MAJOR ARTICLE

RasGRP Exacerbates Lipopolysaccharide-Induced Acute Kidney Injury Through Regulation of ERK Activation

Wen Tang, Lu Wang, Yan Liu, and Dong Xiao

Department of Critical Care Medicine, People's Hospital of Xinjiang Uygur Autonomous Region, Tianshan District, Urumqi, Xinjiang Uygur Autonomous Region, China

Background. Excessive inflammatory activities are reported to be the primary cause of sepsis-induced acute kidney injury (AKI). Ras guanyl nucleotide-releasing protein (RasGRP) could prevent inflammatory response. However, its role in the regulation of inflammatory response in sepsis-associated AKI remains unclear.

Methods. Wild-type or RasGRP1-deficient mice were treated with lipopolysaccharide intraperitoneally in combination with D-galactosamine to establish a mouse model of sepsis-associated AKI. Serum inflammatory cytokines were measured using enzyme-linked immunosorbent assay. The messenger RNA (mRNA) levels of interleukin 6, tumor necrosis factor, nitric oxide synthase 2, and interleukin 1β were measured using quantitative reverse-transcription polymerase chain reaction. The morphological change in kidney tubule was determined by hematoxylin-and-eosin staining. The protein levels of RasGRP, extracellular signal-regulated kinases 1 and 2 (ERK1/2), and c-Jun N-terminal kinase (JNK) were determined using Western blot.

Results. RasGRP1 mRNA and protein levels were significantly increased in patients with sepsis-related AKI compared to those in healthy subjects. RasGRP knockout markedly reduced inflammatory cytokines induced by AKI in sepsis when compared with wild-type mice. Additionally, RasGRP deficiency inhibited the phosphorylation of ERK1/2 without altering JNK expression. In conclusion, we demonstrate that RasGRP1 plays a pivotal role in sepsis-associated AKI. Downregulation of RasGRP1 could significantly inhibit inflammatory response by inhibiting the activation of ERK1/2 and mitogen-activated protein kinase pathway, thereby reducing AKI induced by sepsis.

Conclusions. Our data suggest that RasGRP exacerbates lipopolysaccharide-induced acute kidney injury through regulating ERK activation, which reveals a potential therapeutic target for the treatment of sepsis-induced AKI.

Keywords. acute kidney injury; ERK/MAPK pathway; inflammation; RasGRP; sepsis.

Sepsis is the most common cause of acute kidney injury (AKI) and is associated with increased risks of poor prognosis [1–4]. Prolonged kidney dysfunction and limited functional renal reserve may result in impairment of ability of the kidney to repair [5]. In clinical, sepsis AKI diagnosis is mainly based on specific conditions. Azotemia and oliguria are still the key criteria for diagnosis [6]. However, AKI is a major factor of mortality in patients with sepsis [1]. The pathogenesis of septic AKI is still lacking, and development of critical care medicine is urgently needed. It has been shown that the excessive release of inflammatory mediators and maladaptive responses to inflammation are considered the primary cause of sepsis-related AKI [7].

Therefore, inhibiting inflammation could serve as an effective strategy to improve sepsis-related AKI. Systematic study of the signaling pathways involved in sepsis-induced kidney damage allows us to gain insights into the pathophysiology of sepsis-related AKI.

The mitogen-activated protein kinase (MAPK) is responsible for transducing extracellular signals into the cell. MAPK pathway transmits cell signaling through the form of a 3-level kinase cascade, thereby regulating cell proliferation and differentiation, apoptosis, inflammatory response and vascular development, and other biological functions [8]. The RAS-RAF-MAPK/ERK kinase (MEK)–extracellular signal-regulated kinase (ERK), also known as the MAPK/ERK pathway, is one of the most characterized MAPK pathways. Mechanistically, the RAS guanosine triphosphatase (RAS GTPase) is a downstream effector of receptor tyrosine kinase (RTK) [9]. The activated RAS binds to the N-terminal domain of RAF kinase to activate RAF, then the activated RAF can further bind to and activate the downstream MEK [9]. Subsequently, ERK is activated by MEK. As a result, the activated ERK translocates into the nucleus and causes a series of physiological and biochemical reactions. Ras is a GTP-binding protein and has been an important target for cancer research so far [10, 11]. Studies have

shown that the inhibition of MEK significantly mitigates AKI [12]. However, little is known on the anti-inflammatory role of RAS in the setting of AKI, particularly sepsis. Thus, this study aims to use Ras guanyl nucleotide-releasing protein (RasGRP) knockout mice to explore its function in reducing inflammatory response in sepsis-induced AKI.

MATERIALS AND METHODS

Patient Consent Statement
Written informed consent was obtained from each participant. The design of the work was approved by the People's Hospital of Xinjiang Uygur Autonomous Region.

