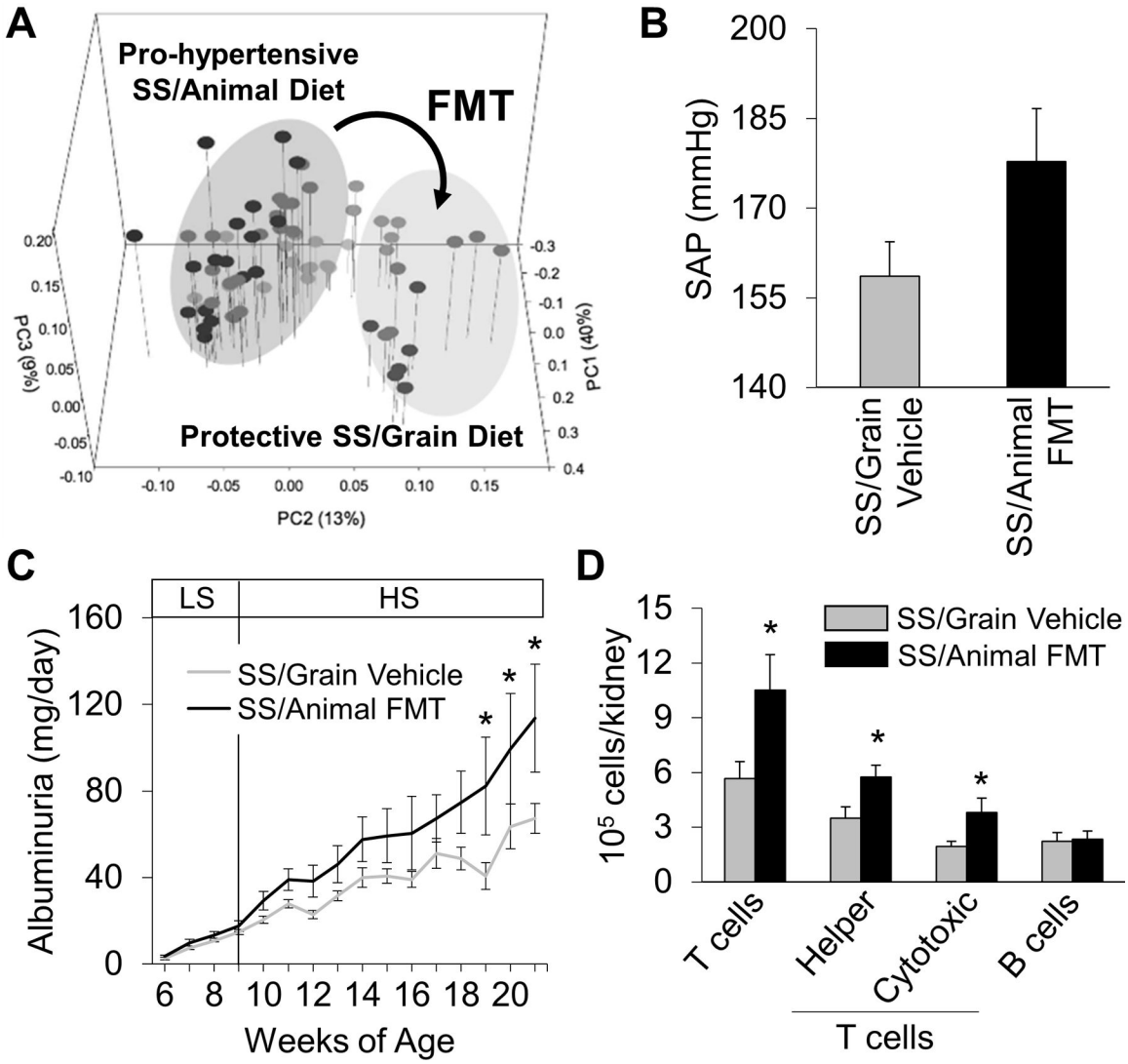

FIGURE 3:

Fecal microbiota transfer (FMT) from SS/Animal into SS/Grain (A) led to a trending increase in systolic arterial pressure in SS/Grain rats receiving FMT after HS (4.0% NaCl) (B), exacerbation of salt-induced albuminuria (C), and increased immune cell infiltration, specifically T cells, into the kidneys (n=5–6/group, *P<.05 vs Vehicle; PC-Principal Coordinate analysis of the weighted Unifrac distance matrix). Reproduced from Abais-Battad and Saravia *et al* [54].

