学位論文

Galectin-9 deficiency exacerbates lipopolysaccharide-induced hypothermia and kidney injury

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ORIGINAL ARTICLE

Galectin-9 deficiency exacerbates lipopolysaccharide-induced hypothermia and kidney injury

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Abstract

Background Galectin-9 (Gal-9) is a multifunctional lectin that moderates inflammation and organ damage. In this study, we tested whether Gal-9 has a protective role in the pathogenesis of endotoxemic acute kidney injury.

Methods We examined the levels of Gal-9 in control mice after lipopolysaccharide (LPS) administration. We developed Gal-9 knockout (KO) mice that lack Gal-9 systemically and evaluated the role of Gal-9 in LPS-induced proinflammatory cytokines, vascular permeability, and renal injury.

Results Gal-9 levels were increased in the plasma, kidney, and spleen within 4 h after LPS administration to wild-type mice. Gal-9 deficiency did not affect the LPS-induced increase in plasma tumor necrosis factor-α levels at 1 h or vascular permeability at 6 h. Lower urine volume and reduced creatinine clearance were observed in Gal-9-KO mice compared with wild-type mice after LPS administration. Gal-9-KO mice had limited improvement in urine volume after fluid resuscitation compared with wild-type mice. LPS reduced the body temperature 12 h after its administration. Hypothermia had disappeared in wild-type mice by 24 h, whereas it was sustained until 24 h in Gal-9-KO mice. Importantly, maintaining body temperature in Gal-9-KO mice improved the response of urine flow to fluid resuscitation.

Conclusion Deficiency in Gal-9 worsened LPS-induced hypothermia and kidney injury in mice. The accelerated hypothermia induced by Gal-9 deficiency contributed to the blunted response to fluid resuscitation.

Keywords Lipopolysaccharide (LPS) · Acute kidney injury (AKI) · Galectin-9 · Hypothermia

Introduction

Sepsis, defined as a condition in which life-threatening organ damage is caused by an uncontrolled host response to infection, is a common cause of acute kidney injury (AKI) [1]. AKI also increases the risk of death in septic patients [2]. Sepsis-related AKI is a complex condition, in which hemodynamic changes, systemic inflammation, and tubular damage are associated with the development of AKI [3, 4]. Currently, there are no effective treatments for septic AKI. In addition, fever and hypothermia are characteristic symptoms of sepsis and AKI occurs frequently in hypothermic patients and may be associated with increased mortality [5]. However, a molecular link between sepsis, body temperature, and AKI has yet to be clarified.

Galectin-9 (Gal-9) is a lectin that binds to the β-galactoside structure and induces apoptosis in activated T cells [6]. We previously demonstrated that Gal-9 attenuated inflammation and kidney damage in autoimmune model mice [7]. Gal-9 is expressed in lymphoid organs including the thymus, as well as peripheral organs, kidney, and spleen [8]. Gal-9 was elevated in animal models of sepsis, resulting in lower mortality rates [9, 10]. Moreover, Gal-9 reduced lung damage and was involved in local organ protection in an acute lung injury model induced by lipopolysaccharide

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(LPS) [11]. Therefore, in the current study, we hypothesized that Gal-9 has a regulatory role in the pathogenesis of endotoxemic AKI.

Materials and methods

Animals

All experiments were approved by the Institutional Animal Care and Use Committee of Kagawa University and followed standard guidelines for the humane care and use of animals in scientific research. Animals were housed in an animal house under the following conditions: temperature 24 ± 1 °C, humidity 55 ± 5%, and a 12-h light/dark cycle. Animals received standard chow and drinking water ad libitum. Male C57BL/6 N mice aged 8–12 weeks were purchased from CLEA (Tokyo, Japan) or Charles River (Kanagawa, Japan) and Gal-9 deficient mice were from our breeding colony.

Generation of *Lgals9*$^{-/-}$ mice

Gal-9-deficient (*Lgals9*$^{-/-}$) mice were generated using the CRISPR-Cas9 system. Single guide RNA (sgRNA) targeted to the *Lgals9* exon 1 was designed using CRISPRdirect [12]. *Lgals9*-sgRNA was synthesized using the EnGen™ sgRNA Synthesis Kit, *Streptococcus pyogenes* (New England Biolabs Inc., MA, USA), and purified with an RNeasy Mini kit (QIAGEN, Hilden, Germany). Alt-R™ S.p. Cas9 Nuclease 3NLS (Integrated DNA Technologies, Inc., IA, USA) was used to form the RNP complex. The sgRNA (770 nM) and Cas9 proteins (385 nM) were mixed in 10 mM Tris–HCl (pH 7.4) and incubated for 10 min at room temperature. The resulting RNP complex was mixed with 1 μM SCR7 pyrazine (Sigma–Aldrich, St Louis, MO, USA) and centrifuged at 20,000*g* for 5 min at room temperature. The injection mixture was then passed through Millipore Centrifugal Filter units (UFC30LG25, Millipore, MA, USA). The final injection mixture was injected into the pronuclei of one-cell stage zygotes (C57BL/6 N background). No mice were crossed with wild-type (WT) C57BL/6 N mice and intercrossed to obtain homogenous mutant mice. The mutation was detected by PCR and sequencing (Supplementary Figure S1). The sequences of primers used in this study are shown in Supplementary Table S1. Gal-9-deficiency was confirmed by a mouse Gal-9 ELISA kit (MyBioSource, San Diego, CA, USA).