Sepsis Animal Model
Female C57BL/6 wild-type and RasGRP C57BL/6 mice at 8 weeks old were purchased from Cyagen Biosciences (Suzhou, China). Mice were housed in a specific pathogen–free facility, and all animal experiments were carried out according to the protocol approved by the Institutional Animal Care and Use Committee in the People's Hospital of Xinjiang Uygur Autonomous Region.

The mice were injected intraperitoneally with lipopolysaccharide (LPS; 0.1 µg per mouse) plus D-galactosamine (0.5 mg/g body weight). Animals were monitored every hour for 24 hours. Blood was obtained from challenged mice at various times, and the serum concentrations of tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) were measured by enzyme-linked immunosorbent assay (ELISA; R&D Biosystems, Minneapolis, Minnesota).

Immunoblot Assay
The samples were homogenized in radioimmunoprecipitation buffer (10 mM Tris-HCl, pH 7.0, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% sodium dodecyl sulfate). Followingly, the supernatants were saved after centrifuge, and the protein concentrations were measured using Bradford BCA (Bio-Rad, Hercules, California). Antibodies targeting ERK (K-23, 1:5000), pERK (E-4, 1:3000), and JNK1 (C-17, 1:1000) were from Santa Cruz Biotechnology (Dallas, Texas). Antibodies targeting pJNK (Thr180/Tyr185, 1:1000) were purchased from Cell Signaling Technology (Danvers, Massachusetts). Antibodies targeting RasGRP (ab96293, 1:1000) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab9485) were purchased from Abcam (Shanghai, China).

ELISA and Quantitative Reverse-Transcription Polymerase Chain Reaction
Supernatant cell cultures were collected and analyzed using ELISA (eBioScience, San Diego, California). Total RNA was extracted using TRIzol reagent (Qiagen, Valencia, California). Complementary DNA synthesis was performed using RNase H-reverse transcriptase (Invitrogen, Waltham, Massachusetts) and oligo (dT) primers. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed with iQTM SYBR Green Supermix (Bio-Rad, Hercules, California) using the iCycler Sequence Detection System (Bio-Rad). The expression of target genes was quantified by a standard curve method and normalized to *Actb*.

The primers were as follows: IL-6: 5′-CTGATGCTGGTGACAACCAC-3′ (forward), 5′-CAGACTTGCCATTGCACAAC-3′ (reverse); TNF: 5′-CATCTTCTCAAAATTCGAGTGACAA-3′ (forward), 5′-CCAGCTGCTCCTCCACTTG-3′ (reverse); IL-1β: 5′-TGGACCTTCCAGGATGAGGACA-3′ (forward), 5′-GTTCATCTCGGAGCCTGTAGTG-3′ (reverse); Nos2: 5′-GAGACAGGGAAGTCTGAAGCAC-3′ (forward), 5′-CCAGCAGTAGTTGCTCCTCTTC-3′ (reverse); and RasGRP1: 5′-CTTCAACACGCTGATGGCTGTG-3′ (forward), 5′-GGACAGCAGTTCAGTCATCTCG-3′ (reverse).

Statistical Analysis
Statistical analysis was performed using Prism software (GraphPad Prism version 6.01). Two-tailed unpaired Student t tests were performed, and data are presented as mean ± standard error of the mean. One-way analysis of variance, where applicable, was performed to determine whether an overall statistically significant change existed before Student t test to analyze the difference between any 2 groups. A P value < .05 was considered statistically significant.

RESULTS

The Protein and Messenger RNA Level of RasGRP Is Increased in Patients With Sepsis
To evaluate whether RasGRP is involved in the inflammatory response caused by sepsis, we tested the messenger RNA (mRNA) and protein levels of RasGRP in the peripheral blood mononuclear cells (PBMCs) of healthy people and patients with sepsis using qRT-PCR and Western blot. PBMCs were obtained from 37 healthy people and 37 patients with sepsis. We found that in patients with sepsis, the expression level of RasGRP was significantly increased (Figure 1A and 1B). Consistently, we found that after LPS stimulation, the level of RasGRP continued to increase over time in mouse macrophages (Figure 1C and 1D). These results suggest that RasGRP plays a key role in response to inflammation.