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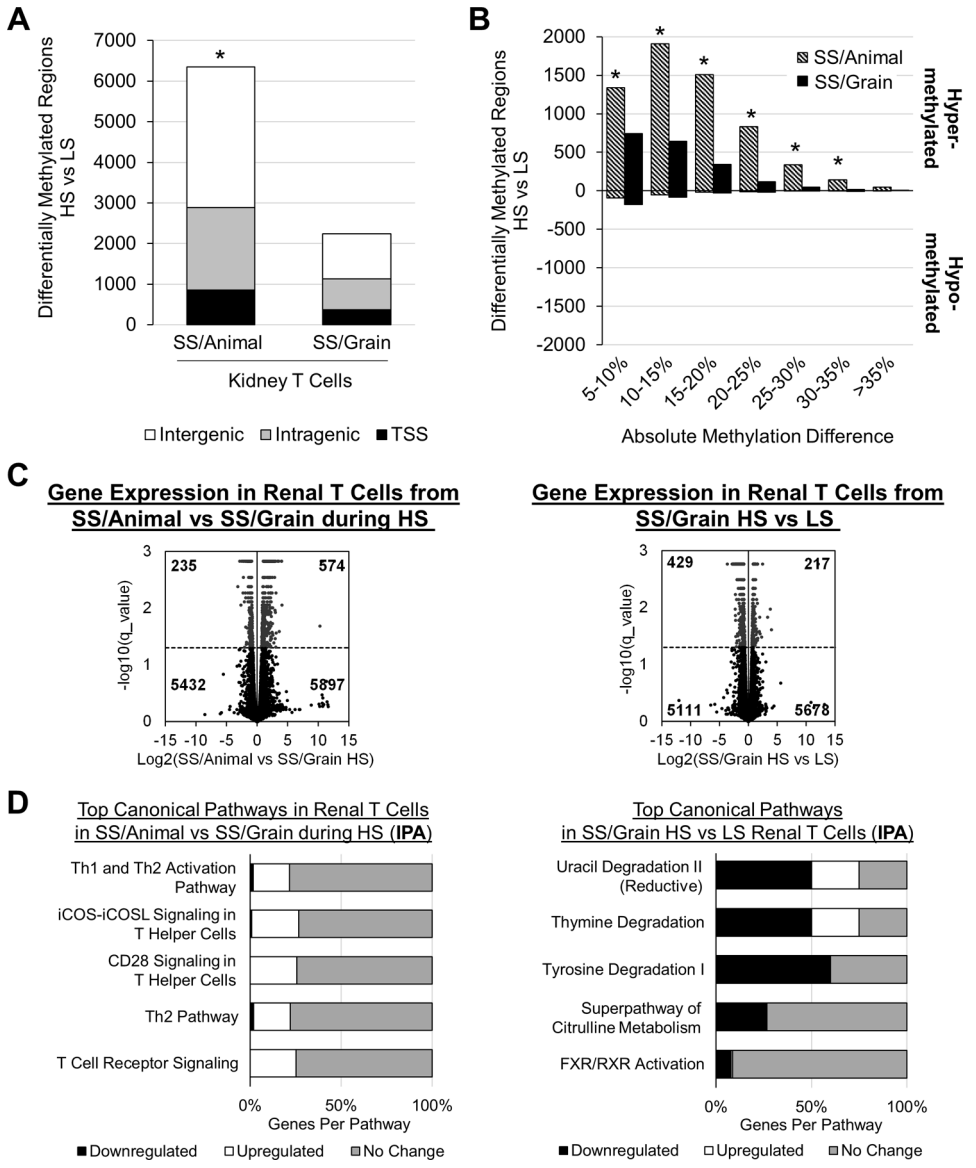

