

KIDNEY

Sexual Dimorphic Role of CD14 (Cluster of Differentiation 14) in Salt-Sensitive Hypertension and Renal Injury

Daniel J. Fehrenbach¹, Justine M. Abais-Battad, John Henry Dasinger, Hayley Lund, Theodore Keppel¹, Jeylan Zemaj, Mary Cherian-Shaw, Rebekah L. Gundry¹, Aron M. Geurts¹, Melinda R. Dwinell, David L. Mattson

ABSTRACT: Genomic sequence and gene expression association studies in animals and humans have identified genes that may be integral in the pathogenesis of various diseases. CD14 (cluster of differentiation 14)—a cell surface protein involved in innate immune system activation—is one such gene associated with cardiovascular and hypertensive disease. We previously showed that this gene is upregulated in renal macrophages of Dahl salt-sensitive animals fed a high-salt diet; here we test the hypothesis that CD14 contributes to the elevated pressure and renal injury observed in salt-sensitive hypertension. Using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat-associated 9), we created a targeted mutation in the *CD14* gene on the Dahl SS (SS/JrHSDMcwi) background and validated the absence of CD14 peptides via mass spectrometry. Radiotelemetry was used to monitor blood pressure in wild-type and CD14^{-/-} animals challenged with high salt and identified infiltrating renal immune cells via flow cytometry. Germline knockout of CD14 exacerbated salt-sensitive hypertension and renal injury in female animals but not males. CD14^{-/-} females demonstrated increased infiltrating macrophages but no difference in infiltrating lymphocytes. Transplant of CD14^{+/+} or CD14^{-/-} bone marrow was used to isolate the effects of CD14 knockout to hematopoietic cells and confirmed that the differential phenotype observed was due to knockout of CD14 in hematopoietic cells. Ovariectomy was used to remove the influence of female sex hormones, which completely abrogated the effect of CD14 knockout. These studies provide a novel treatment target and evidence of a new dichotomy in immune activation between sexes within the context of hypertensive disease where CD14 regulates immune cell activation and renal injury. (**Hypertension. 2021;77:228–240. DOI: 10.1161/HYPERTENSIONAHA.120.14928.**) • **Data Supplement**

Key Words: bone marrow ■ hypertension ■ immunity ■ kidney ■ sex characteristics

Association studies, such as GWASs, have identified various genes of interest that may be involved in cardiovascular disease.¹ Working from this list of genes, we have previously shown that the identified genes *SH2B3* (LNK) and *CD247* play mechanistic roles in immunity and the development of salt-sensitive hypertension.^{2–4} Herein, we investigated an alternatively identified gene *CD14* (cluster of differentiation 14) using the Dahl SS (SS/JrHSDMcwi) rat animal model. Previous studies have implicated a clinical significance for CD14 in the context of hypertension and renal injury. It was

first demonstrated that *CD14* was associated with cardiovascular disease in 1999,⁵ and this observation has been repeatedly confirmed by others in genome-wide association studies.^{6,7} Additionally, a specific C(–260)T CD14 promoter variant associates with acute coronary syndrome, where patients produce greater amounts of the inflammatory cytokine IL (interleukin)-6,⁸ which we have previously shown to be important in salt-sensitive hypertension.⁹ These observations imply that individuals with variants in the *CD14* gene have an increased risk for developing cardiovascular disease; however, this

Correspondence to: Daniel J. Fehrenbach, Department of Medicine, Division of Clinical Pharmacology, Vanderbilt University Medical Center, MRB4 (Langford), p410 2213 Garland Ave, Nashville, TN 37232. Email daniel.fehrenbach@vumc.org

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Novelty and Significance

What Is New?

- Salt-sensitive Dahl SS rats exhibit increased renal CD14 (cluster of differentiation 14) when on a high-salt diet.
- Global knockout of CD14 results in a female-specific exacerbation of salt-sensitive phenotypes including salt-induced elevation in blood pressure, renal damage, and renal macrophage infiltration.
- Ovariectomy abrogates the effect of CD14 knockout implicating female sex hormones in potentiating innate immune system activation in this model.

What Is Relevant?

- Mutations in CD14 have been associated with hypertension in genome-wide association and gene expression studies in human populations. These results point to a sexually dimorphic mechanism by which the immune system affects salt-sensitive phenotypes.

Summary

In this study, we demonstrate that CD14—a protein involved innate immune system activation—interacts with female sex hormone signaling to modulate the inflammatory activity contributing to salt-sensitive hypertension and renal damage in Dahl SS rats.

Nonstandard Abbreviations and Acronyms

CD14	cluster of differentiation 14
CRISPR/Cas9	clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat—associated 9
E2	estradiol
IL	interleukin
iNOS	inducible NO synthase
KIM-1	kidney injury molecule-1
NFκB	nuclear factor kappa B
SLE	systemic lupus erythematosus
SS	SS/JrHSDMcwi
TLR	Toll-like receptor
TNFα	tumor necrosis factor alpha

does not provide information on whether variant *CD14* actively contributes to the disease or is unable to provide an appropriate compensatory response.

Canonically, CD14 is a glycosylphosphatidylinositol-anchored cell surface protein¹⁰ that functions in the TLR (Toll-like receptor) 4 complex to initiate proinflammatory signaling events in response to gram-negative bacteria through recognition of lipopolysaccharide.¹¹ The few studies using a CD14 knockout mouse have shown altered response to lipopolysaccharide,¹² attenuation in obesity-related cardiovascular dysfunction,¹³ and an attenuation of necrosis in hepatic ischemia reperfusion injury.¹⁴ CD14 has also been shown to be upregulated during renal ischemia reperfusion injury¹⁵ and in tubular

epithelial cells of the kidney after unilateral ureteral obstruction.¹⁶ In human populations, soluble CD14 protein in the circulation is inversely correlated with estimated glomerular filtration rate¹⁷ and can also be used as a predictor of hypertension in patients with HIV.¹⁸ Mutations in the *CD14* gene are associated with increased arterial stiffness¹⁹ and prevalence of hypertension in patients with IgA nephropathy.²⁰ In addition, independent of hypertensive state, CD14 polymorphisms can be used to predict progression of IgA nephropathy.²¹