RasGRP Is Required for the Induction of Various Proinflammatory Cytokines in Bone Marrow–Derived Macrophages Induced by Toll-Like Receptor Stimulation
To further explore the function of RasGRP in sepsis-induced inflammation, we measured cytokines, such as IL-6, TNF-α, Nos2, and IL-1β, using a RasGRP knockout mice model of sepsis. The results showed that bone marrow–derived macrophages from RasGRP knockout mice showed lower mRNA expression of
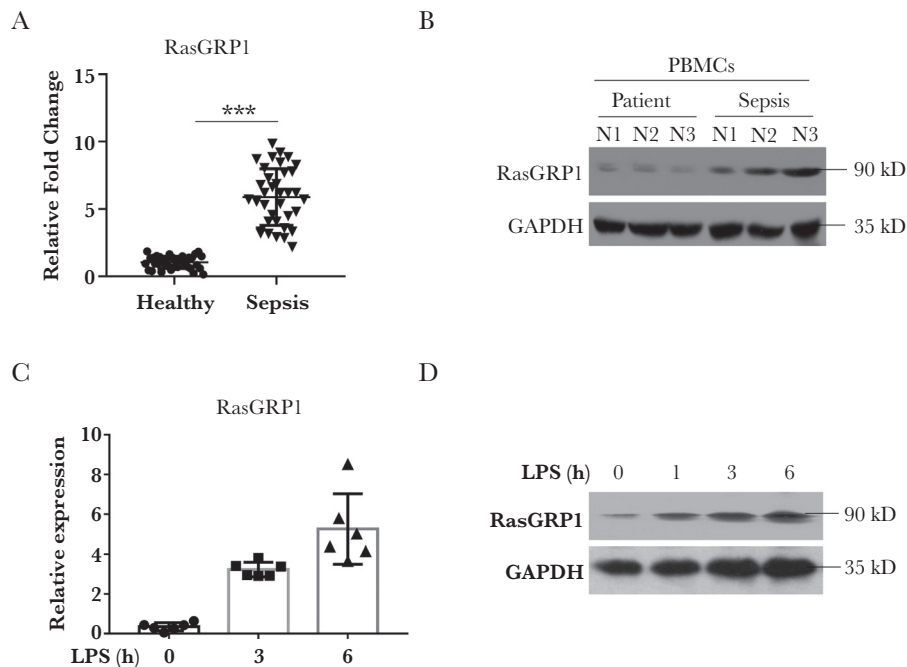
Figure 1. The protein and messenger RNA (mRNA) level of Ras guanyl nucleotide-releasing protein (RasGRP) is increased in the patients with sepsis. *A*, Quantitative polymerase chain reaction (qPCR) analysis of RasGRP in peripheral blood mononuclear cells from healthy donors and patients with sepsis. Actin was used as loading control and for the relative normalization (n = 37). *B*, The protein level of RasGRP was evaluated by immunoblot assay (IB). *C*, The mRNA level of RasGRP in bone marrow–derived macrophages (BMDMs) stimulated with lipopolysaccharide (LPS) (100 ng/mL) was detected by qPCR for the indicated time points (n = 6). *D*, IB analysis of RasGRP in whole-cell lysates of LPS-stimulated BMDMs. Data are shown as the mean ± standard error of the mean based on 3 independent experiments. Statistical analyses represent variations in experimental biological replicates. Two-tailed Student *t* tests were performed. ***$P < .001$. Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LPS, lipopolysaccharide; PBMCs, peripheral blood mononuclear cells; RasGRP, Ras guanyl nucleotide-releasing protein.

all inflammatory cytokines mentioned above under LPS stimulation (Figure 2A). Similarly, significantly elevated levels of cytokines were detected after cytosine-phosphate-guanosine (CpG) stimuli, which were abrogated in RasGRP-deficient mice (Figure 2B). Furthermore, we found that the protein levels of IL-6 and TNF-α were significantly lower in RasGRP-deficient compared to the control mice after LPS stimulation (Figure 2C).

RasGRP Deficiency Impaired LPS-Induced Kidney Function and Pathological Changes in Mice

To verify the regulation effect of RasGRP on inflammation and sepsis-induced kidney injury in vivo, LPS (0.1 µg per mouse) plus D-galactosamine (0.5 mg/g body weight) were used to induce a mouse model of acute sepsis. Hematoxylin-and-eosin staining showed a significant kidney injury with LPS treatment in wild-type mice, which was notably reduced in RasGRP-deficient mice (Figure 3A). Serum creatinine (SCr) and blood urea nitrogen (BUN) are widely used indictors of AKI. As shown in Figure 3B, SCr and BUN were significantly increased at 12 hours after LPS treatment, and this elevation continued through 24 hours in the wild-type mice. Remarkably, SCr and BUN levels were significantly reduced in RasGRP-deficient mice after LPS treatment when compared with wild-type mice (Figure 3B). Furthermore, we evaluated the effects of RasGRP on serum levels of inflammatory cytokines after LPS treatment

using ELISA. The results showed that RasGRP knockout mice significantly inhibited LPS-induced inflammatory response. Correspondingly, the levels of inflammatory cytokines IL-6, TNF-α, and IL-1β in the serum were markedly reduced in RasGRP knockout mice compared to control animals (Figure 3C). The above results suggest that RasGRP may serve as a potential new drug target for the treatment of sepsis and renal injury.