FIGURE 4:

T cells from the kidneys of Dahl SS/Animal exhibit significantly more differentially methylated regions (DMRs) in T cells isolated from the SS/Animal compared to SS/Grain (A). The DMRs in the T cells isolated from the kidney were primarily hypermethylated (B). Comparison of genes up- and downregulated in T cells isolated from the kidney of SS/Animal and SS/Grain rats fed the high salt (4.0% NaCl) diet (C-F) showed upregulation of inflammatory, T-cell signaling pathways in SS/Animal (C-D). Comparison of genes up- and down-regulated in T cells isolated from the kidney of SS/Grain fed the low salt (LS, 0.4% NaCl) or the HS diet demonstrated downregulation of pathways related primarily to metabolic processes during HS (E-F). TSS=transcription start site, n=4 pools/group, 3 rats/pool, *P<.05 vs SS/Grain. Reproduced from Abais-Battad *et al* [33] and Dasinger *et al* [50].

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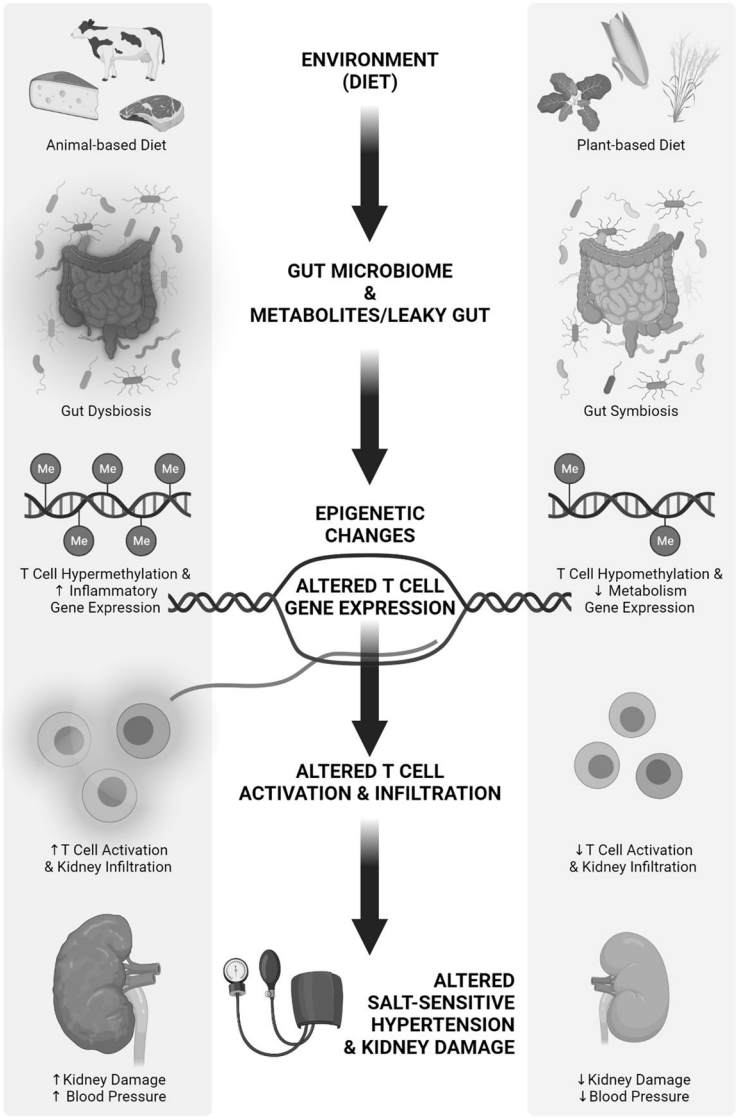

FIGURE 5:

Hypothesized effects of animal- and plant-based diets to alter the gut microbiome and gut metabolites with downstream effects on DNA methylation, gene expression, immune activation, and salt-sensitive hypertension and renal damage. Created with BioRender.com.