Triggering Receptor Expressed on Myeloid Cells-2 (TREM2) Inhibits Steroidogenesis in Adrenocortical Cell by Macrophage-derived Exosomes in Lipopolysaccharide-induced Septic Shock

Hui Ye
Zhejiang University School of Medicine First Affiliated Hospital
Pinhao Li
Zhejiang University School of Medicine First Affiliated Hospital
Qian Zhai
Xi'an Jiaotong University Medical College First Affiliated Hospital
Ping Fang
Ningbo Medical Treatment Centre Li Huili Hospital
Shiyue Yang
Zhejiang University School of Medicine First Affiliated Hospital
Yaqi Sun
Zhejiang University School of Medicine First Affiliated Hospital
Shuijing Wu
Zhejiang University School of Medicine First Affiliated Hospital
Ruoqiong Huang
Zhejiang University School of Medicine Children's Hospital
Qixing Chen
Zhejiang University School of Medicine Children's Hospital
Xiangming Fang (xmfang@zju.edu.cn)
Department of Anesthesiology and Intensive Care, the First Hospital, School of Medicine, Zhejiang University https://orcid.org/0000-0002-2283-1863

Research

Keywords: Septic shock, TREM2, Macrophage, Adrenocortical Steroidogenesis, Exosome, Cell cross-talk

Posted Date: August 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-57909/v1

Version of Record: A version of this preprint was published at Molecular and Cellular Endocrinology on April 1st, 2021. See the published version at https://doi.org/10.1016/j.mce.2021.111178.

Abstract

Background: Endogenously produced glucocorticoids exhibit immunomodulating properties and are of pivotal importance for sepsis outcome. Uncontrolled activation of the immune-adrenal crosstalk increases the risk of sepsis-related death. Triggering receptor expressed on myeloid cells-2 (TREM2) is richly expressed on macrophages and has been demonstrated to improve outcome of sepsis by enhancing elimination of pathogens. However, the role and mode of action of macrophage TREM2 on adrenocortical steroidogenesis remains unclear in septic shock.

Methods: The acute septic shock model was established by intraperitoneally challenging wild-type (WT) and TREM2 knock-out (*Trem2*$^{-/-}$) mice with lipopolysaccharide (30 mg/kg). The mice were assessed for TREM2 expression and local inflammation in adrenal gland and synthesis of corticotropin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) *in vivo.* Bone marrow-derived macrophages or macrophage-derived exosomes were isolated from WT and *Trem2*$^{-/-}$ mice and co-cultured with adrenocortical cells. The expression of steroidogenic enzymes and corticosterone production were assessed.

Results: Genetic deficiency of TREM2 caused significantly higher corticosterone levels (326.6 ± 73.0 ng/ml in *Trem2*$^{-/-}$ mice *vs*. 151.1 ± 58.9 ng/ml in WT mice; $p < 0.001$) at the early stage of LPS-induced septic shock. While TREM2 deficiency neither increased CRH and ACTH, nor exacerbated the inflammation in adrenocortical tissue during septic shock. *Ex vivo* study revealed that *Trem2*$^{-/-}$ macrophages significantly promoted the expression of steroidogenic enzymes and increased production of corticosterone (27.73 ± 1.78 ng/ml in *Trem2*$^{-/-}$ mice *vs*. 22.96 ± 1.94 ng/ml in W T mice; $p < 0.01$). Furthermore, *Trem2*$^{-/-}$ macrophage-derived exosomes were able to mimic *Trem2*$^{-/-}$ macrophages in enhancing adrenocortical steroidogenesis.

Conclusions: At the early stage of lipopolysaccharide-induced septic shock, macrophage TREM2 inhibited the steroid synthesis and corticosterone production in adrenocortical cells, which may be partially associated with macrophage-derived exosomes.

Introduction

Sepsis is a life-threatening disease characterized by a dysregulated host response to infection causing organ dysfunction [1]. Annual prevalence of sepsis was estimated at 31.5 million and the annual number of deaths at nearly 11 million worldwide [2]. The high mortality rate of sepsis (15~33.5%) is increased up to 40~70% when further progressing to septic shock or suffering from multiple organ failure [3-5].

In the development of septic shock, microbes and toxins do not only activate the immune system but also initiate a stress response including a rapid activation of the hypothalamic-pituitary-adrenal (HPA) axis, which leads to production of adrenocorticotrophic hormone (ACTH) and amounts of glucocorticoids (corticosterone in rodents and cortisol in humans) releasing into circulation [6]. Glucocorticoids are

steroid hormones synthesized in the adrenal gland from cholesterol precursors [7]. The essential role of endogenous glucocorticoids in regulating homeostatic processes under basal and challenging conditions and protecting the host from detrimental consequences of an overactivated inflammatory immune response including sepsis, has been well established [8]. Genetic defects or surgical interventions in stress response and adrenal gland contributes to the increased mortality rates when suffered from sepsis [9, 10], indicating that an intact function of glucocorticoids production in adrenal gland is of pivotal importance for sepsis surviving.

Among many mechanisms potentially involved, activation of intra-adrenal cellular systems which are composed of adrenocortical and adrenomedullary cells, resident and recruited immune cells plays an important role in adrenocortical hormone production during septic shock conditions [11-13]. A balance among stress systems allows controlling infection while maintaining cardiovascular and metabolic homeostasis during sepsis, which if not sufficiently counteracted may exacerbate the outcome of critically ill patients [14]. It has been demonstrated that prolonged activation of the immune-adrenal crosstalk increases the risk of sepsis-related death [6].

Triggering receptor expressed on myeloid cells-2 (TREM2) is a novel phagocytic receptor of the immunoglobulin superfamily, mainly expressed by microglia in the central nervous system (CNS) and macrophages in the peripheral organs and tissues [15]. Studies confirmed that TREM2 was crucial to maintain microgliosis in response to pathological processes of Alzheimer's disease (AD) and other neurodegenerative diseases [16-18]. Recently, some studies also showed that TREM2 played an important regulatory role in local or systemic inflammatory disorders [19-21]. Despite the reported function of TREM2 in pathological conditions in the CNS, whether TREM2 interacts with the neuroendocrine system and how TREM2 impacts progress of septic shock remains unexplored.