RasGRP Positively Regulated the ERK Pathway

To determine the molecular mechanism of RasGRP regulating inflammation, we analyzed via the STRING Interaction Network and found that RasGRP molecules can bind to K-Ras protein and Ras-related protein R-Ras (RRAS) (Figure 4A). Therefore, we hypothesize that RasGRP also functions via regulation of the phosphorylation and activity of ERKs. We further assessed the mechanism of RasGRP. Western blot detection showed that RasGRP deletion significantly inhibited the activation of the ERK1/2 signaling pathway downstream of Toll-like receptor 4 (TLR4); however, the phosphorylation of other MAPK branches such as c-Jun N-terminal kinase (JNK) was not affected (Figure 4B). We used ERK inhibitors to verify our findings, and the results showed that ERK inhibitors not only inhibit the expression of proinflammatory cytokines, but also remove the phenotype caused by RasGRP deletion (Figure 4C).
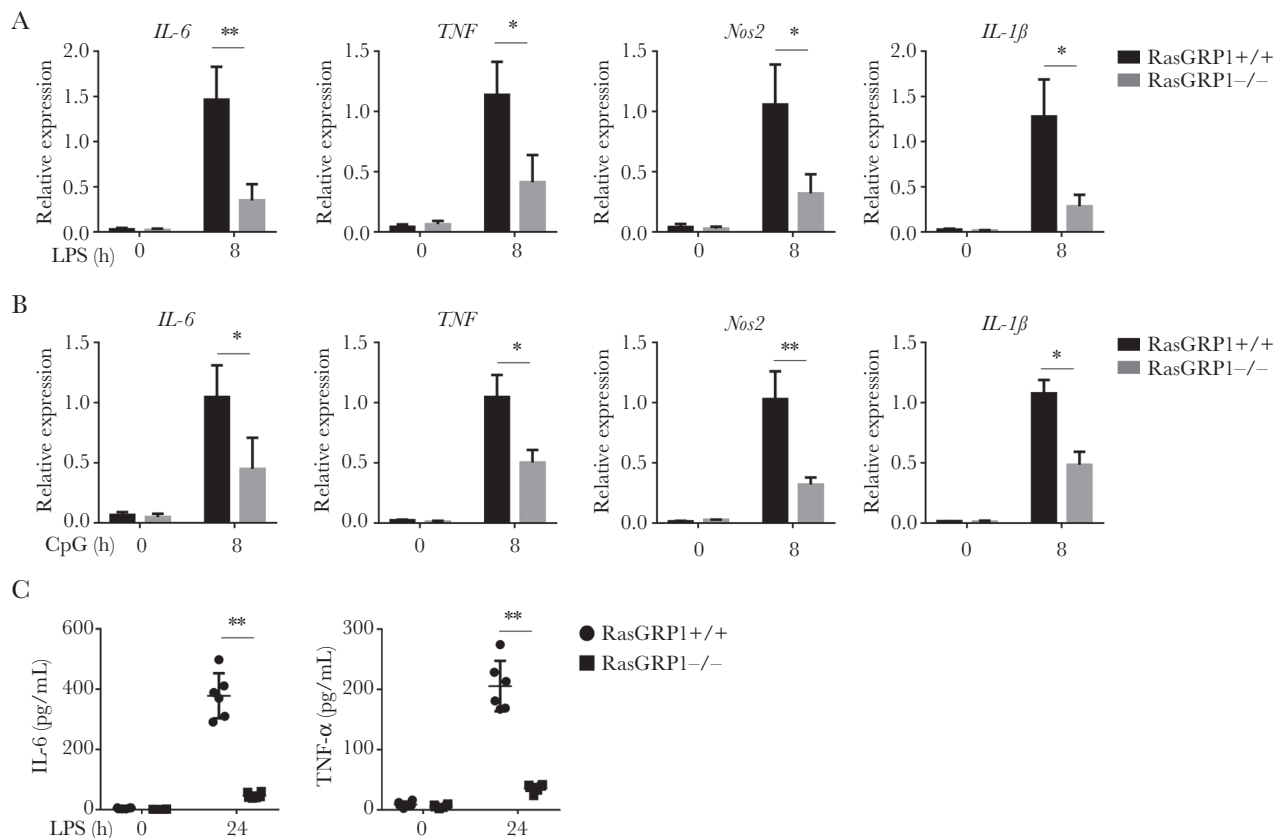
Figure 2. Ras guanyl nucleotide-releasing protein (RasGRP) is required for the induction of various proinflammatory cytokines in bone marrow–derived macrophages (BMDMs) induced by Toll-like receptor stimulation. *A* and *B*, Expression of lipopolysaccharide (LPS; 100 ng/mL)– or CpG (5 nM)–induced cytokines of wild-type and RasGRP knockout BMDMs was measured by quantitative reverse-transcription polymerase chain reaction (n = 6). *C*, Enzyme-linked immunosorbent assay of LPS-induced cytokines in the supernatants of wild-type and RasGRP knockout BMDMs for 24 hours (n = 6). All data are presented as fold relative to the *Actb* messenger RNA level. Data are presented as mean ± standard error of the mean and representative of at least 3 independent experiments. Statistical analyses represent variations in experimental biological replicates. Two-tailed Student *t* tests were performed. ∗*P* < .05; ∗∗*P* < .01. Abbreviations: CpG, cytosine-phosphate-guanosine; IL-1β, interleukin 1β; IL-6, interleukin 6; LPS, lipopolysaccharide; Nos2, nitric oxide synthase 2; RasGRP, Ras guanyl nucleotide-releasing protein; TNF-α, tumor necrosis factor alpha.