Experimental protocol

Mice were injected intraperitoneally (i.p.) with LPS (O-55:B5, Sigma–Aldrich) (5 mg/kg) and sacrificed at 4 or 24 h. The fluid resuscitation group received fluid resuscitation (50 mL/kg saline subcutaneously [s.c.]) at 4 and 18 h after LPS administration [13] (Fig. 1a). Deep body temperature was measured immediately by a rectal thermometer after anesthesia with sevoflurane at the indicated intervals. Separately prepared mice were used for the renal blood flow (RBF), cytokine, and vascular permeability experiments. In the warming experiment, the entire metabolic cage was heated using a heating table to maintain the ambient temperature at 30 °C while maintaining humidity for 1 h prior to LPS administration (Fig. 1b). To induce hypothermia, we used adenosine triphosphate (ATP), which when converted into adenosine, signals through adenosine receptors in the hypothalamus to induce hypothermia [14, 15]. Mice were injected i.p. with ATP (500 mg/kg; Sigma–Aldrich) and their deep body temperature was measured at the indicated intervals.

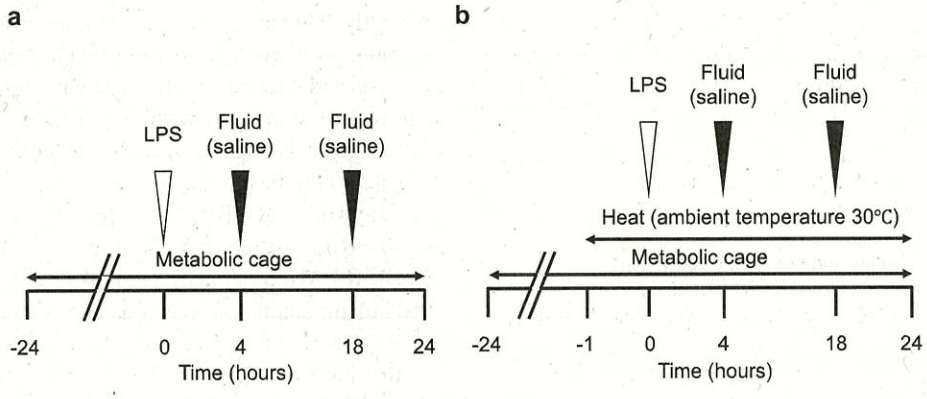

Fig. 1 Experimental protocols. **a** Wild-type and knockout mice were injected intraperitoneally (i.p.) with LPS (5 mg/kg). The fluid resuscitation group received fluid resuscitation (50 mL/kg saline subcutaneously [s.c.]) at 4 and 18 h. **b** In the warming experiment, the ambient temperature was maintained at 30 °C by heating the entire metabolic cage 1 h before LPS administration. Wild-type and knockout mice were injected i.p. with LPS (5 mg/kg) and fluid resuscitation s.c. (50 mL/kg) at 4 and 18 h. *LPS* lipopolysaccharide

Urine collection and tissue preparation

Mice were acclimated in metabolic cages for 24 h prior to urine collection. Urine volume was measured by weight (1 g/mL). In the heating experiment, a small amount of oil was added to the collection bottle to prevent evaporation of the urine. For analysis, animals were euthanized after LPS administration, and then blood, kidney, and spleen samples were collected. Kidney tissues were fixed in neutral formalin solution, thinly sliced, and stained with hematoxylin and eosin for histological evaluation.

Renal function, Gal-9, and cytokine measurements

Blood urea nitrogen (BUN) and creatinine (Cr) in samples were measured using commercial assay kits (LabAssay Creatinine [Wako, Osaka, Japan] and DetectX Urea Nitrogen Colorimetric Detection Kit [Arbor Assays, Ann Arbor, MI, USA]). Creatinine clearance (CCr, μl/min) was calculated using the following formula: plasma Cr (mg/dl) × urine volume (μl/day)/urine Cr (mg/dl) × 1440. Gal-9, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in plasma were measured using commercially available assay kits (Mouse Galectin-9 ELISA Kit [MyBioSource] and Mouse TNF-alpha and Mouse IL-6 ELISA Kits [Proteintech, Chicago, IL, USA]) according to the manufacturer's instructions. All samples were measured in duplicate and appropriately diluted for accurate measurements within a standard curve.