A recent report from the Systolic Blood Pressure Intervention Trial trial demonstrated that intensive blood pressure-lowering treatment was accompanied by a distinctly greater decrease in albumin-to-creatinine ratio and urinary inflammatory markers compared with standard treatment, illustrating the direct relationship between hypertensive phenotype, renal inflammation, and renal damage.²² Of hypertensives, ≈50% are considered salt sensitive—a term used to describe those whose blood pressure responds to changes in dietary salt.²³ These individuals are at a greater risk for developing cardiovascular disease²⁴ and demonstrate reduced survival.²⁵ The Dahl SS rat is an animal model that demonstrates salt-sensitive hypertension accompanied by distinct renal damage and renal immune cell infiltration.²⁶ We have shown that immunosuppression reduces this infiltration, concurrent with an attenuation in the hypertensive response to dietary salt.²⁷ A transcriptomic analysis of renal outer medullary tissue in the Dahl SS rat revealed that a number of genes related to innate immunity pathways are upregulated when these animals are on a high-salt diet and develop renal damage.²⁸ We further confirmed that innate immunity genes in TLR signaling pathways are

upregulated specifically in infiltrating macrophages in the kidneys²⁹ of this model. Of particular note, *CD14*, *Ly96*, and *TLR4*, all encoding proteins that function in the TLR4 protein complex, were upregulated in these renal infiltrating macrophages in response to a high-salt diet.

We hypothesize that *CD14*—a gene that associates with hypertension in human populations and upregulated in renal macrophages of the Dahl SS rat on a high-salt diet—contributes to the pathogenesis of salt-sensitive hypertension. To investigate this, we have created a genetic knockout of *CD14* on the Dahl SS rat background, which we have validated by mass spectrometry. We have performed studies on these SS-*CD14*^{−/−} rats using a high-salt diet model of salt-sensitive hypertension and renal injury.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animals

Some experiments were performed on age-matched male and female, inbred Dahl SS rats (SS/JrHSDMcwi). Other experiments were performed on littermate male and female, wild type, or homozygous knockout for *CD14* animals born from heterozygous breeders (*CD14*^{em2Mcwi}; Rat Genome Database ID:12790610). This strain of SS rats was created by the MCW Gene Editing Rat Resource Center using CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat-associated 9) mutagenesis as we have described previously.^{30,31} Briefly, these animals were produced by injecting a pX330 plasmid³² encoding SpCas9 and sgRNA targeting the *CD14* coding sequence GGAGTACCTTCCTAAAGCGTGTGG (protospacer adjacent motif underlined) into SS rat embryos. Founder animals were genotyped by Cel-1 assay and confirmed by Sanger sequencing to identify a founder animal harboring a net 7-bp deletion (deleted TAAAGCGT, inserted C; Figure 2A) within the CRISPR target site. This founder was backcrossed to the parental SS strain, and a breeding colony was established. Offspring will be referred to as *CD14*^{+/+} or *CD14*^{−/−}. The colony of breeders was maintained on a 0.4% NaCl diet (AIN-76A; Dyets). For high-salt challenge studies, animals received indwelling carotid catheter radiotelemetry implantation at 7 weeks of age as described below. After recovery and baseline pressure recordings, rats were switched to a 4.0% NaCl diet (AIN-76A) for 21 days. At the end of the study period, animals were deeply anesthetized under isoflurane gas inhalation and the kidneys flushed and excised. For bone marrow transplant experiments, recipients received total body irradiation (11 Gy) as described previously.^{2,33} Donor animals were euthanized by barbiturate overdose (beuthanasia-D; Midwest Veterinary Supply), both femurs extracted, and bone marrow collected in Dulbecco's phosphate buffered saline. Bone marrow solution (0.3 mL/recipient) was transplanted into the recipients via conscious

tail vein injection. Animals were allowed to recover for 2 weeks before being implanted with telemeters as in the high-salt challenge protocol. To assess the role of female sex hormones, ovariectomy was performed at 6 weeks of age and the female rats allowed to recover before receiving radiotelemetry implantation and high-salt challenge. All experimental animal procedures were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee.

Surgical Procedures

Blood Pressure Monitoring

At 7 weeks of age, rats underwent a carotid telemetry implantation surgery as described previously.^{4,34} Briefly, the rats were deeply anesthetized under isoflurane gas inhalation. Using aseptic technique, the carotid artery was exposed, and a telemetry catheter was inserted into the artery. Telemetry units (HDS10; Data Sciences International, St. Paul, MN) were placed under the skin at the nape of the neck. Analgesics (0.3 mg/kg buprenorphine SR) and antibiotics (25 mg/kg cefazolin) were administered postsurgically.

Ovariectomy

For some experiments, female *CD14*^{+/+} and *CD14*^{−/−} rats received an ovariectomy in the sixth week of age. To do this, animals were deeply anesthetized under isoflurane gas inhalation, bilateral flank incisions made, the ovaries located, and a ligature tied tightly around the superior-most end of each horn of the uterus. The ovaries were excised, uterus horns returned to the peritoneum, muscle layer sutured, and the skin closed with absorbable suture. Analgesics (0.3 mg/kg buprenorphine SR) and antibiotics (25 mg/kg cefazolin) were administered postsurgically.

Urinalysis

For experiments in SS parental animals, overnight urine collections were performed while on the low-salt diet or at the end of the high-salt period, whereas in experiments on *CD14* wild-type and knockout animals, collections were performed on days-1, 7, 14, and 21 of the experimental protocol. Urine creatinine values were measured by an autoanalyzer (ACE; Alfa Wasserman, Fairfield, NJ) with an assay based on the Jaffé reaction. Urine albumin was quantified with a fluorescent assay that utilized Albumin Blue 580 dye (Sigma-Aldrich, St. Louis, MO) and a fluorescent plate reader (FL-600; BioTek, Winooski, VT). Urinary protein was quantified utilizing Weichselbaum biuret reagent and an autoanalyzer (Alfa Wasserman). Urine *CD14* (Lifespan Bioscience), KIM-1 (kidney injury molecule-1; R&D Systems), and nephrin (Ethos Biosciences) were measured by ELISA according to the manufacturers' instructions.

Immune Cell Isolation From the Kidney

Immune cells in the kidney were isolated as described previously.^{34,35} Briefly, the left kidney was minced and incubated in RPMI 1640 media containing L-glutamine, HEPES, collagenase type IV, and DNase. The solution was then passed through a series of 100, 70, and 40-μm filters. Mononuclear cells were separated by Percoll density gradient centrifugation (400g×30 minutes at room temperature) and washed with a wash buffer (2% HI-FBS, 5 mmol/L EDTA DPBS). Cells from the kidney

were then pelleted and resuspended in the wash buffer solution, and the concentration of cells was determined by counting on a hemocytometer.