DISCUSSION

Sepsis-associated AKI is a significant cause of prolonged hospitalization and increases the risk of mortality [6]. There is an urgent need to develop an effective strategy to reduce sepsis-associated AKI. In this study, we assessed the alteration of RasGRP in healthy subjects and patients with sepsis-associated AKI. Furthermore, to confirm the protective effects of RasGRP deficiency in AKI, we used a RasGRP knockout mice model of LPS-associated AKI to determine the involvement of the RAS-ERK pathway in reducing inflammatory response during AKI.

RasGRP is mainly expressed in blood cells, contributing to facilitate GTP hydrolysis and activate Ras [13–16]. In this study, the mRNA and protein levels of RasGRP1 were significantly elevated in PBMCs obtained from patients with sepsis compared with healthy subjects. It was found that RasGRP1 binds to the orphan nuclear receptor transcription factor Nurr1 and regulates the inflammatory response [17]. Moreover, upregulated RasGRP1 has been associated with less inflammation in patients with cancer [18, 19]. However, the effect of RasGPR1 on the

inflammatory response in kidney remains unknown. Therefore, we examined the serum RasGRP1 level in a mice model of inflammation induced by LPS; we found that the RasGRP1 level was increased after 1 hour of LPS stimulation and continuously increased through 6 hours of LPS treatment by utilizing a mouse model of LPS-induced AKI. These findings indicate that RasGRP1 is closely related to the inflammatory response.

Inflammatory response is a primary factor causing complications during sepsis-associated AKI [20–24]. It was found that RasGRP1 could prevent an inflammatory response. For example, it was shown that suppressing RasGRP1 inhibited neuroinflammation [25]. Additionally, Baars et al showed that decreased RasGRP1 reduced active inflammatory disease [26]. However, the impact of RasGRP1 on sepsis-induced AKI is still unclear. To determine the role in regulating inflammatory response in sepsis-associated AKI, we measured mRNA and protein levels of inflammatory mediators including IL-6, TNF-α, Nos2, and IL-1β. We found that the protein levels of inflammatory cytokines were significantly increased in sepsis-associated AKI in wild-type mice but markedly inhibited in RasGRP1
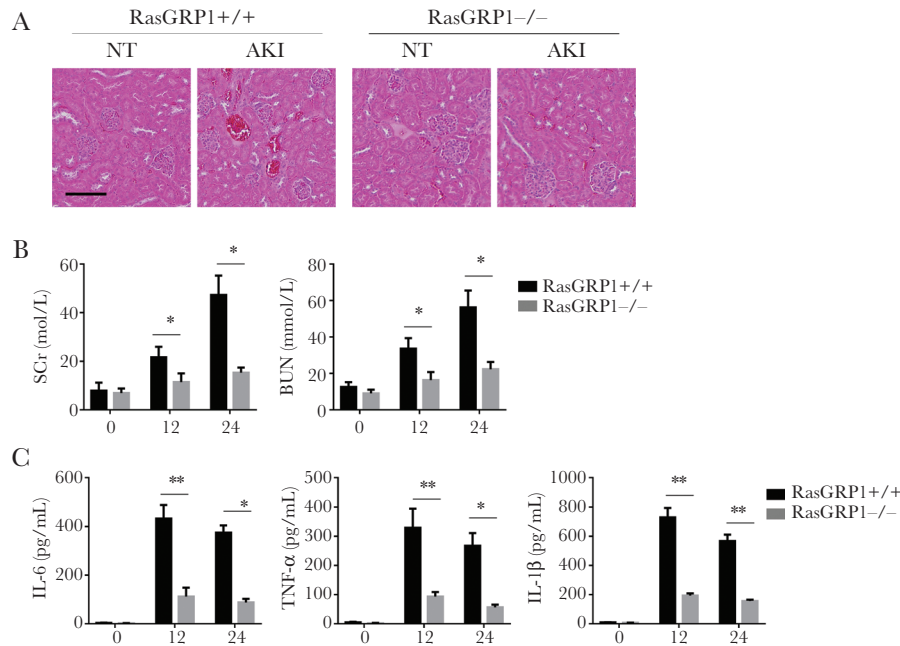
Figure 3. Ras guanyl nucleotide-releasing protein (RasGRP) deficiency impaired lipopolysaccharides (LPS)-induced kidney function and pathological changes in mice. *A*, Kidney tissue sections were subjected to hematoxylin-and-eosin staining and histopathological observation. After LPS treatment, the degree of kidney injury and pathological changes were observed in wild-type and RasGRP knockout female mice at 24 hours. Magnification: ×100; scale bar, 50 μm. *B*, Levels of serum creatinine and blood urea nitrogen in mice analyzed at different time points by the fully automatic biochemical analyzer (n = 8). *C*, Levels of interleukin 1β, interleukin 6, and tumor necrosis factor alpha were assessed through the corresponding enzyme-linked immunosorbent assay kits (n = 8). Data are presented as mean ± standard error of the mean and representative of at least 3 independent experiments. Statistical analyses represent variations in experimental biological replicates. Two-tailed Student t tests were performed. *$P < .05$; **$P < .01$. Abbreviations: AKI, acute kidney injury; BUN, blood urea nitrogen; IL-1β, interleukin 1β; IL-6, interleukin 6; NT, non-treatment; RasGRP, Ras guanyl nucleotide-releasing protein; SCr, serum creatinine; TNF-α, tumor necrosis factor alpha.