Vascular permeability study

Vascular permeability was evaluated 6 h after LPS administration. Mice were injected intravenously with Evans blue (2%, 4 mL/kg; Sigma–Aldrich) 1 h before evaluation. Then, 6 h after LPS administration, 3 mL of saline was injected into the abdominal cavity, and ascites samples were collected after gently rubbing the abdomen [16]. The absorbance of the formamide extract contained in the samples was measured with a spectrophotometer (SH-9000; Corona, Ibaraki, Japan).

Measurement of RBF

Renal artery diameter and arterial velocity were measured using ultrasonography and a pulsed Doppler (LOGIQ e, G.E. Health Care, Chicago, IL, USA) to determine the RBF [17–19]. RBF was calculated using the following equation: $RBF = \pi\ (0.5 \times \text{renal artery diameter})^2 \times (\text{time-averaged flow velocity of the renal artery})$.

After anesthetizing mice with 1–2% sevoflurane, a target heart rate of 400–450 (beats per minute) was measured on a 37 °C heating table to minimize data variability.

Real-time PCR

Collected kidneys were soaked overnight in RNA later, RNA was extracted using ISOGEN (Nippon Gene, Tokyo, Japan), and complementary DNA was prepared using ReverTra Ace® (Toyobo, Osaka, Japan). The mRNA levels of 18S rRNA, neutrophil gelatinase-associated lipocalin (NGAL; a renal tubular damage marker), and Gal-9 were determined using ABI Step One Plus Real-Time PCR System with a Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). The primer sequences (forward and reverse) used in this study are shown in Supplementary Table S2. The mRNA level of each gene was normalized to the level of 18S rRNA. Relative mRNA concentrations were determined using the $2^{-\Delta\Delta Ct}$ method, and the ΔΔCt value was calculated using data from control mice.

Statistical analysis

Results are expressed as the mean and standard error of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA) following the Tukey's multiple comparison test for comparisons among more than three groups. Two-way ANOVA with Tukey's multiple comparisons was used to compare groups with two factors, i.e., Gal-9 and fluid resuscitation, and renal parameters at 24 h after LPS (Fig. 4), or RBF and body temperature at each time point after LPS. (Fig. 5, Supplementary Figure S3a). The relationship between RBF and body temperature at each time point was examined using linear regression. The means of two groups were compared using the Student's *t* test. All values were normally distributed in the normal control group, whereas skewing was observed for oliguric groups. All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) with p values < 0.05 considered statistically significant.

Results

Rapid elevation of systemic and local Gal-9 after LPS administration

Figure 2 shows the change in Gal-9 levels at 4 or 24 h after saline or LPS administration in WT mice. LPS administration increased the Gal-9 levels in plasma and Gal-9 mRNA expression levels in the kidney and spleen, both of which were significantly elevated at 4 h compared with 24 h. These results suggested that LPS treatment increased plasma Gal-9

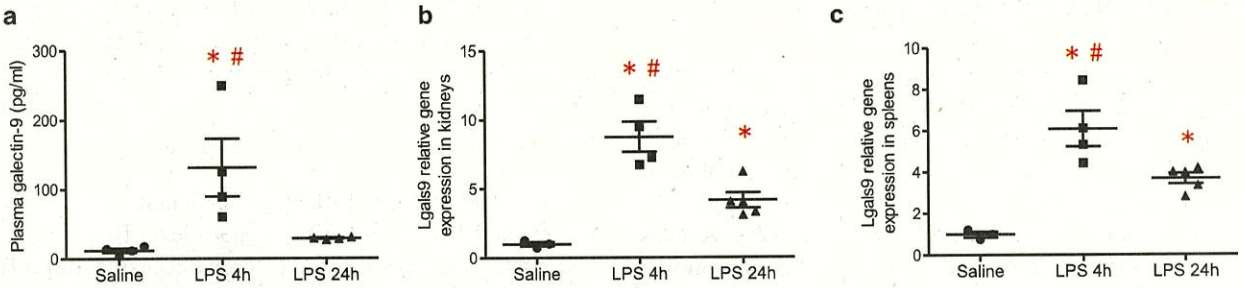

Fig. 2 LPS induces the early elevation of systemic and local Gal-9. Change in Gal-9 in C57BL/6 N mice 4 or 24 h after saline or LPS administration ($n=3$–5): **a** Gal-9 levels in plasma, **b** Gal-9 mRNA levels in kidneys, and **c** Gal-9 mRNA in spleens. Data show the mean ± SEM ($P<0.05$). *$p<0.05$ versus saline group; #$p<0.05$ versus LPS 24-h group. *LPS* lipopolysaccharide, *Gal-9* galectin-9

levels systemically as well as local Gal-9 levels in the kidney and spleen.