In the present study, we tested the hypothesis that whether TREM2 expressed on macrophage was involved in the activation of the adrenal glucocorticoid response at the early stage of septic shock and interacted with adrenocortical cells via macrophage-derived exosomes in an inflammatory microenvironment. We used wild-type (WT) and TREM2 knock-out (*Trem2*$^{-/-}$) mice to establish a septic shock model via intraperitoneally administration of lipopolysaccharide (LPS). *Invivo* TREM2 expression, synthesis of corticotropin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) and local inflammation in adrenal gland were determined. Furthermore, primary macrophages isolated from WT or *Trem2*$^{-/-}$ mice or macrophage-derived exosomes were co-cultured with adrenocortical cells in an inflammatory microenvironment to confirm intercelluar interaction, and process of adrenocortical steroid synthesis *ex vivo* was assessed.

Material And Methods

Animals

WT C57BL/6 mice were obtained from Experimental Animal Center of Zhejiang University (Hangzhou, China). *Trem2*$^{-/-}$ mice were kindly provided by Professor Macro Colonna from Washington University in St. Louis and bred as described [22]. For the study, 6- to 8-week old male mice (weighing 20 to 25 g) were used. Mice were housed using a 12-hour light-dark cycle (lights on at 7:00 AM) under constant temperature (21-23°C). All mice were maintained on a standard chow pellet diet and tap water ad libitum. All experiments using animals were performed under the approval of the Animal Care and Use Committee of Zhejiang University (Hangzhou, China).

LPS-induced Septic Shock Model

WT and *Trem2*$^{-/-}$ mice were intraperitoneally injected with LPS (*Escherichia coli* serotype O111:B4, 30 mg/kg; Sigma-Aldrich, USA) or an equal volume of the sterile saline vehicle to elicit septic shock as previously described [23]. Blood, hypothalamus and adrenal gland were collected postmortem 0, 3, 9 h later for analysis. The mice were randomly assigned to experimental conditions. All further experiments were blinded to murine genotypes and treatments.

Isolation of Bone Marrow-Derived Macrophages (BMDMs)

The murine BMDMs of WT and *Trem2*$^{-/-}$ mice were isolated from the femur and tibia in single cell suspension and seeded in 6-well plates (2.5×10^6 cells per well) with Dulbecco's modified eagle medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Biological Industries, USA), 1% penicillin/streptomycin and 20 ng/ml mouse granulocyte macrophage-colony stimulating factor (PeproTech, USA). These cells were differentiated into macrophage for another 6~7 days with replacement of fresh growth media in every 3 days. Cells were maintained in a humidified incubator (Thermo Fisher Scientific, USA) with 5% CO_2 at 37 °C. Then BMDMs were harvest or stimulated for further experiments.

Primary Adrenocortical Cell Culture

Primary mouse adrenocortical cells were isolated from WT mice as previously reported with some modifications [24, 25]. Briefly, adrenal glands of WT mice were excised and freed from perirenal fat. The adrenal was carefully halved and enucleated to eliminate medullas parts. Repeated digestions (thrice to quartic) were performed for 1 h in buffer containing 0.1% collagenase I (Gibco, USA), 1% hyaluronidase (Sigma-Aldrich, USA) and 0.1% bovine serum albumin (Sigma-Aldrich, USA) at 37 ºC on an orbital shaker. The adrenocortical suspension was pressed through a 70 μM cell strainer, centrifuged at 800 g for 5 min at 4 ºC and the cell pellet was resuspended in DMEM/nutrient mix F-12 (Gibco, USA) supplemented with 10% FBS and 1% penicillin/streptomycin. Suspended cells were placed in culture dishes at 37 ºC for 1 h to enable selective attachment of fibroblasts. Cell viability (> 90%) was assessed by trypan blue exclusion test. Adrenocortical cells collected from supernatants were counted and seeded in a 24-well culture plate (Corning, USA) at a density of 1×10^5 cells/ml, the medium being changed every 48 h. Cells were then cultured in media for 5~6 days for the subsequent treatments.

Macrophage-Adrenocortical Cell Coculture

Differentiated BMDMs were co-cultured with matured adrenocortical cells using a trans-well plate (Corning, USA), with BMDMs cultured in the upper chamber (membrane pore = 0.4 μm) and primary adrenocortical cells in the lower chamber, allowing a contact-independent communication by secreted components. To evaluate the effect of WT and *Trem2*$^{-/-}$ BMDMs on adrenocortical cells in an inflammatory condition, the experimental system was stimulated with ACTH (100 nM; Bioss, China) and LPS (100 ng/ml) for 6 h. The culture medium was collected for corticosterone detection and adrenocortical cells were harvested for RNA extraction after washing with phosphate buffer saline (PBS) twice.

Purification of Macrophage-derived Exosomes

To harvest exosomes, WT and *Trem2*$^{-/-}$ BMDMs were changed for fresh media and stimulated with LPS (100 ng/ml) for 24 h. The cell culture supernatants were centrifuged sequentially at 1,500 g for 15 min and 16,500 g for 30 min to eliminate the cell debris and large vesicles. The pellet was washed once with PBS by ultracentrifugation for 2 h at 118,000 g at 4 °C in a swinging bucket rotor (Beckman Coulter, USA). The exosome-containing pellet was resuspended in 100~200 μl of PBS for further experiments.

Nanoparticle Tracking Analysis

The size distribution of exosomes was measured by nanoparticle tracking analysis technology using ZetaView PMX 110 (Particle Metrix, Germany) and Software ZetaView 8.04.02 SP2. Samples were diluted in PBS to obtain an appropriated concentration and subsequently assessed according to the operating instructions.

Electron Microscopic Examination

Fresh exosomes samples were resuspended with 100 μl PBS and then placed on 200-mesh formvar-coated copper grids. Samples were stained with 2% aqueous uranyl acetate and air-dried. Transmission electron microscopy (Thermo Fisher Scientific, USA) was performed to view the physical characterization of exosomes.