knockout mice. In line with the protein data, the mRNA levels of inflammatory cytokines appeared to show similar trends. These results suggest that downregulation of RasGRP1 could effectively reduce inflammation induced by sepsis.

To confirm the protective role of RasGRP1 in sepsis-induced AKI, we used a mouse model of sepsis-associated AKI by treatment with LPS in combination with D-galactosamine. First, the kidney damage was confirmed by histological staining. Morphological changes in glomerular and kidney tubule demonstrated a marked injury in wild-type mice, and less injury was observed in RasGRP1 knockout mice. Consistently, SCr and BUN, commonly used clinical biomarkers of kidney function, showed that renal injury was significantly augmented in wild-type mice. In contrast, RasGRP effectively reduced renal injury to the level of that of controls. These data imply that RasGRP1 plays a crucial role, in particular, in sepsis-associated AKI. Thus, we further investigated the inhibitory role of suppressing RasGRP1 in sepsis-induced AKI. The serum IL-6, TNF-α, and IL-1β levels were significantly elevated in wild-type mice as expected. On the other hand, RasGRP1 knockout dramatically reduced inflammation, suggesting that decreasing RasGPR1 is closely related to anti-inflammation in the setting of sepsis-induced AKI.

RasGPR plays an essential role in regulating MAPK pathways in response to inflammation. To explore the mechanisms of RasGRP in sepsis-related AKI, we carried out STRING Interaction Network analysis. We found that RasGRP could bind to KRAS and RRAS proteins, thereby regulating ERK/MAPK pathway. Notably, Western blot data showed that RasGRP deficiency significantly inhibited the activation of TLR4 and the downstream mediator ERK1/2. Interestingly, no significant change was observed in JNK phosphorylation. In line with our findings, Molineros et al reported that abnormally expressed RasGRP1 is responsible for the phosphorylation of the ERK/MAPK pathway in systemic lupus erythematosus [27]. Ko et al further suggested that RasGRP regulates the ERK1/2/MAPK pathway through activating the H-Ras isoform of Ras [28]. Overall, these findings indicate that RasGRP1 deficiency inhibits the activation of the MAPK pathway by suppressing the phosphorylation of ERK1/2 in sepsis-associated AKI.

There are limitations to this study. First, the role of other isoforms of RasGRPs in sepsis-associated AKI have not been studied. The mechanisms of other isoforms of RasGRP need to be studied in future research. Second, whether RasGRP1 deficiency affects cell apoptosis and kidney function, such as glomerular filtration rate and urinary protein, will need to be included in future studies.