Gal-9 deficiency does not exacerbate systemic proinflammatory cytokines or vascular permeability after LPS administration

Next, we investigated the effect of Gal-9 on LPS-induced systemic effects. Serum levels of Gal-9 were below the detection limit (<7.8 pg/mL) in Gal-9-KO mice. We measured inflammatory cytokines in WT and Gal-9-KO mice after LPS administration. Plasma TNF-α levels at 1 h after LPS administration were increased; however, there was no significant difference in TNF-α levels between the groups (Fig. 3a). Plasma IL-6 levels were increased at 4 h after LPS administration compared with the saline injection group (mean (SEM) 986 (60) pg/mL); however, there was no significant difference in IL-6 levels between the groups (mean (SEM): WT + LPS; 11,280 (2534) pg/mL vs KO + LPS; 15,690 (2478) pg/mL, 95%CI: − 13,520 to 4700, $p=0.27$). Time points for the measurement of these cytokines were based on a previous report [20]. The values of these cytokines are known to be almost normalized at 24 h after LPS administration.

Next, we assessed vascular permeability in WT and Gal-9-KO mice after LPS administration. LPS increased the leakage of Evans blue into ascites, but there was no significant difference in leakage between the groups (Fig. 3b).

Gal-9 deficient induces AKI after LPS administration

Previous studies have shown that LPS reduced urine output and the glomerular filtration rate (GFR) [21]; therefore, we investigated the effect of Gal-9 on LPS-induced AKI. At the early time point of 4 h after LPS administration, renal parameters (urine volume, CCr, BUN, and NGAL) were not significantly different between the WT and Gal-9-KO mice. WT mice had increased urine volume, improved CCr, and decreased BUN in response to fluid resuscitation at 24 h after LPS administration. In the KO group, fluid

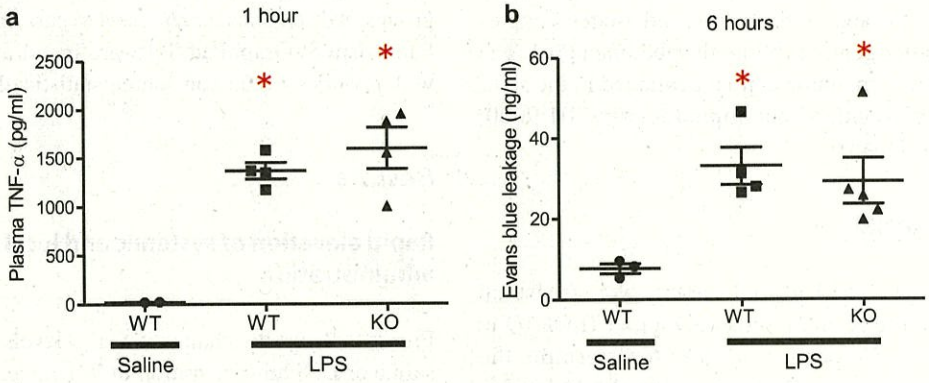

Fig. 3 Gal-9 deficiency does not exacerbate systemic inflammation after LPS administration. **a** Plasma TNF-α at 1 h after LPS administration ($n=2$–4). **b** Leakage of Evans blue into ascites at 6 h after LPS administration ($n=3$–5). Data show the mean ± SEM ($p<0.05$). *$p<0.05$ versus WT saline group. *Gal-9* galectin-9, *LPS* lipopolysaccharide, *WT* C57BL/6 N mice, *KO* galectin-9 knockout mice, *TNF-α* tumor necrosis factor-α