Flow Cytometry

General characterization of immune cell types was performed as described previously.^{34,35} Mononuclear cells were incubated with Fc receptor blocking CD32 for 5 minutes followed by an incubation for 30 minutes in a solution containing antibodies for the following extracellular markers: anti-CD3 (eBioscience) for T cells, anti-CD4 (BioLegend) for helper T cells, anti-CD8 (BioLegend) for cytotoxic T cells, anti-CD45R (BD Bioscience) for B cells, and anti-CD11b/c (eBioscience) for monocytes and macrophages. All cells were then analyzed by flow cytometry (LSRII Becton Dickinson) with FACSDIVA software (Becton Dickinson) and FlowJo Software (TreeStar). The gating strategy can be found in Figure S2 in the [Data Supplement](#).

Mass Spectrometry Validation of CD14 Knockout on the Dahl SS Genetic Background

Peritoneal macrophages were isolated from CD14^{+/+} and CD14^{-/-} animals and flash frozen. Cells were lysed and proteins digested with trypsin. The resulting peptide mixture was analyzed by data-dependent acquisition to inform identifiable peptides corresponding to CD14. Subsequently, peptide sequences identified as matching wild-type or mutant CD14 sequences were then assayed by parallel reaction monitoring for targeted peptide detection and relative abundance determination. A detailed description of these methods is provided in the [Data Supplement](#).^{36–38}

Statistical Analysis

One-way ANOVA with a Holm-Sidak post hoc, 2-way ANOVA, 2-way Repeated Measures-ANOVA, or Student *t* test was used where appropriate. Data are expressed as means±SE. A value of *P* < 0.05 was considered statistically significant. SigmaPlot 12.5 software (Systat Software, Inc) or Prism 8 software (GraphPad) was used for all statistical analyses.

RESULTS

Urinary and Renal CD14 Protein Is Increased in Response to a High-Salt Diet in Parental Dahl SS Rats

Parental Dahl SS males and females on a high-salt diet demonstrated lower serum CD14 compared with those maintained on a low-salt diet (Figure 1A; *P* < 0.01 in males, *P* < 0.05 in females). Males also exhibited elevated renal outer medullary CD14 when on the high-salt diet (Figure 1B; *P* < 0.01). Urinary CD14 was increased in male rats fed high salt relative to urinary albumin (Figure 1C; *P* < 0.001) and creatinine (Figure 1E; *P* < 0.001)—a trend consistent in females (Figure 1D and 1F).

CRISPR/Cas9 Knockout of CD14 on the Dahl SS Genetic Background Validated by Mass Spectrometry

CRISPR/Cas9 was used to target CD14 early in the second exon, which caused an indel resulting from an 8-base pair deletion and 1-base pair insertion (Figure 2A). This frameshift deletion at bases 248 to 254 results in a predicted premature stop codon. Targeted analysis of peptide sequences by parallel reaction monitoring demonstrated that genetic knockout of CD14 eliminated detectable CD14 protein as seen by loss of almost all CD14 tryptic peptides (Figure 2B).

Knockout of CD14 in Female but Not Male Animals of the Dahl SS Background Confers Exacerbated Salt-Sensitive Renal Damage and Hypertension

CD14 knockout did not significantly affect the salt-sensitive increase in blood pressure (Figure 3A; *P* > 0.05) nor the amount of renal damage as assessed by proteinuria and albuminuria (Figure 3C and 3E; *P* > 0.05) in male animals. In contrast, CD14^{-/-} females demonstrated an augmentation of the salt-induced blood pressure response (Figure 3B; *P* < 0.05) and an exacerbation in renal damage assessed by albuminuria and proteinuria (Figure 3D and 3F; *P* < 0.05) compared with CD14^{+/+} littermates. At the end of the 21-day high-salt challenge, urinary nephron excretion was increased in CD14^{-/-} females (*P* < 0.01) but not CD14^{-/-} male animals (Figure S1A and S1B) indicating damage to the glomerulus. Excretion of KIM-1 was not statistically different between the CD14^{+/+} and CD14^{-/-} rats of either sex (Figure S1C and S1D).

Increased Macrophages in Female CD14^{-/-} Dahl SS Rats

After the high-salt challenge, the number of infiltrating immune cells in the kidney was evaluated by flow cytometry. CD14^{+/+} and CD14^{-/-} male animals demonstrated a similar number of infiltrating renal immune cells (Figure 4A through 4C; *P* > 0.05). In contrast, knockout of *CD14* in females resulted in an elevation in infiltrating macrophages (Figure 4E; *P* < 0.01) independent of changes to the number of infiltrating lymphocytes (Figure 4F; *P* > 0.05).

CD14 Knockout in Hematopoietic Cells of Female Dahl SS Rats Exacerbates Salt-Sensitive Renal Damage and Hypertension

As *CD14* is a gene expressed across various tissues,³⁹ our next goal was to determine whether loss of CD14 specifically in circulating immune cells was responsible

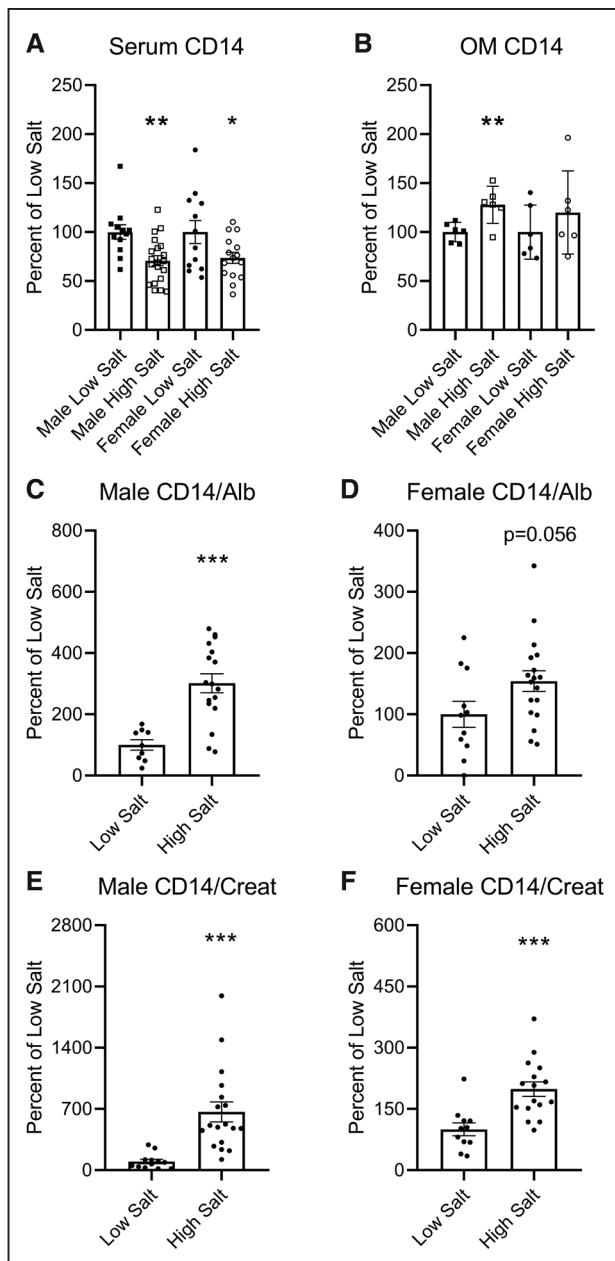


Figure 1. Levels of serum and urinary CD14 in male and female Dahl SS rats on either a low or high-salt diet.