In conclusion, we demonstrated that RasGRP1 plays a pivotal role in sepsis-associated AKI. Downregulation of RasGRP1 could significantly inhibit inflammatory response by inhibiting
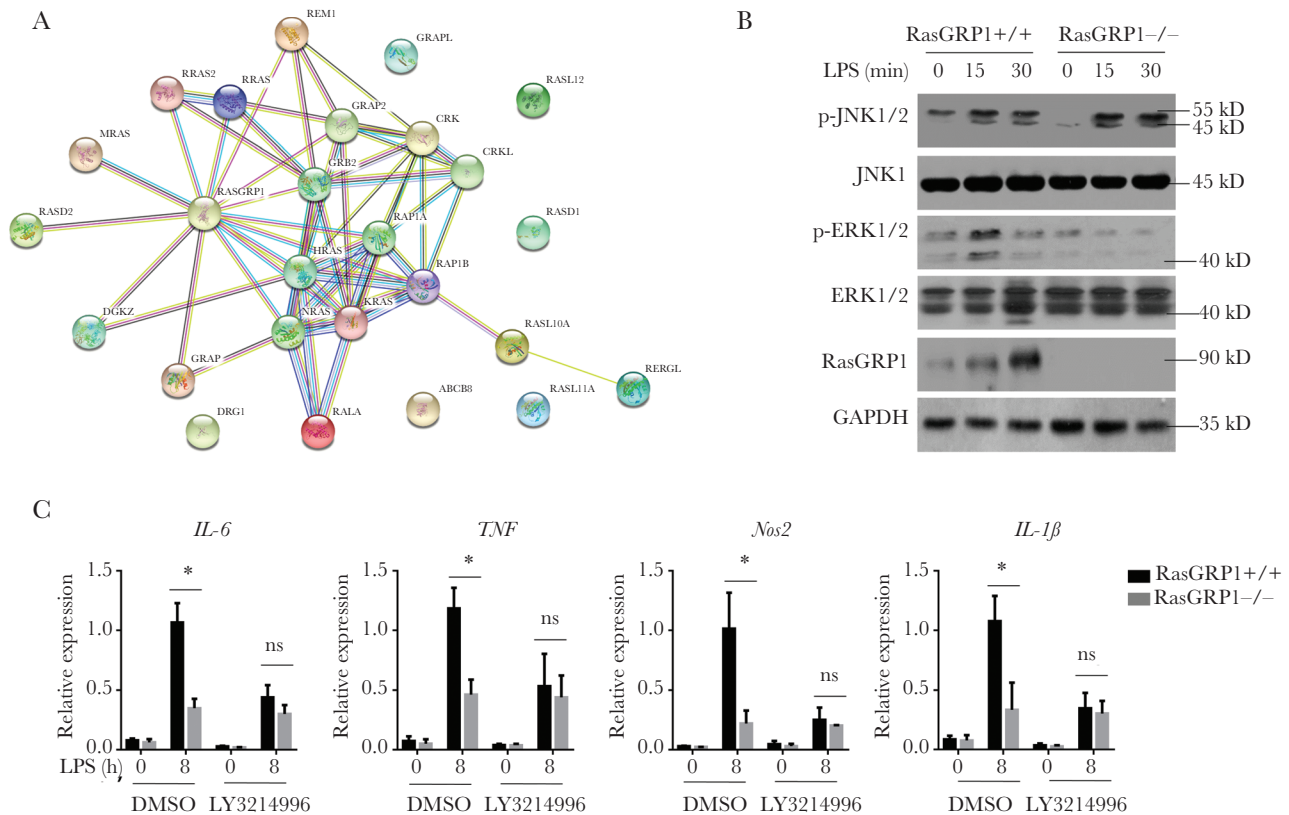
Figure 4. Ras guanyl nucleotide-releasing protein (RasGRP) positively regulates the ERK pathway. *A*, Interacted proteins of RasGRP were presented in the STRING Interaction Network. *B*, Wild-type and RasGRP knockout bone marrow–derived macrophages (BMDMs) were stimulated with lipopolysaccharide (LPS; 100 ng/mL) for indicated time points. Immunoblot analysis of the indicated phosphorylated (p-) and total indicated proteins in whole-cell lysates. *C*, Wild-type and RasGRP knockout BMDMs were stimulated with LPS (100 ng/mL) and ERK inhibitor LY3214996 (10 nM) for indicated time points. The expression of induced cytokines of wild-type and RasGRP knockout BMDMs were measured by quantitative reverse-transcription polymerase chain reaction (n = 3). All data are presented as fold relative to the *Actb* messenger RNA level. Data are presented as mean ± standard error of the mean and representative of at least 3 independent experiments. Statistical analyses represent variations in experimental biological replicates. Two-tailed Student *t* tests were performed. ∗$P < .05$. Abbreviations: DMSO, dimethyl sulfoxide; IL-1β, interleukin 1β; IL-6, interleukin 6; LPS, lipopolysaccharide; Nos2, nitric oxide synthase 2; ns, not significant; RasGRP, Ras guanyl nucleotide-releasing protein; TNF, tumor necrosis factor.

the activation of the ERK1/2 and MAPK pathways, thereby reducing AKI induced by sepsis. Our data reveal the potentiality of targeting RasGRP to develop a novel approach for treatment of sepsis-induced AKI.