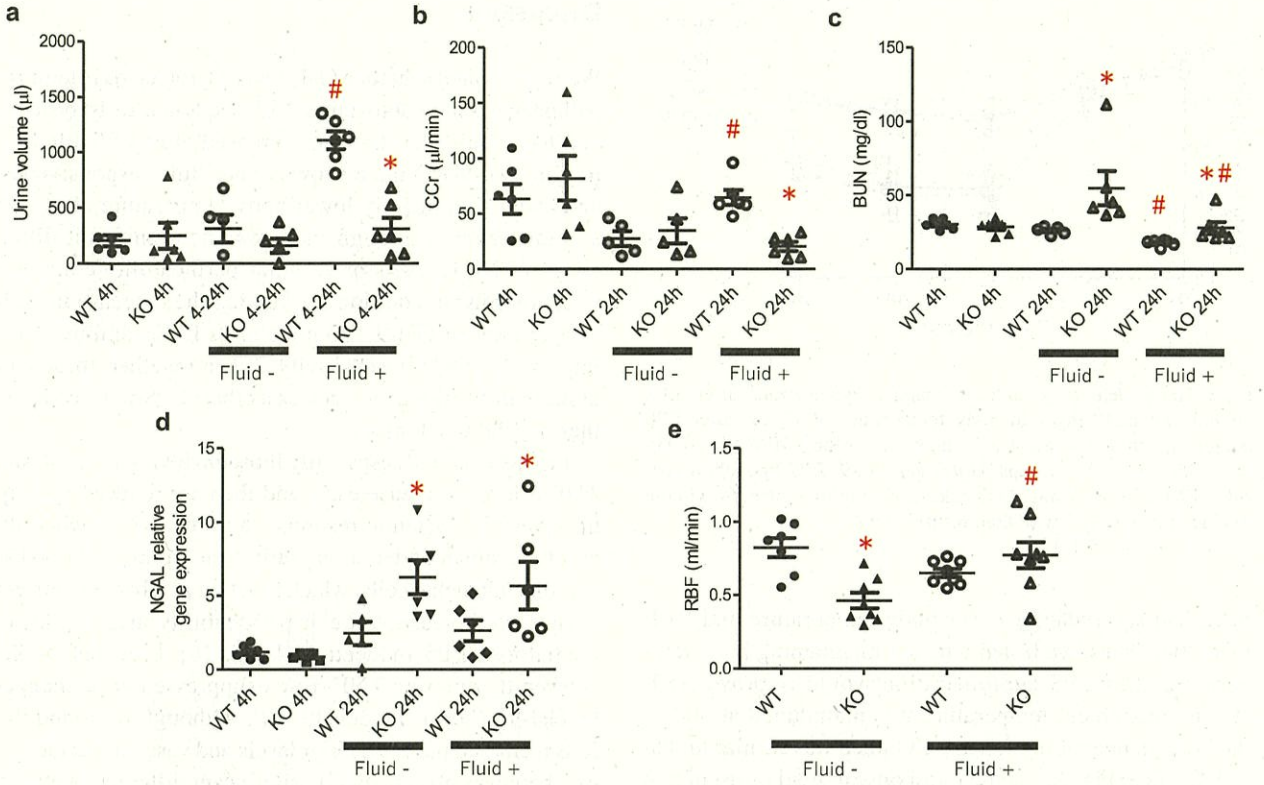

Fig. 4 Gal-9 deficiency exacerbates renal injury after LPS administration. Changes in renal parameters with and without fluid resuscitation after LPS administration ($n=5$–6). **a** Urine volume at 4 h and between 4 and 24 h. **b** CCr, **c** BUN, and **d** NGAL mRNA in kidneys ($n=5$–6). Renal parameters (urine volume, CCr, BUN, NGAL) were not significantly different between WT and KO groups at 4 h after LPS administration. **e** RBF data were obtained 24 h after LPS administration and were obtained from a separate mouse set ($n=7$–8). Data show the mean ± SEM ($p<0.05$). *$p<0.05$ versus WT groups; #$p<0.05$ versus without fluid groups. *Gal-9* galectin-9, *LPS* lipopolysaccharide, *WT* C57BL/6 N mice, *KO* galectin-9 knockout mice, *CCr* creatinine clearance, *BUN* blood urea nitrogen, *NGAL* neutrophil gelatinase-associated lipocalin; RBF, renal blood flow

resuscitation did not increase urine volume or CCr at 24 h, although BUN was decreased (Fig. 4a–c). NGAL mRNA expression after LPS administration was increased in the KO group compared with the WT group at 24 h, regardless of whether fluid resuscitation was performed (Fig. 4d). There was no remarkable histological change in the kidneys after LPS administration to WT or KO mice (Supplementary Figure S2).

RBF was decreased and was lowest at 12 h after LPS administration in both groups. It did not change significantly with fluid resuscitation in the WT group. However, RBF in the KO group was lower than that in the WT group, and it improved with fluid resuscitation at 24 h after LPS (Fig. 4e, Supplementary Figure S3a).

Gal-9 deficiency exacerbates LPS-induced hypothermia

Because LPS affects body temperature [22, 23], we examined the body temperature in both groups after the administration of LPS. The body temperature of mice began to decrease at around 6 h after LPS administration in both groups and reached the lowest temperature at 12 h. The KO group had markedly lower body temperatures than the WT group at 12 h after LPS administration, which persisted even after 24 h (Fig. 5).

Recovery of fluid responsiveness in Gal-9-KO mice by maintaining body temperature

The correlation between RBF and body temperature was examined because RBF and body temperature were markedly decreased at 12 h after LPS administration and they followed a similar time course. There was a positive correlation between body temperature and RBF at each time point examined after LPS administration (Supplementary Figure S3b–d).