Exosome Treatment *ex vivo*

For *ex vivo* treatment, WT or *Trem2*$^{-/-}$ BMDM-derived exosomes (20 μg/ml) were added to 1×10^5 cells/ml adrenocortical cells. After 6 h, adherent cells and culture medium were collected for corticosterone production and mRNA expression measurements, respectively.

Measurement of ACTH and Corticosterone Concentrations

The serum concentrations and cell culture supernatant levels of corticosterone (Cayman, USA) and ACTH (Mybiosource, USA) were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

Analysis of Serum Cytokines

The serum cytokines tumor necrosis factor (TNF)-α, interleukin (IL) -6, IL-1β and IL-10 were measured by corresponding ELISA kits (all from eBioscience, USA) according to the manufacturer's instructions.

Evaluation of Organ Functions

The serum concentrations of creatinine (CRE), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and plasma concentrations of lactic acid were determined using commercial assay kits (NanJing KeyGen Biotech, China).

Immunofluorescence Analysis

The adrenal gland was fixed in a 4% formaldehyde solution and embedded in OCT Tissue-Tek for 10-μm frozen sections. Frozen sections were mounted on slides, fixed and permeabilized in PBS-0.3% Triton X-100. Adrenal glands were incubated with primary antibodies for rat anti-mouse F4/80 (clone Cl:A3-1; 10 μg/ml; AbD Serotec, USA) and goat anti-mouse TREM2 antibody (10 μg/ml; Abcam, USA) overnight at 4°C, followed by incubation of Alexa Fluor 488 conjugated donkey-anti-rat IgG (4 μg/ml; Invitrogen, USA) for F4/80 and Alexa Fluor 594-conjugated donkey-anti-goat IgG (4 μg/ml; Invitrogen, USA) for TREM2 for one hour at room temperature. Coverslips were mounted with VECTASHIELD mounting medium with DAPI (Vector Labs, USA) to detect all cell nuclei. Images were evaluated using a Leica DM5000B microscope (Leica, Germany) or Olympus confocal laser scanning microscope (Olympus, Japan). At least 3 fields in a randomly selected section of each mouse were captured for analysis of $F4/80^{+}TREM2^{+}$ double-labeled cells in the adrenal gland.

Quantitative Real-time Polymerase Chain Reaction Analysis (qRT-PCR)

The mRNA was extracted from homogenates of the hypothalamus and adrenal cortex, and cultured cells using TRIzol reagent (Life Technologies, USA) as instructions described. qRT-PCR was applied to detect relative expression level of mRNA from tissue samples by using SYBR PCR Master Mix (TaKaRa, Japan) and standard oligonucleotides. β-actin was used as the endogenous control gene. Primers designed to quantify specific transcripts were listed in **Table S1**. Amplification data were analyzed using Roche 480 PCR System (Roche, USA). Data analysis was performed using the comparative threshold cycle method with arithmetic formulae ($2^{-\triangle\triangle Ct}$), and the mRNA levels were expressed as a fold change compared to the control group.

Statistical Analysis

Statistical evaluations were employed by GraphPad Prism 6.00 (GraphPad Software Inc., USA). A two-tailed paired or unpaired Student's *t* test was used to compare difference between two independent groups. The covariance efficiency factor was also assessed for each endogenous control (covariate) to ensure that there were no effects of treatment on the endogenous controls. Survival between two independent groups was analyzed using a log-rank (Mantel-Cox) test. All data shown in the figures and text are represented as mean ± standard error of the mean (SEM). Differences were accepted as statistically significant if a *p* value was less than 0.05.

Results

TREM2 Deficiency on Macrophages in Adrenal Cortex Induced Increased Serum Corticosterone Concentration during LPS-induced Septic Shock.

To investigate the role of TREM2 in the local immune-adrenal crosstalk during septic shock, WT mice were challenged intraperitoneally with LPS from *E. coli*. Double immunofluorescence stained with F4/80 and TREM2 antibodies was used to locate the expression of TREM2 in the adrenal gland. It was found that TREM2 was co-localized with tissue macrophages which were recognized by F4/80 marker in the adrenal cortex rather than medulla tissue (fig. 1A). Followed by LPS challenge for 9 h, tissue macrophages showed a lower level of TREM2 fluorescent intensity ($p < 0.05$), although the number of double-positive ($F4/80^+$ and $TREM2^+$) cell in adrenal cortex did not significantly decreased compared that of control group (fig. 1B). In accordance, mRNA levels of TREM2 were significantly decreased after LPS administration ($p < 0.01$) (fig. 1C).

Then, WT and *Trem2*$^{-/-}$ mice were used to further explore the effect of TREM2 on adrenal glucocorticoid response during septic shock by intraperitoneally injected with LPS and serum levels of corticosterone were detected. Following LPS challenge, WT mice exhibited 6-fold (151.1 ± 22.2 ng/mL at 3 h, $p < 0.01$) and 8-fold (208.3 ± 8.6 ng/mL at 9 h, $p < 0.001$) increases in serum corticosterone levels at 3 h and 9 h, as compared to control group (26.3 ± 1.4 ng/mL at 0 h). The serum corticosterone levels were similar between 3 h and 9 h groups in WT mice. In contrast to the decreased mRNA levels of TREM2, serum corticosterone levels in *Trem2*$^{-/-}$ mice at 3 h and 9 h after LPS administration were significantly higher than those in corresponding WT mice (3 h: 326.6 ± 23.1 ng/mL in *Trem2*$^{-/-}$ mice *vs.* 151.1 ± 22.2 ng/mL in WT mice, $p < 0.001$; 9 h: 388.2 ± 15.4 ng/mL in *Trem2*$^{-/-}$ mice *vs.* 208.3 ± 8.6 ng/mL in WT mice, $p < 0.01$). Interestingly, TREM2-deficient mice produced more corticosterone at rest (0 h: 53.3 ± 3.7 ng/mL in *Trem2*$^{-/-}$ mice *vs.* 26.3 ± 1.4 ng/mL in WT mice, $p < 0.01$) (fig. 1D). Taken together, these data suggest that TREM2 deficiency elicited activation of adrenal glucocorticoid responses and affected the process of hormone synthesis or release in the adrenal gland, the major output of which is corticosterone production in rodents, at the early stage of LPS-induced septic shock.