Serum (A), kidney outer medulla (OM) homogenate (B), and urine (C–F) were collected from male and female, parental SS (SS/JrHSDMcwi) rats maintained on either a low-salt or high-salt diet for 3 wk and CD14 (cluster of differentiation 14) protein measured by ELISA. Alb indicates Albumin; and Creat, Creatinine. $n=6$ to 20. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs low salt.

for the augmented salt-sensitive response observed in the $CD14^{-/-}$ female rats. $CD14^{+/+}$ female recipients received total body irradiation to eliminate their host immune system followed by bone marrow transfer from either $CD14^{+/+}$ or $CD14^{-/-}$ donor females. The resultant chimeras were $CD14^{+/+}$ in all solid tissue but either $CD14^{+/+}$ or $CD14^{-/-}$ in hematopoietic cells. Consistent with what was observed in the germline-whole body knockout, those deficient in CD14 only in hematopoietic

cells demonstrated exacerbated renal damage (Figure 5A; $P<0.05$), augmented blood pressure response (Figure 5B; $P<0.05$), and renal macrophage infiltration (Figure 5D; $P<0.01$). An additional elevation in infiltrating B cells was also observed (Figure 5E; $P<0.05$). Representative flow cytometry plots of these renal infiltrating immune cell populations can be found in Figure S2.

Exacerbation of Salt-Sensitive Phenotypes in $CD14^{-/-}$ Female Dahl SS Rats Is Female Sex Hormone Dependent

Due to the sexual dimorphic effect of knocking out CD14, we assessed whether female sex hormones contribute to this dichotomy. Ovariectomy was performed on $CD14^{+/+}$ and $CD14^{-/-}$ females who were then subjected to a high-salt challenge. Unlike what was observed in Figures 3 through 5, $CD14^{+/+}$ and $CD14^{-/-}$ OVX females no longer demonstrated differential renal damage (Figure 6A; $P>0.05$), elevation in blood pressure (Figure 6B; $P>0.05$), or immune cell infiltration (Figure 6C through 6E; $P>0.05$).

DISCUSSION

Due to an established relationship between CD14 and hypertension in human populations, we aimed to understand its role in a model of salt-sensitive hypertension, the Dahl SS rat. An initial experiment demonstrated that Dahl SS rats on a high-salt diet have depressed levels of soluble CD14 in the serum compared with those on a low-salt diet. This was accompanied by a reciprocal effect on the urinary levels of CD14, where a high-salt diet in SS rats raised urinary CD14. The increase in renal CD14 is consistent with our previous reports^{28,29} and is similar to the reported increased urinary soluble CD14 protein observed in patients with rheumatoid arthritis, another condition of sterile inflammation.^{40,41} In these studies, a highly sensitive mass spectrometry technique was used to confirm a robust and complete knockout of the CD14 protein by CRISPR/Cas9. This approach provided antibody-independent evidence that truncated protein products were not being produced—a frequent concern that arises in genetic knockout studies.

This work demonstrated that genetic deletion of CD14 on the Dahl SS background exacerbates salt-sensitive phenotypes including renal damage, hypertension, and macrophage infiltration, establishing a regulatory role for CD14-dependent signaling. It is notable that this exacerbation was only observed in female animals, which would implicate that the regulatory role of CD14 may be estrogen dependent. This conclusion is consistent with the fact that ovariectomy eliminated the differential response to the salt challenge observed in $CD14^{-/-}$ females. These studies have also shown that CD14-dependent signaling