Gal-9 had no effect on AKI in the early phase because there was no significant difference in the renal parameters in WT and Gal-9-KO groups within 6 h. However, Gal-9-KO

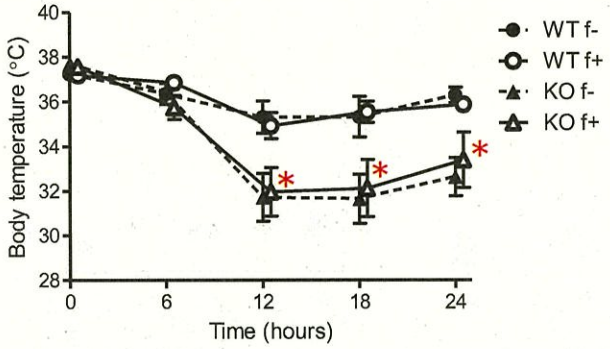

Fig. 5 Gal-9 deficiency induces marked hypothermia after LPS administration. Changes in body temperature over time after LPS administration ($n=7$–8). Data show the mean ± SEM ($p<0.05$). *$p<0.05$ versus WT groups. *Gal-9* galectin-9, *LPS* lipopolysaccharide, *WT* C57BL/6 N mice, *KO* galectin-9 knockout mice, *f*− without fluid resuscitation, *f*+ with fluid resuscitation

mice had significantly lower body temperature and RBF thereafter. Thus, we tested whether maintaining body temperature after LPS administration would improve AKI. When the ambient temperature was maintained at 30 °C, the body temperature of Gal-9 KO mice was similar to that of WT mice (Fig. 6a), and the response to fluid resuscitation was restored in Gal-9 KO mice after LPS administration (Fig. 6b).

Adenosine and its phosphates, such as ATP, induce hypothermia in the thermoregulatory center. Thus, we investigated whether Gal-9 was involved in this pathway. ATP-induced hypothermia in Gal-9-KO mice within 30 min after ATP administration, but there was no significant difference compared with control mice (Supplementary Figure S4).

Discussion

We investigated whether Gal-9 has a protective role in the pathogenesis of endotoxemic AKI. We found early systemic and local Gal-9 levels were increased after LPS administration. Gal-9-KO mice showed poor fluid responsiveness and had a significantly lower body temperature after LPS administration, although there was no significant difference in TNF-α levels or vascular permeability compared with controls. In addition, preventing the decrease in body temperature in Gal-9-KO mice after LPS administration improved their fluid sensitivity. Taken together, these data indicate that Gal-9 deficiency exacerbate LPS-induced hypothermia and renal injury.

In sepsis models, especially those involving LPS, plasma TNF-α levels increase early and then are reduced rapidly; however, the systemic response continued even when the cytokine storm ended at an early time point [24]. TNF-α acts on endothelial cells, which function as a barrier between tissues, to increase vascular permeability and it is also a mediator of LPS-induced AKI [25, 26]. Previous studies showed that plasma TNF-α was suppressed or unchanged by Gal-9 in sepsis models [9, 10]. Although we found that LPS increased plasma TNF-α levels and vascular permeability, there was no statistically significant difference between the two groups, suggesting Gal-9 may not be involved in the early cytokine storm induced by TNF-α.

Although fever is generally observed in patients with sepsis, it was reported that patients with hypothermia often have a worse general condition and AKI than those without hypothermia [5]. Mice experienced a peak decrease in body temperature at 8–11 h after LPS administration [23], and there was a correlation between body temperature and

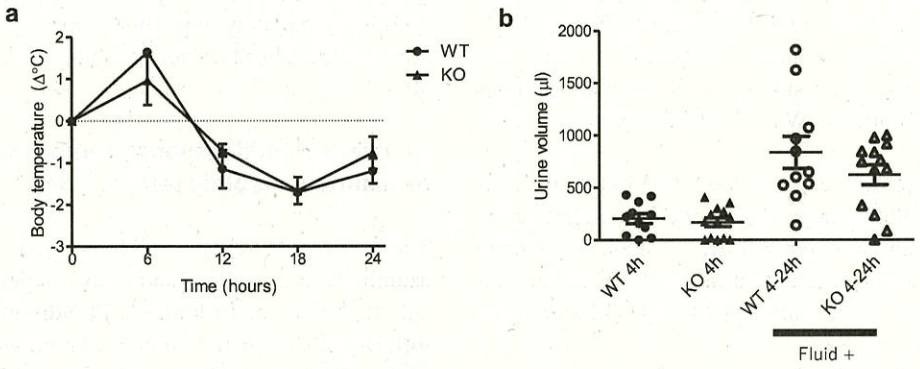

Fig. 6 Recovery of fluid responsiveness in Gal-9-KO mice by maintaining body temperature after LPS administration. **a** Body temperature between WT and KO groups by setting the ambient temperature to 30 °C. There was no significant difference in body temperature between the two groups. **b** Urine volume between WT and KO groups with fluid resuscitation after LPS administration when setting the ambient temperature to 30 °C ($n=11$–13). Urine volume between 4 and 24 h was increased by fluid resuscitation in both groups when setting the ambient temperature to 30 °C. Data show the mean ± SEM ($p<0.05$). *Gal-9* galectin-9, *LPS* lipopolysaccharide, *WT* C57BL/6 N mice, *KO* knockout

RBF; the lower the body temperature, the lower the RBF [23]. In addition, LPS decreased urine volume and GFR, whereas fluid infusion ameliorated the reduction in RBF, GFR, and urine volume [21, 27]. However, Gal-9 deficiency had no effect on urine volume with fluid resuscitation after LPS administration, although RBF was improved. LPS-induced filtrate leakage from the tubules to the interstitium is also involved in AKI [13]. Thus, increased RBF and a potential increase in GFR might not result in increased urine volume in Gal-9-KO mice. Although Gal-9 was increased in the kidneys, renal Gal-9 is unlikely to directly contribute to the decrease in RBF and it is unclear whether it contributes to AKI pathophysiology in the current study.