Deficiency of TREM2 Neither Altered CRH/ACTH Synthesis nor Increased Inflammatory Response in Adrenocortical Tissue During Septic Shock

It is generally accepted that adrenocortical hormone production is regulated by hypothalamic CRH production and pituitary ACTH secretion during course of systemic inflammatory response syndrome or sepsis [14]. We next explored whether increased corticosterone levels were associated with increased CRH/ACTH synthesis following sepsis. Compared to those in control groups (0 hour), serum ACTH levels did not exhibit significant alteration when encountered with sepsis for 3 hours. ACTH levels were significantly reduced at 9 h after septic shock in both of the groups (9 h: 7940.0 ± 597.0 pg/mL in WT mice and 9924.0 ± 759.0 pg/mL in *Trem2*$^{-/-}$ mice), when compared to those at 3 h after septic shock in the respective groups (3 h: 15553.0 ± 2605.0 pg/mL in WT mice and 15847.0 ± 1185.0 pg/mL in *Trem2*$^{-/-}$ mice, $p < 0.05$, respectively), but there was no difference between WT and *Trem2*$^{-/-}$ groups at the early stage of septic shock (fig. 2A). In the hypothalamus, CRH expression level was higher at 3 h and 9 h after LPS administration compared with that in control groups, but this result was not statistically significant between WT and *Trem2*$^{-/-}$ groups during septic shock (fig. 2B). In all, sharply elevated corticosterone in a murine model of LPS-induced septic shock is not dependent on hypothalamic CRH production and pituitary ACTH secretion.

In addition to the elevated CRH and ACTH synthesis during sepsis, a critical involvement of adrenal inflammation and local immune-adrenal cross talk was also implicated in sepsis. Recent studies reported that massive inflammatory mediators such as cytokines within adrenal glands affected steroidogenic process [12, 13]. Thus, expression of the inflammatory cytokines (TNF-α, IL-6, IL-1β and IL-10) in adrenal cortex tissue after systemic administration of LPS was determined. As expected, stimulation with LPS promoted an increase in cytokines mRNA expression in adrenal cortex, which peaked at 3 h after septic shock. However, no difference of those inflammatory cytokines was observed between WT and *Trem2*$^{-/-}$ mice at the observing time points (fig. 3, A-D). Taken together, TREM2 deficiency-induced steroidogenesis is not related with intra-adrenal inflammatory microenvironment during septic shock.

Deficiency of Macrophage TREM2 Increased Steroidogenesis of Adrenocortical Cells

Emerging evidence using genetically manipulated mice suggest that local immune-endocrine crosstalk plays a substantial role in sustained adrenal glucocorticoid production during sepsis [11-13]. We next used trans-well coculture system to assess the effect of macrophage on steroidogenesis in primary WT adrenocortical cells after LPS stimulation simultaneously with ACTH (fig. 4A). We found that *Trem2*$^{-/-}$ macrophage induced an augment of corticosterone production (27.73 ± 1.78 ng/ml in *Trem2*$^{-/-}$ macrophages *vs.* 22.96 ± 1.94 ng/ml in WT macrophages; $p < 0.01$) (fig. 4B) and increased the expression of key steroidogenic genes (StAR, P450Scc and CYP21) compared with WT macrophages (fig. 4C, D, F). In addition, 3β-HSD mRNA level was comparable between WT and *Trem2*$^{-/-}$ macrophage groups (fig. 4E). These results indicate that TREM2-deficient macrophages induced steroidogenesis in adrenocortical tissue through local immune-adrenal cross talk.

Exosomes Derived from TREM2-Deficient Macrophages Enhanced Steroidogenesis of Adrenocortical Cells

Exosomes (30-150 nm in size) released by almost all types of cells are increasingly recognized as vital mediators of intercellular communication for critical illness, including acute lung injury and sepsis [26]. Therefore, we tested whether WT and *Trem2*$^{-/-}$ macrophage-derived exosomes were involved in communication between macrophages and adrenocortical cells. A schematic illustration of the *ex vivo* experiment is shown in fig. 5A. Electron microscopy observed typical and spherical sizes in exosomes isolated from the culture medium of LPS-activated WT and *Trem2*$^{-/-}$ macrophages (fig. 5B). Nanoparticle tracking analysis showed that both WT and *Trem2*$^{-/-}$ macrophage-derived exosomes had similar size (fig. 5C). Exosomes secreted by *Trem2*$^{-/-}$ macrophages significantly increased concentrations of corticosterone in supernatant of adrenocortical cells with ACTH stimulation (162.60 ± 13.19 ng/ml in *Trem2*$^{-/-}$ BMDM-derived exosomes *vs.* 109.30 ± 18.88 ng/ml in WT BMDM-derived exosomes; $p < 0.05$) (fig. 5D). In accordance, the mRNA levels of four key steroidogenic genes (StAR, P450Scc, 3β-HSD and CYP21) were all increased when exposed to *Trem2*$^{-/-}$ macrophage-secreted exosomes (fig. 5, E-H). As summarized from the *ex vivo* results, TREM2 can inhibit process of steroidogenesis in adrenocortical cells mediated by macrophage-derived exosomes at the early stage of septic shock.