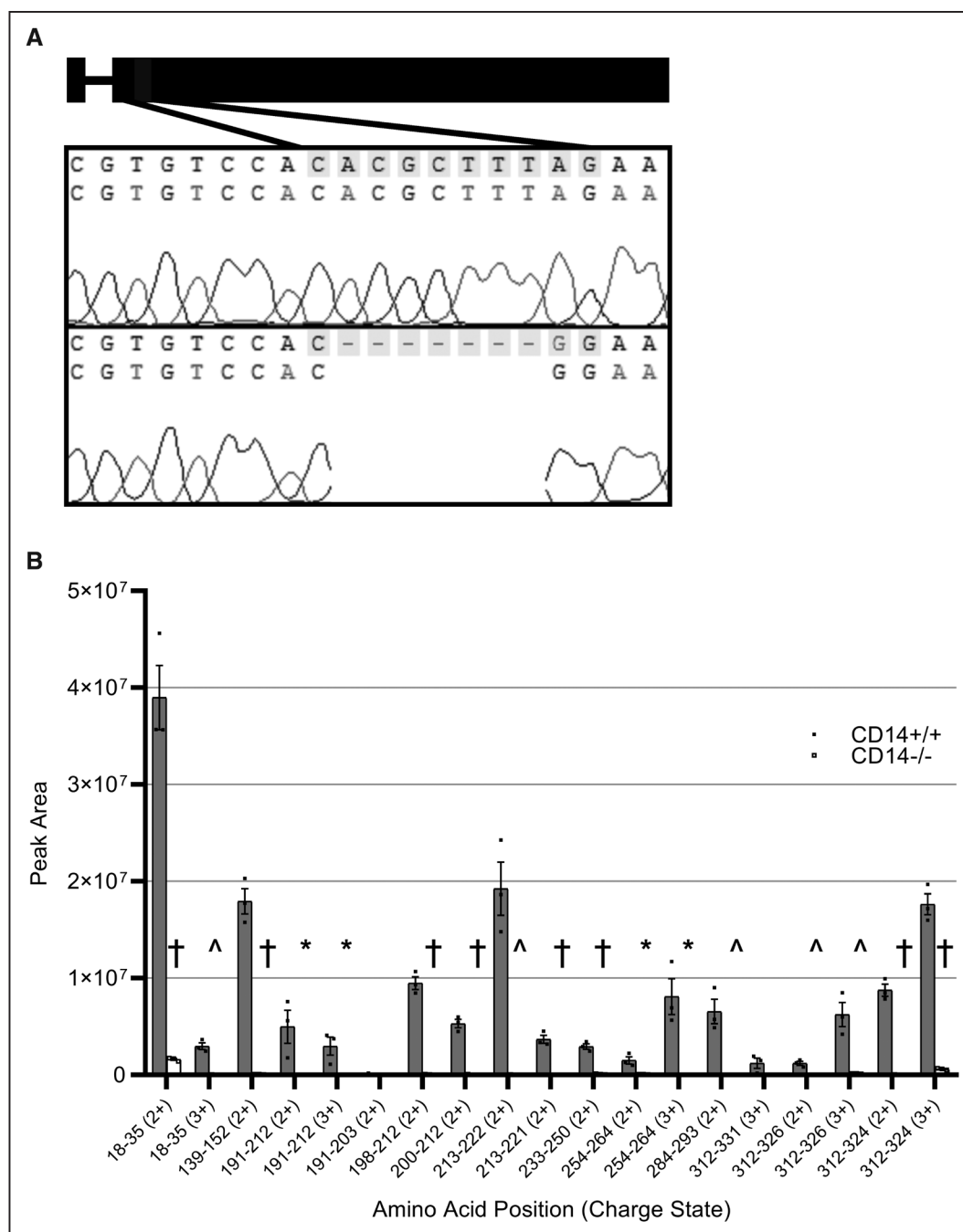


Figure 2. Sequencing and protein validation of CD14 knockout.

Schematic for the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeat-associated 9) target site and the resultant 8 base pair deletion and 1 base pair insertion, which causes transcription of a predicted premature stop codon in the CD14 (cluster of differentiation 14) gene on the Dahl SS (SS/JrHSDMcwi) background (A). Parallel reaction monitoring was used to measure the presence of peptide sequences from the CD14 protein in isolated peritoneal macrophages (B). n=3/3. Charge state (2+, 3+) is indicated for each peptide monitored. †P<0.001, *P<0.01, *P<0.05.

specifically in hematopoietic cells plays this regulatory role. It is interesting to note that mean arterial pressure in female CD14^{-/-} rats reached similar levels to that of male rats even though the absolute magnitude of urinary renal damage markers and renal immune cell infiltration was approximately half of what was observed in males. As female rats are more often smaller than males, we

were not surprised to observe a smaller magnitude in albumin excretion even when blood pressures reached similar levels, consistent with previous reports of Dahl SS rats.⁴² Likewise, female kidneys are considerably smaller than male kidneys, which is reflected in a smaller absolute number of infiltrating immune cells in females. Nonetheless, the effect of CD14 knockout in females is clear

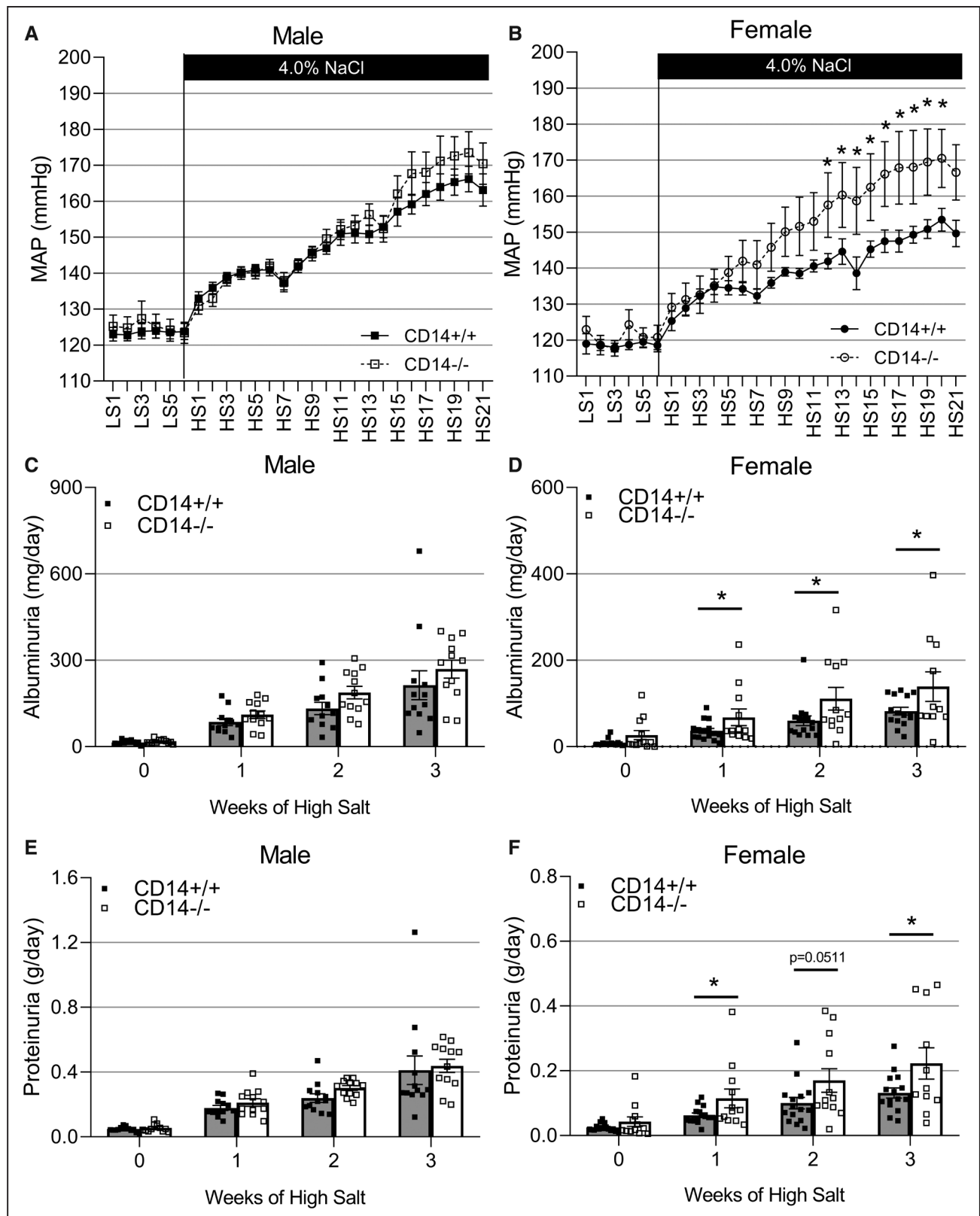


Figure 3. Effect of CD14 knockout on salt-sensitive hypertension in male and female Dahl SS rats.