We did not determine how Gal-9 deficiency exacerbated the LPS-induced hypothermia. Abnormal body temperature occurs during sepsis, and body temperature is controlled by the hypothalamic thermoregulatory center. Regarding the mechanism of fever, inflammatory cytokines (TNF-α, IL-6) produced during sepsis act on the hypothalamus to induce fever via prostaglandin E2 [22]. However, ATP induces hypothermia after its conversion to adenosine diphosphate, adenosine monophosphate, and adenosine, which act on adenosine receptors in the hypothalamus [14, 15]. Furthermore, the extracellular concentration of ATP and that in the blood was elevated in sepsis models [28, 29]. However, the present results demonstrated no difference in ATP-induced hypothermia in the Gal-9-KO groups compared with controls. Therefore, Gal-9 may not be involved in the adenosine receptor-mediated thermoregulation mechanism in response to LPS-induced hypothermia but may be involved in other pathways via the thermoregulatory center.

There are several methods (direct and indirect) to evaluate RBF in mice [30–32], including the use of an ultrasound device [17–19]; however, their results are generally difficult to evaluate. In the present study, the data of RBF at 0 h were comparable between all groups; therefore, we could evaluate each group relative to each other.

We did not measure the systemic blood pressure in the present study and, therefore, cannot eliminate the possibility that Gal-9 contributes to it. LPS at the dosage used in this study decreased the blood pressure at about 10 h [21], which was the time point where RBF was reduced. However, we did not obtain data that could be used to investigate the time course association between blood pressure, RBF, and body temperature.

Conclusion

We demonstrated that Gal-9 has an important role in LPS-induced hypothermia and AKI.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10157-021-02152-2.

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Author contributions KO contributed to the study conception and design. Material preparation, data collection, and analysis were performed by KO and then HS, KH, and MH developed the transgenic mouse line. The first draft of the manuscript was written by KO and then TS and HF commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors have declared that no conflict of interest exists.

Ethical approval This study was approved by the Institutional Animal Care and Use Committee of Kagawa University (#20616) and followed standard guidelines for the humane care and use of animals in scientific research.

References

1. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. J Am Med Assoc. 2005;294:813–8.
2. Coca SG, Yusuf B, Shlipak MG, Garg AX, Parikh CR. Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. Am J Kidney Dis. 2009;53:961–73.
3. Doi K. Role of kidney injury in sepsis. J Intensive Care. 2016;4:17.
4. Nakano D. Septic acute kidney injury: a review of basic research. Clin Exp Nephrol. 2020;24:1091–102.
5. Wiewel MA, Harmon MB, van Vught LA, Scicluna BP, Hoogendijk AJ, Horn J, et al. Risk factors, host response and outcome of hypothermic sepsis. Crit Care. 2016;20:1–9.
6. Hirashima M, Niki T, Masaki T. Galectin-9 changes its function to maintain homeostasis. Trends Glycosci Glycotechnol. 2018;30:SE09-18.
7. Moritoki M, Kadowaki T, Niki T, Nakano D, Soma G, Mori H, et al. Galectin-9 ameliorates clinical severity of MRL/lpr lupus-prone mice by inducing plasma cell apoptosis independently of Tim-3. PLoS ONE. 2013;8:e60807.
8. Nio-Kobayashi J. Histological mapping and subtype-specific functions of galectins in health and disease. Trends Glycosci Glycotechnol. 2018;30:SE89-96.
9. Tsuboi Y, Abe H, Nakagawa R, Oomizu S, Watanabe K, Nishi N, et al. Galectin-9 protects mice from the Shwartzman reaction by attracting prostaglandin E2-producing polymorphonuclear leukocytes. Clin Immunol. 2007;124:221–33.