Enhanced Steroidogenesis in *Trem2*$^{-/-}$ Mice Related to Alleviated Plasma Lactic Acid during LPS-induced Septic Shock

Studies found that glucocorticoids can modulate innate immunity to promote the resolution of inflammation and organs failure, so we evaluated the impact of enhanced steroidogenesis of adrenal gland on the phenotype of septic shock in TREM2-deficient mice. Mortality analysis showed that there was no statistical significance between WT and *Trem2*$^{-/-}$ mice after LPS-induced septic shock (fig. 6A). LPS-induced septic shock markedly increased serum levels of proinflammatory cytokine TNF-α and anti-inflammatory cytokine IL-10, but there was no difference between WT and *Trem2*$^{-/-}$ mice at the observing time-points (fig. 6B, C). Then we explored organ functions by analyzing serum levels of CRE, AST and ALT. There was no difference in these variables between WT and *Trem2*$^{-/-}$ mice at the early stage of septic shock (fig. 6D-F). The immune-modulating proprieties of glucocorticoids give the biologic plausibility that corticosteroids may improve tissue perfusion [8, 27]. Thus, we measured plasma lactic acid level. The data showed similar baseline levels of plasma lactic acid between WT and *Trem2*$^{-/-}$ mice (2.11 ± 0.41 nmol/ml in *Trem2*$^{-/-}$ mice *vs* in 1.73 ± 0.24 nmol/ml in WT mice, respectively). However, plasma lactic acid levels were significantly reduced in *Trem2*$^{-/-}$ mice after septic shock (3 h: 3.64 ± 0.22 nmol/ml in *Trem2*$^{-/-}$ mice *vs.* 4.90 ± 0.42 nmol/ml in WT mice, $p < 0.05$; 9 h: 3.62 ± 0.12 nmol/ml in *Trem2*$^{-/-}$ mice *vs.* 5.74 ± 0.48 nmol/ml in WT mice, $p < 0.01$) (fig. 6G). These results revealed that enhanced synthesis and release of corticosterone into circulation in TREM2-deficient mice improved tissue perfusion during acute endotoxic shock.

Discussion

Adjunctive glucocorticoids have been used to treat patients for more than half a century because of an increased demand for patients suffered from adrenal insufficiency [7, 28-31]. Adrenal insufficiency

especially occurred in patients whose endogenous adrenal response was inadequate for the degree of severe stress response during acute conditions including sepsis, septic shock and acute respiratory distress syndrome [32, 33]. However, the complicated interactions among components of neuroendocrine network lead to the poor understanding of the pathogenesis underlining adrenal insufficiency (fig. 7).

TREM2 is extensively studied in central chronic degenerative diseases especially Alzheimer's disease, and plays an important regulatory role in local or systemic inflammatory disorders [19, 21, 34]. Genetic or functional interventions of TREM2 result in reduced accumulation of surrounding plaques, impaired microglial phagocytosis as well as marked adjacent neuronal dystrophy [20, 35, 36]. Although TREM2 has been known to be involved in the anti-inflammatory response and osteoclast development, recent work demonstrated that overexpression of TREM2 caused the development of obesity coupled with insulin resistance and hepatic steatosis [37, 38]. Furthermore, TREM2 signaling is newly identified as a major driver of adipose tissue macrophage responses during obesity and characteristic of detecting extracellular pathogenic lipids across multiple tissues [17]. TREM2 expressed on macrophage not only affects inflammation via toll-like receptor (TLR)-dependent pathway, but also participates in metabolic processes in different disease microenvironments via mechanisms still unknown [17, 38, 39]. However, it has not been examined whether TREM2 was involved in neuroendocrine system such as the adrenal gland during sepsis.

We observed that TREM2 was co-localized with tissue macrophages in the adrenal cortex rather than medulla tissue, with decreased fluorescent intensity and mRNA expression level in a time-dependent pattern in an endotoxic model of septic shock. This was consistent with the *in vitro* study which showed a rapid decline of TREM-2 protein level upon LPS stimulation in selected macrophage populations [40]. Previous studies has showed that endotoxemic model induces an initial activation of the HPA axis with significantly higher circulating corticosterone levels when subjected to LPS [41]. In *in vivo* experiment employing WT and *Trem2*$^{-/-}$ mice, we found that TREM2 deficiency induced dramatically increase of corticosterone production at the early stage of LPS-induced septic shock. In addition, the mRNA expression level of TREM2 in adrenal cortex demonstrated an opposite trend against corticosterone levels among WT mice during septic shock. These data presented that TREM2 played an important role in the regulation of steroidogenesis in the adrenal gland during septic shock.

Various studies found that the activation of intra-adrenal cellular systems, composed of adrenocortical, resident and recruited immune-inflammatory cells, plays a pivotal role in adrenocortical glucocorticoid response during septic shock conditions [11-13]. However, CRH/ACTH pathway and intra-adrenal inflammatory response was found not to be involved in remarkable elevation of corticosterone levels. Interestingly, LPS-activated *Trem2*$^{-/-}$ macrophages promoted corticosterone release when co-cultured with primary adrenocortical cells in a trans-well system. We also showed a robust increase in key steroidogenic genes in this *ex vivo* condition. Taken together, these findings show that *Trem2*$^{-/-}$ macrophages might produce some factors can be transferred into adrenocortical cells.

Complex cell-cell communication mediated by paracrine molecules is essential to maintain the physiologic tissue environment necessary to respond to stresses [42]. Exosomes are small extracellular vesicles transferring molecular components such as proteins, DNA, and microRNAs (miRNAs) [43]. Exosomes represent an emerging carrier in the delivery of endogenous molecules to regulate gene expression and relevant physiological and pathophysiological processes in recipient cells [26, 42, 44]. Previous studies demonstrated that suppression of adrenocortical steroid synthesis by a macrophage-derived product might be a new regulatory pathway in host response to LPS [45, 46]. Our data showed that corticosterone secretion and steroidogenesis process were both significantly increased when adrenocortical cells were treated with exosomes from LPS-stimulated *Trem2*$^{-/-}$ macrophage. These results provide an explanation, at least in part, for increased steroids synthesis and release into circulation in *Trem2*$^{-/-}$ mice during septic shock (fig. 7).