Radiotelemetry was used to monitor 6 d of low-salt baseline and 21 d of high-salt diet mean arterial blood pressure (MAP) recordings in male ($n=11/12$, A) and female ($n=7/11$, B) CD14^{+/+} and CD14^{-/-} Dahl SS (SS/JrHSDMcwi) rats. Weekly overnight urine collections were used to measure albuminuria (C and D) and proteinuria (E and F; male, $n=12/12$; female, $n=12/15$; 2-way Repeated Measures-ANOVA; * $P<0.05$). CD14 indicates cluster of differentiation 14; HS, high salt; and LS, low salt.

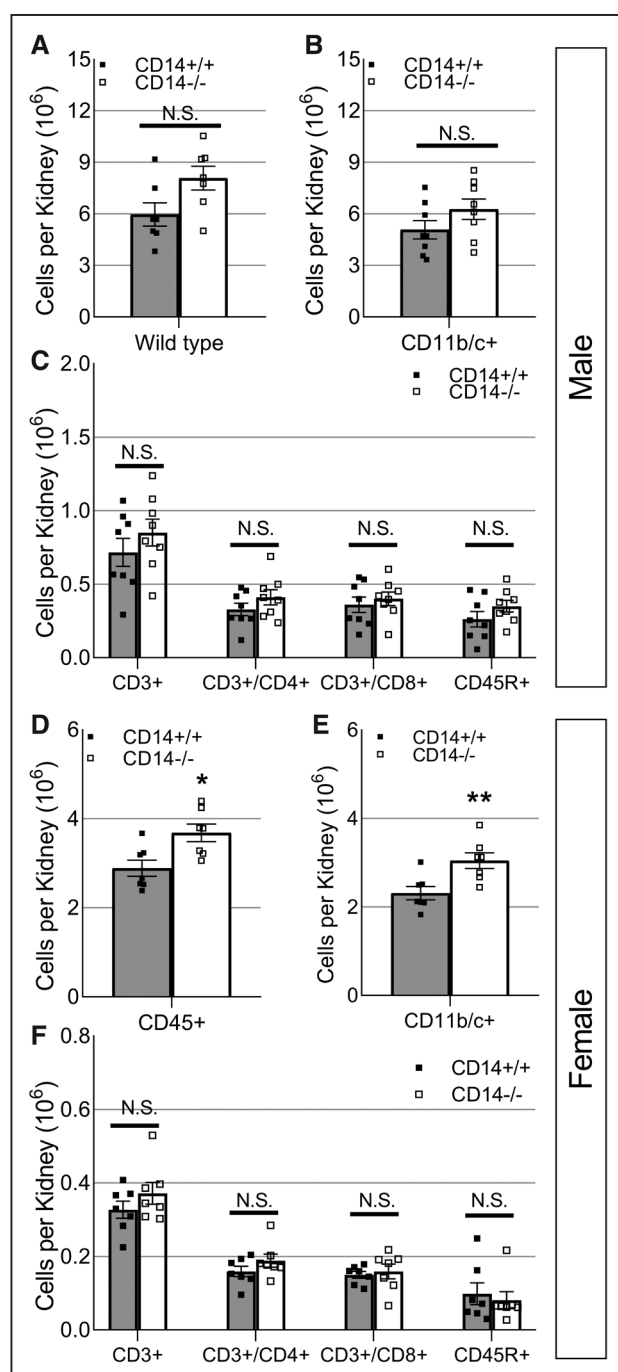


Figure 4. Effect of CD14 knockout on the number of infiltrating renal immune cells in male and female Dahl SS rats in response to a high-salt diet.

Infiltrating immune cells in the kidneys of male ($n=8/8$, A–C) and female ($n=7/7$, D–F) CD14^{+/+} and CD14^{-/-} Dahl SS (SS/JrHSDMwi) rats including CD45⁺ leukocytes (A and D), CD11b/c⁺ macrophages (C and E), CD3⁺ T cells, CD3⁺/CD4⁺ helper T cells, CD3⁺/CD8⁺ cytotoxic T cells, and CD45R⁺ B cells (C and F). CD14 indicates cluster of differentiation 14; and NS, not significant. * $P<0.05$, ** $P<0.01$ vs CD14^{+/+}.

and eliminates, to some degree, the protection normally observed.

Though not assessed in these studies, CD14^{-/-} mice show enhanced neutrophil recruitment to sites of

infection, which provides a beneficial clearance of bacteria.⁴³ It is possible that, in our model, CD14^{-/-} female rats may show increased renal neutrophil recruitment, which may cause sterile inflammation to persist. Additional literature has shown that CD14 plays a regulatory role in host immunity. CD14^{-/-} macrophages show enhanced TNF α (tumor necrosis factor alpha) production in response to *Borrelia burgdorferi* and importantly, a failure to upregulate negative regulators such as SOCS genes through the p38/MAPK (mitogen-activated protein kinases) pathway.⁴⁴ Interestingly, *SOCS3* is one of those negative regulators that is upregulated in the renal outer medulla²⁸ and renal T cells⁴⁵ when Dahl SS animals are on a high-salt diet. Work by these authors has also shown that CD14-null animals show persistent inflammation in Lyme disease⁴⁶ and postulate that CD14-independent signaling is more “destructive” than CD14-dependent signaling.