10. Kadowaki T, Morishita A, Niki T, Hara J, Sato M, Tani J, et al. Galectin-9 prolongs the survival of septic mice by expanding tim-3-expressing natural killer T cells and PDCA-1+ CD11c+ macrophages. Crit Care. 2013;17:1–11.
11. Kojima K, Arikawa T, Saita N, Goto E, Tsumura S, Tanaka R, et al. Galectin-9 attenuates acute lung injury by expanding CD14-plasmacytoid dendritic cell-like macrophages. Am J Respir Crit Care Med. 2012;184:328–39.
12. Naito Y, Hino K, Bono H, Ui-Tei K. CRISPRdirect: software for designing CRISPR/Cas guide RNA with reduced off-target sites. Bioinformatics. 2015;31:1120.
13. Nakano D, Kitada K, Wan N, Zhang Y, Wiig H, Wararat K, et al. Lipopolysaccharide induces filtrate leakage from renal tubular lumina into the interstitial space via a proximal tubular Toll-like receptor 4–dependent pathway and limits sensitivity to fluid therapy in mice. Kidney Int. 2020;97:904–12.
14. Swoap SJ, Rathvon M, Gutilla M. AMP does not induce torpor. Am J Physiol. 2007;293:468–73.
15. Carlin JL, Jain S, Duroux R, Suresh RR, Xiao C, Auchampach JA, et al. Activation of adenosine A2A or A2B receptors causes hypothermia in mice. Neuropharmacology. 2018;139:268.
16. Kitamura H, Nakano D, Sawanobori Y, Asaga T, Yokoi H, Yanagita M, et al. Guanylyl Cyclase A in both renal proximal tubular and vascular endothelial cells protects the kidney against acute injury in rodent experimental endotoxemia models. Anesthesiology. 2018;129:296–310.
17. Leisman DE, Fernandes TD, Bijol V, Abraham MN, Lehman JR, Taylor MD, et al. Impaired angiotensin II type 1 receptor signaling contributes to sepsis-induced acute kidney injury. Kidney Int. 2021;99:148–60.
18. Wu J, Agbor LN, Fang S, Mukohda M, Nair AR, Nakagawa P, et al. Failure to vasodilate in response to salt loading blunts renal blood flow and causes salt-sensitive hypertension. Cardiovasc Res. 2021;117:308.
19. Xu H, Ma Z, Lu S, Li R, Lyu L, Ding L, et al. Renal resistive index as a novel indicator for renal complications in high-fat diet-fed mice. Kidney Blood Press Res. 2017;42:1128–40.
20. Hong Y-H, Chao W-W, Chen M-L, Lin B-F. Ethyl acetate extracts of alfalfa (Medicago sativa L.) sprouts inhibit lipopolysaccharide-induced inflammation in vitro and in vivo. J Biomed Sci. 2009;16:64.
21. Nakano D, Doi K, Kitamura H, Kuwabara T, Mori K, Mukoyama M, et al. Reduction of tubular flow rate as a mechanism of oliguria in the early phase of endotoxemia revealed by intravital imaging. J Am Soc Nephrol. 2015;26:3035–44.
22. Billeter AT, Hellmann J, Roberts H, Druen D, Gardner SA, Sarojini H, et al. MicroRNA-155 potentiates the inflammatory response in hypothermia by suppressing IL-10 production. FASEB J. 2014;28:5322.
23. Broman LM, Carlström M, Källskog Ö, Wolgast M. Effect of nitric oxide on renal autoregulation during hypothermia in the rat. Pflugers Arch. 2017;469:669.
24. van Deventer S, Buller H, ten Cate J, Aarden L, Hack C, Sturk A. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood. 1990;76:2520–6.
25. Xu C, Wu X, Hack BK, Bao L, Cunningham PN. TNF causes changes in glomerular endothelial permeability and morphology through a Rho and myosin light chain kinase-dependent mechanism. Physiol Rep. 2015;3:e12636.
26. Cunningham PN, Dyanov HM, Park P, Wang J, Newell KA, Quigg RJ. Acute renal failure in endotoxemia is caused by TNF acting directly on TNF receptor-1 in kidney. J Immunol. 2002;168:5817–23.
27. Aksu U, Bezemer R, Demirci C, Ince C. Acute effects of balanced versus unbalanced colloid resuscitation on renal macrocirculatory and microcirculatory perfusion during endotoxemic shock. Shock. 2012;37:205–9.
28. Sakaki H, Tsukimoto M, Harada H, Moriyama Y, Kojima S. Autocrine regulation of macrophage activation via exocytosis of ATP and activation of P2Y11 receptor. PLoS ONE. 2013;8:59778.
29. Dosch M, Zindel J, Jebbawi F, Melin N, Sanchez-Taltavull D, Stroka D, et al. Connexin-43-dependent ATP release mediates macrophage activation during sepsis. Elife. 2019;8:e42670.
30. Boffa J-J, Just A, Coffman TM, Arendshorst WJ. Thromboxane receptor mediates renal vasoconstriction and contributes to acute renal failure in endotoxemic mice. J Am Soc Nephrol. 2004;15:2358–65.
31. Kirkby NS, Sampaio W, Etelvino G, Alves DT, Anders KL, Temponi R, et al. Cyclooxygenase-2 selectively controls renal blood flow through a novel PPARβ/δ-dependent vasodilator pathway. Hypertension. 2018;71:297–305.
32. Hunter RW, Craigie E, Homer NZM, Mullins JJ, Bailey MA. Acute inhibition of NCC does not activate distal electrogenic Na+ reabsorption or kaliuresis. Am J Physiol - Ren Physiol. 2014;306:F457.