Prior studies have confirmed that TREM2 played a protective role in the host defense response to polymicrobial sepsis by enhanced clearance of microbes, and activation of TREM2 promoted microglial switching from the detrimental M1 phenotype to the beneficial M2 phenotype in a mouse model of middle cerebral artery occlusion [19, 21]. However, the role of TREM2 when encountered with an acute inflammatory challenge is still controversial. Gawishit et al. found TREM2 as a potential target to prevent TLR-4 mediated overwhelming inflammation in mice with gram-negative sepsis [40]. Liu et al. showed that TREM-2 gene silencing significantly aggravated the pathological change and inflammatory responses of endotoxin-induced acute lung injury in mice [47]. However, Leyns et al. reported that defect in TREM2 signaling attenuated neuroinflammation and protect against pure tau pathology [18]. Therefore, TREM2 could not turn the tide of systemic or overwhelming inflammatory cascade in LPS-induced acute sepsis and the role of TREM2 as a phagocytic receptor for bacteria might be more crucial in innate immune response during bacterial sepsis. Additionally, regarding of the immunomodulatory effect of glucocorticoids, we found that TREM2 deficiency with higher levels of corticosterone alleviated plasma lactic acids, suggesting to some extent, endogenous corticosteroids hastened the resolution of shock during sepsis.

In current study, we used a high dose of LPS (30 mg/kg) for intraperitoneal injection to induce a septic shock model. Both WT mice and *Trem2*$^{-/-}$ mice were died within 36 hours after injection of LPS. The overwhelming endotoxic response and high lethality may hamper the real function of TREM2 on the pathophysiology of septic shock. In spite of this, a mechanism to involve *Trem2*$^{-/-}$ macrophages-derived exosomes in adrenocortical steroidogenesis during progress of septic shock was strongly suggested. Future studies to confirm these findings in an appropriate sepsis model are needed and may provide deep insight onto these preliminary findings.

Conclusions

In conclusion, our study reports that TREM2 expressed on macrophage plays a crucial role in regulating adrenal steroidogenesis by macrophage-derived exosomes at the early stage of LPS-induced septic shock, which provides a deeper understanding of the biology of TREM2-expressing macrophages and

may yield novel therapeutic handles to target adjunctive corticosteroid treatment during septic shock in clinic.

Abbreviations

TREM2: Triggering receptor expressed on myeloid cells-2; *Trem2*$^{-/-}$: TREM2 knock-out; CRH: Corticotropin releasing hormone; ACTH: Adrenocorticotropic hormone; HPA axis: Hypothalamic-pituitary-adrenal axis; CNS: Central nervous system; AD: Alzheimer's disease; LPS: Lipopolysaccharide; BMDs: Bone marrow-derived macrophage; DMEM: Dulbecco's modified eagle medium; FBS: Fetal bovine serum; PBS: Phosphate buffer saline; ELISA: enzyme-linked immunosorbent assay; TNF: tumor necrosis factor; IL: interleukin; CRE: creatinine; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; qRT-PCR: Quantitative real-time polymerase chain reaction analysis; SEM: Standard error of the mean; TLR: Toll-like receptor; miRNA: microRNA; StAR: Steroidogenic acute regulatory protein; P450Scc: P450 side-chain cleavage; 3β-HSD: 3β hydroxydehydrogenase; CYP21: 21-hydroxylase

Declarations

1. **Ethics approval and consent to participate:** All experiments using animals were performed under the approval of the Animal Care and Use Committee of Zhejiang University, Hangzhou, China.
2. **Consent for publication:** Not applicable.
3. **Availability of data and materials:** All data generated or analysed during this study are included in this published article and its supplementary information files.
4. **Competing interests:** The authors declare that they have no competing interests.
5. **Funding:** This work was supported by Grant No. 2018YFC2001904 from the National Key R&D Program of China (Beijing, China) and Grant No. 872008025 from the National Natural Science Foundation of China (Beijing, China).
6. **Authors' contributions:** Hui Ye and Pinhao Li performed the experiments, wrote the manuscript and contributed equally to this work . Qian Zhai, Ping Fang, Shiyue Yang and Yaqi Sun collected the samples analyzed the data. Shuijing Wu and Ruoqiong Huang contributed to the conception and design. Qixing Chen helped to revise the manuscript. Xiangming Fang revised the manuscript and supervised all the steps of this study. All authors read and approved the final manuscript for publication.
7. **Acknowledgements:** Not applicable.
8. **Authors' information:** [1]Department of Anesthesiology and Intensive Care Unit, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003 China. [2]Department of Anesthesiology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061 China. [3]Department of Anesthesiology, Lihuili Hospital, Ningbo Medical Center, Ningbo, 315041 China. [4]Department of Clinical Research Center, Children's Hospital, Zhejiang University School of Medicine, Hangzhou, 310003 China.

References

1. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017; 43(3): 304-377.
2. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kissoon N, Finfer S, Fleischmann-Struzek C, Machado FR, Reinhart KK, Rowan K, Seymour CW, Watson RS, West TE, Marinho F, Hay SI, Lozano R, Lopez AD, Angus DC, Murray CJL, Naghavi M. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet. 2020; 395(10219): 200-211.
3. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, Angus DC, Rubenfeld GD, Singer M. Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016; 315(8): 775-787.
4. Cheng B, Li Z, Wang J, Xie G, Liu X, Xu Z, Chu L, Zhao J, Yao Y, Fang X. Comparison of the Performance Between Sepsis-1 and Sepsis-3 in ICUs in China: A Retrospective Multicenter Study. Shock. 2017; 48(3): 301-306.
5. Rhee C, Dantes R, Epstein L, Murphy DJ, Seymour CW, Iwashyna TJ, Kadri SS, Angus DC, Danner RL, Fiore AE, Jernigan JA, Martin GS, Septimus E, Warren DK, Karcz A, Chan C, Menchaca JT, Wang R, Gruber S, Klompas M. Incidence and Trends of Sepsis in US Hospitals Using Clinical vs Claims Data, 2009-2014. JAMA. 2017; 318(13): 1241-1249.
6. Kasahara E, Inoue M. Cross-talk between HPA-axis-increased glucocorticoids and mitochondrial stress determines immune responses and clinical manifestations of patients with sepsis. Redox Rep. 2015; 20(1): 1-10.
7. Venkatesh B, Finfer S, Cohen J, Rajbhandari D, Arabi Y, Bellomo R, Billot L, Correa M, Glass P, Harward M, Joyce C, Li Q, McArthur C, Perner A, Rhodes A, Thompson K, Webb S, Myburgh J. Adjunctive Glucocorticoid Therapy in Patients with Septic Shock. N Engl J Med. 2018; 378(9): 797-808.
8. Heming N, Sivanandamoorthy S, Meng P, Bounab R, Annane D. Immune Effects of Corticosteroids in Sepsis. Front Immunol. 2018; 9: 1736.
9. Gilibert S, Galle-Treger L, Moreau M, Saint-Charles F, Costa S, Ballaire R, Couvert P, Carrie A, Lesnik P, Huby T. Adrenocortical scavenger receptor class B type I deficiency exacerbates endotoxic shock and precipitates sepsis-induced mortality in mice. J Immunol. 2014; 193(2): 817-826.