One of the more striking observations in these studies was that CD14^{-/-} females showed an exacerbated salt-sensitive response, whereas knockout in male animals had no effect, indicating that female sex hormones may be involved. This was then confirmed by performing ovariectomy before the high-salt challenge, which eliminated the differential response to salt between CD14^{+/+} and CD14^{-/-} females. A possible explanation for these results is that female sex hormones augment inflammatory mechanisms and CD14-dependent signaling regulates this enhancement, where these 2 factors play opposing roles in regulating inflammatory activation. This contradicts the well-established protective role of estrogens observed in the Dahl SS rat.⁴⁷

Estradiol (E2) has been shown to upregulate surface expression of the CD14 protein.⁴⁸ Treating primary microglia (a type of macrophage) with E2 enhances the proinflammatory response to lipopolysaccharide in ovariectomized female rats.⁴⁹ E2 also promotes M1 proinflammatory activation upregulating iNOS (inducible NO synthase) and IL-1 β and prevents M2 activation by downregulating IL-10 and arginase. This increase in inflammation seen with E2 treatment may be due to increased cadherin-11-dependent signaling,⁵⁰ another gene upregulated in the renal outer medulla of Dahl rats on a high-salt diet.²⁸ Two important studies by Calippe et al^{51,52} demonstrated that the inflammatory response to E2 was via estrogen receptor alpha, which increased NF κ B (nuclear factor kappa B) p65 transcriptional activity, downregulated the inhibitory PI3K/AKT pathway, and increased the production of IL-1 β , IL-6, and TNF α . Notably, these effects were observed with chronic E2 treatment but not with acute treatment. Though these results are from animal models, peritoneal macrophages isolated from human patients showed the exact same effects of enhanced IL-6 and TNF α production with E2 treatment. This was even more pronounced if macrophages came from patients with endometriosis—a state where there is chronic inflammation.⁵³

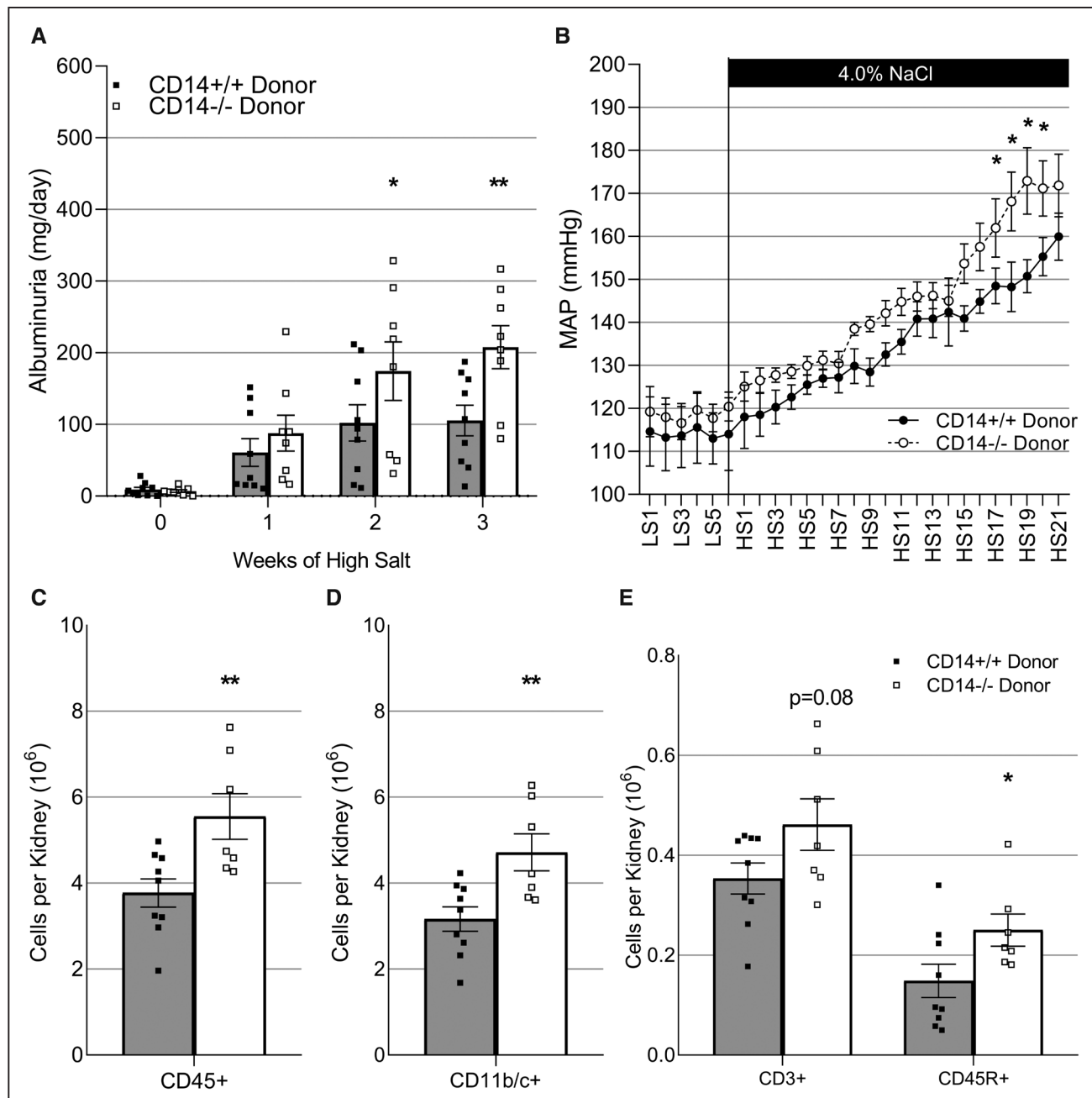


Figure 5. Bone marrow from either CD14^{+/+} or CD14^{-/-} Dahl SS (SS/JrHSDMcwi) donors was transferred into total body irradiated CD14^{+/+} recipients who were then subjected to a high-salt challenge.

Albuminuria (A). Mean arterial pressure (MAP; B). Infiltrating CD45⁺ leukocytes (C), CD11b/c⁺ macrophages (D), CD3⁺ T cells, and CD45R⁺ B cells (E). n=6 to 9. CD14 indicates cluster of differentiation 14; HS, high salt; and LS, low salt. **P*<0.05, ***P*<0.01 vs CD14^{+/+} donor.

Though female sex is associated with protection from cardiovascular disease, females are more likely to be diagnosed with an autoimmune disease. Various investigators have documented that polymorphisms in the human *CD14* gene are associated with increased incidence of autoimmune diseases such as autoimmune thyroid disease,⁵⁴ Parkinson disease,⁵⁵ celiac disease,^{56–58} and systemic lupus erythematosus (SLE).⁵⁹ Interestingly, multiple reports have shown there is no such relationship between *CD14* polymorphisms and

rheumatoid arthritis.^{59–63} Women with SLE have reduced monocyte surface expression of CD14 compared with healthy controls, and expression is also reduced when comparing females with SLE to males with SLE.⁶⁴ This is consistent with the results of the present studies where loss of CD14 enhanced the disease process. As reviewed by Moulton,⁶⁵ estrogen signaling, particularly through ER α , appears to be proinflammatory and contributes to the inflammatory activity of SLE, again consistent with the present results. Though there is