Notes

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Gomez H, Kellum JA. Sepsis-induced acute kidney injury. Curr Opin Crit Care **2016**; 22:546–53.
2. Godin M, Murray P, Mehta RL. Clinical approach to the patient with AKI and sepsis. Semin Nephrol **2015**; 35:12–22.
3. Lentini P, de Cal M, Clementi A, D'Angelo A, Ronco C. Sepsis and AKI in ICU patients: the role of plasma biomarkers. Crit Care Res Pract **2012**; 2012:856401.
4. Prowle JR. Sepsis-associated AKI. Clin J Am Soc Nephrol **2018**; 13:339–42.
5. Sharma A, Mucino MJ, Ronco C. Renal functional reserve and renal recovery after acute kidney injury. Nephron Clin Pract **2014**; 127:94–100.
6. Zarjou A, Agarwal A. Sepsis and acute kidney injury. J Am Soc Nephrol **2011**; 22:999–1006.
7. Zarbock A, Gomez H, Kellum JA. Sepsis-induced acute kidney injury revisited: pathophysiology, prevention and future therapies. Curr Opin Crit Care **2014**; 20:588–95.
8. Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. Cell Res **2002**; 12:9–18.
9. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene **2007**; 26:3291–310.
10. Formica V, Roselli M. Targeted therapy in first line treatment of RAS wild type colorectal cancer. World J Gastroenterol **2015**; 21:2871–4.
11. Hegewisch-Becker S, Nopel-Dunnebacke S, Hinke A, et al. Impact of primary tumour location and RAS/BRAF mutational status in metastatic colorectal cancer treated with first-line regimens containing oxaliplatin and bevacizumab: prognostic factors from the AIO KRK0207 first-line and maintenance therapy trial. Eur J Cancer **2018**; 101:105–13.
12. Teuma C, Pelletier S, Amini-Adl M, et al. Adjunction of a MEK inhibitor to vemurafenib in the treatment of metastatic melanoma results in a 60% reduction of acute kidney injury. Cancer Chemother Pharmacol **2017**; 79:1043–9.
13. Stone JC. Regulation and function of the RasGRP family of Ras activators in blood cells. Genes Cancer **2011**; 2:320–34.
14. Calixto AR, Moreira C, Pabis A, et al. GTP hydrolysis without an active site base: a unifying mechanism for Ras and related GTPases. J Am Chem Soc **2019**; 141:10684–701.
15. Novelli ET, First JT, Webb LJ. Quantitative measurement of intrinsic GTP hydrolysis for carcinogenic glutamine 61 mutants in H-Ras. Biochemistry **2018**; 57:6356–66.
16. Topol IA, Cachau RE, Nemukhin AV, Grigorenko BL, Burt SK. Quantum chemical modeling of the GTP hydrolysis by the RAS-GAP protein complex. Biochim Biophys Acta **2004**; 1700:125–36.
17. Oh M, Kim SY, Gil JE, et al. Nurr1 performs its anti-inflammatory function by regulating RasGRP1 expression in neuro-inflammation. Sci Rep **2020**; 10:10755.

18. Ksionda O, Limnander A, Roose JP. RasGRP Ras guanine nucleotide exchange factors in cancer. Front Biol (Beijing) **2013**; 8:508–32.

19. Gbenedio OM, Bonnans C, Grun D, et al. RasGRP1 is a potential biomarker to stratify anti-EGFR therapy response in colorectal cancer. JCI Insight **2019**; 5:e127552.

20. Bellomo R, Kellum JA, Ronco C, et al. Acute kidney injury in sepsis. Intensive Care Med **2017**; 43:816–28.

21. Dirkes S. Sepsis and inflammation: impact on acute kidney injury. Nephrol Nurs J **2013**; 40:125–32; quiz 133.

22. Ferre S, Deng Y, Huen SC, et al. Renal tubular cell spliced X-box binding protein 1 (Xbp1s) has a unique role in sepsis-induced acute kidney injury and inflammation. Kidney Int **2019**; 96:1359–73.

23. Lu SJ, Xu JH, He ZF, Wu P, Ning C, Li HY. Innate immune molecule surfactant protein D attenuates sepsis-induced acute kidney injury through modulating apoptosis and NFkappaB-mediated inflammation. Int Wound J **2020**; 17:100–6.

24. Shen J, Liu L, Zhang F, Gu J, Pan G. LncRNA TapSAKI promotes inflammation injury in HK-2 cells and urine derived sepsis-induced kidney injury. J Pharm Pharmacol **2019**; 71:839–48.

25. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders HJ. Acute kidney injury. Nat Rev Dis Primers **2021**; 7:52.

26. Baars MJD, Douma T, Simeonov DR, et al. Dysregulated RASGRP1 expression through RUNX1 mediated transcription promotes autoimmunity. Eur J Immunol **2021**; 51:471–82.

27. Molineros JE, Singh B, Terao C, et al. Mechanistic characterization of RASGRP1 variants identifies an hnRNP-K-regulated transcriptional enhancer contributing to SLE susceptibility. Front Immunol **2019**; 10:1066.

28. Ko B, Joshi LM, Cooke LL, et al. Phorbol ester stimulation of RasGRP1 regulates the sodium-chloride cotransporter by a PKC-independent pathway. Proc Natl Acad Sci U S A **2007**; 104:20120–5.