10. Kanczkowski W, Sue M, Zacharowski K, Reincke M, Bornstein SR. The role of adrenal gland microenvironment in the HPA axis function and dysfunction during sepsis. Mol Cell Endocrinol. 2015; 408: 241-248.
11. Chen LS, Singh SP, Müller G, Bornstein SR, Kanczkowski W. Transcriptional Analysis of Sepsis-Induced Activation and Damage of the Adrenal Endothelial Microvascular Cells. Front Endocrinol (Lausanne). 2019; 10: 944.
12. Jennewein C, Tran N, Kanczkowski W, Heerdegen L, Kantharajah A, Drose S, Bornstein S, Scheller B, Zacharowski K. Mortality of Septic Mice Strongly Correlates With Adrenal Gland Inflammation. Crit Care Med. 2016; 44(4): e190-199.
13. Kanczkowski W, Alexaki VI, Tran N, Großklaus S, Zacharowski K, Martinez A, Popovics P, Block NL, Chavakis T, Schally AV, Bornstein SR. Hypothalamo-pituitary and immune-dependent adrenal regulation during systemic inflammation. Proc Natl Acad Sci U S A. 2013; 110(36): 14801-14806.
14. Annane D. The Role of ACTH and Corticosteroids for Sepsis and Septic Shock: An Update. Front Endocrinol (Lausanne). 2016; 7:70.
15. Paradowska-Gorycka A, Jurkowska M. Structure, expression pattern and biological activity of molecular complex TREM-2/DAP12. Hum Immunol. 2013; 74(6): 730-737.
16. Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, Loboda AA, Zhou Y, Cairns NJ, Kambal A, Loginicheva E, Gilfillan S, Cella M, Virgin HW, Unanue ER, Wang Y, Artyomov MN, Holtzman DM, Colonna M. TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. Cell. 2017; 170(4): 649-663.e613.
17. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, Lundgren P, Bleriot C, Liu Z, Deczkowska A, Keren-Shaul H, David E, Zmora N, Eldar SM, Lubezky N, Shibolet O, Hill DA, Lazar MA, Colonna M, Ginhoux F, Shapiro H, Elinav E, Amit I. Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner. Cell. 2019;178(3): 686-698.
18. Leyns CEG, Ulrich JD, Finn MB, Stewart FR, Koscal LJ, Remolina Serrano J, Robinson GO, Anderson E, Colonna M, Holtzman DM. TREM2 deficiency attenuates neuroinflammation and protects against neurodegeneration in a mouse model of tauopathy. Proc Natl Acad Sci U S A. 2017; 114(43): 11524-11529.
19. Chen Q, Zhang K, Jin Y, Zhu T, Cheng B, Shu Q, Fang X. Triggering receptor expressed on myeloid cells-2 protects against polymicrobial sepsis by enhancing bacterial clearance. Am J Respir Crit Care Med. 2013; 188(2): 201-212.
20. Filipello F, Morini R, Corradini I, Zerbi V, Canzi A, Michalski B, Erreni M, Markicevic M, Starvaggi-Cucuzza C, Otero K, Piccio L, Cignarella F, Perrucci F, Tamborini M, Genua M, Rajendran L, Menna E, Vetrano S, Fahnestock M, Paolicelli RC, Matteoli M. The Microglial Innate Immune Receptor TREM2 Is Required for Synapse Elimination and Normal Brain Connectivity. Immunity. 2018; 48(5): 979-991.e978.
21. Zhai Q, Li F, Chen X, Jia J, Sun S, Zhou D, Ma L, Jiang T, Bai F, Xiong L, Wang Q. Triggering Receptor Expressed on Myeloid Cells 2, a Novel Regulator of Immunocyte Phenotypes, Confers

Neuroprotection by Relieving Neuroinflammation. Anesthesiology. 2017; 127(1): 98-110.