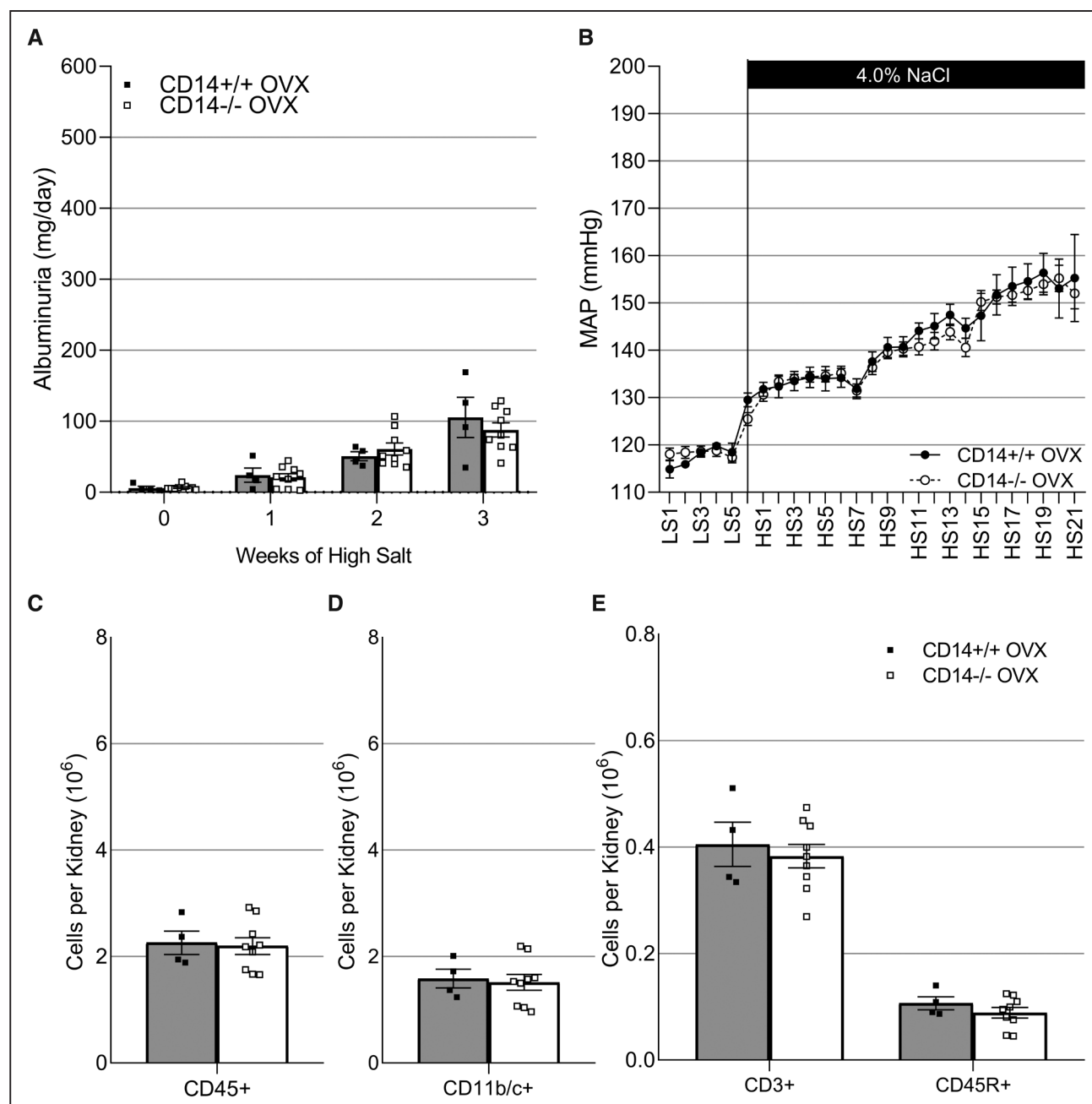


Figure 6. Ovariectomy (OVX) was performed on CD14^{+/+} and CD14^{-/-} Dahl SS (SS/JrHSDMcwi) rats who were then subjected to a high-salt challenge.

Albuminuria (**A**). Mean arterial pressure (MAP; **B**). Infiltrating CD45⁺ leukocytes (**C**), CD11b/c⁺ macrophages (**D**), CD3⁺ T cells, and CD45R⁺ B cells (**E**). n=4/8. No significant differences were identified. CD14 indicates cluster of differentiation 14.

a pronounced increased incidence of hypertension in women with SLE,⁶⁶ it is unclear how or whether CD14 contributes to this relationship. The results of the present studies may be informative for understanding the relationship between biological sex and prevalence of autoimmune diseases. The mechanistic connection between CD14 and female sex hormones is not yet clear, and we do not yet know whether the effects of these signaling pathways on inflammatory balance are through shared or parallel pathways. Importantly, these

studies reinforce the relationship between increased arterial blood pressure, renal immune cell infiltration, and renal injury. Consistent with previous work,⁶⁷ increased renal perfusion pressure drives immune cells to infiltrate into the kidney interstitium, which we speculate is in response to increasing pressure-induced tissue damage. These infiltrating immune cells then contribute to the accumulating tissue damage and renal dysfunction, further potentiating the hypertensive phenotype.

PERSPECTIVES

CD14—a gene associated with cardiovascular disease in human populations—is also upregulated in Dahl SS rats when on a high-salt diet. We created a genetic knock-out of CD14 on the Dahl SS background to demonstrate a novel regulatory role for CD14-dependant signaling in hematopoietic cells. In addition, we show that this is female specific, and the exacerbation of disease seen in CD14^{-/-} animals is eliminated by ovariectomy. This sexual dimorphism in innate immune system activation was previously unappreciated and provides a new understanding of the difference in pathogenesis between males and females, as well as a targetable pathway.

ARTICLE INFORMATION

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Affiliations

Department of Physiology (D.J.F., H.L., J.Z., A.M.G., M.R.D.), Center for Biomedical Mass Spectrometry Research (T.K., R.L.G.), and Genomic Sciences and Precision Medicine Center (A.M.G., M.R.D.), Medical College of Wisconsin, Wauwatosa, WI. Department of Physiology, Augusta University and the Medical College of Georgia, Augusta, GA (D.J.F., J.M.A.-B., J.H.D., M.C.-S., D.L.M.). CardioMics Program, Center for Heart and Vascular Research (R.L.G.), Division of Cardiovascular Medicine (R.L.G.), and Department of Cellular and Integrative Physiology (R.L.G.), University of Nebraska Medical Center, Omaha, NE.

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Disclosures

None.

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