22. Turnbull IR, Gilfillan S, Cella M, Aoshi T, Miller M, Piccio L, Hernandez M, Colonna M. Cutting edge: TREM-2 attenuates macrophage activation. J Immunol. 2006; 177(6): 3520-3524.
23. Zaman MM, Masuda K, Nyati KK, Dubey PK, Ripley B, Wang K, Chalise JP, Higa M, Hanieh H, Kishimoto T. Arid5a exacerbates IFN-gamma-mediated septic shock by stabilizing T-bet mRNA. Proc Natl Acad Sci U S A. 2016; 113(41): 11543-11548.
24. Wang CN, Liu YJ, Duan GL, Zhao W, Li XH, Zhu XY, Ni X. CBS and CSE are critical for maintenance of mitochondrial function and glucocorticoid production in adrenal cortex. Antioxid Redox Signal. 2014; 21(16): 2192-2207.
25. Ziolkowska A, Macchi C, Trejter M, Rucinski M, Nowak M, Nussdorfer GG, Malendowicz LK. Effects of neuromedin-U on immature rat adrenocortical cells: in vitro and in vivo studies. Int J Mol Med. 2008; 21(3): 303-307.
26. Terrasini N, Lionetti V. Exosomes in Critical Illness. Crit Care Med. 2017; 45(6): 1054-1060.
27. Cohen J, Venkatesh B. Adjunctive Corticosteroid Treatment in Septic Shock. Anesthesiology. 2019; 131(2): 410-419.
28. Fang F, Zhang Y, Tang J, Lunsford LD, Li T, Tang R, He J, Xu P, Faramand A, Xu J, You C. Association of Corticosteroid Treatment With Outcomes in Adult Patients With Sepsis: A Systematic Review and Meta-analysis. JAMA Intern Med. 2019; 179(2): 213-223.
29. Annane D, Renault A, Brun-Buisson C, Megarbane B, Quenot JP, Siami S, Cariou A, Forceville X, Schwebel C, Martin C, Timsit JF, Misset B, Ali Benali M, Colin G, Souweine B, Asehnoune K, Mercier E, Chimot L, Charpentier C, François B, Boulain T, Petitpas F, Constantin JM, Dhonneur G, Baudin F, Combes A, Bohé J, Loriferne JF, Amathieu R, Cook F, Slama M, Leroy O, Capellier G, Dargent A, Hissem T, Maxime V, Bellissant E. Hydrocortisone plus Fludrocortisone for Adults with Septic Shock. N Engl J Med. 2018; 378(9): 809-818.
30. Keh D, Trips E, Marx G, Wirtz SP, Abduljawwad E, Bercker S, Bogatsch H, Briegel J, Engel C, Gerlach H, Goldmann A, Kuhn SO, Huter L, Meier-Hellmann A, Nierhaus A, Kluge S, Lehmke J, Loeffler M, Oppert M, Resener K, Schadler D, Schuerholz T, Simon P, Weiler N, Weyland A, Reinhart K, Brunkhorst FM. Effect of Hydrocortisone on Development of Shock Among Patients With Severe Sepsis: The HYPRESS Randomized Clinical Trial. JAMA. 2016; 316(17): 1775-1785.
31. Rygård SL, Butler E, Granholm A, Møller MH, Cohen J, Finfer S, Perner A, Myburgh J, Venkatesh B, Delaney A. Low-dose corticosteroids for adult patients with septic shock: a systematic review with meta-analysis and trial sequential analysis. Intensive Care Med. 2018; 44(7): 1003-1016.
32. Annane D, Pastores SM, Rochwerg B, Arlt W, Balk RA, Beishuizen A, Briegel J, Carcillo J, Christ-Crain M, Cooper MS, Marik PE, Umberto Meduri G, Olsen KM, Rodgers SC, Russell JA, Van den Berghe G. Guidelines for the Diagnosis and Management of Critical Illness-Related Corticosteroid Insufficiency (CIRCI) in Critically Ill Patients (Part I): Society of Critical Care Medicine (SCCM) and European Society of Intensive Care Medicine (ESICM) 2017. Crit Care Med. 2017; 45(12): 2078-2088.

33. Annane D, Pastores SM, Arlt W, Balk RA, Beishuizen A, Briegel J, Carcillo J, Christ-Crain M, Cooper MS, Marik PE, Meduri GU, Olsen KM, Rochwerg B, Rodgers SC, Russell JA, Van den Berghe G. Critical Illness-Related Corticosteroid Insufficiency (CIRCI): A Narrative Review from a Multispecialty Task Force of the Society of Critical Care Medicine (SCCM) and the European Society of Intensive Care Medicine (ESICM). Crit Care Med. 2017; 45(12): 2089-2098.
34. Raha AA, Henderson JW, Stott SR, Vuono R, Foscarin S, Friedland RP, Zaman SH, Raha-Chowdhury R. Neuroprotective Effect of TREM-2 in Aging and Alzheimer's Disease Model. J Alzheimers Dis. 2017; 55(1): 199-217.
35. Wang YM, Ulland TK, Ulrich JD, Song W, Tzaferis JA, Hole JT, Yuan P, Mahan TE, Shi Y, Gilfillan S, Cella M, Grutzendler J, DeMattos RB, Cirrito JR, Holtzman DM, Colonna M. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. J Exp Med. 2016; 213(5): 667-675.
36. Yuan P, Condello C, Keene CD, Wang Y, Bird TD, Paul SM, Luo W, Colonna M, Baddeley D, Grutzendler J. TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. Neuron. 2016; 90(4): 724-739.
37. Park M, Yi JW, Kim EM, Yoon IJ, Lee EH, Lee HY, Ji KY, Lee KH, Jang JH, Oh SS, Yun CH, Kim SH, Lee KM, Song MG, Kim DH, Kang HS. Triggering receptor expressed on myeloid cells 2 (TREM2) promotes adipogenesis and diet-induced obesity. Diabetes. 2015; 64(1): 117-127.
38. Perugorria MJ, Esparza-Baquer A, Oakley F, Labiano I, Korosec A, Jais A, Mann J, Tiniakos D, Santos-Laso A, Arbelaiz A, Gawish R, Sampedro A, Fontanellas A, Hijona E, Jimenez-Agüero R, Esterbauer H, Stoiber D, Bujanda L, Banales JM, Knapp S, Sharif O, Mann DA. Non-parenchymal TREM-2 protects the liver from immune-mediated hepatocellular damage. Gut. 2019; 68(3): 533-546.
39. Ito H, Hamerman JA. TREM-2, triggering receptor expressed on myeloid cell-2, negatively regulates TLR responses in dendritic cells. Eur J Immunol. 2012; 42(1): 176-185.
40. Gawish R, Martins R, Bohm B, Wimberger T, Sharif O, Lakovits K, Schmidt M, Knapp S. Triggering receptor expressed on myeloid cells-2 fine-tunes inflammatory responses in murine Gram-negative sepsis. FASEB J. 2015; 29(4): 1247-1257.
41. Besnier E, Clavier T, Tonon MC, Selim J, Lefevre-Scelles A, Morin F, Tamion F, Dureuil B, Castel H, Compere V. Ketamine and Etomidate Down-regulate the Hypothalamic-Pituitary-Adrenal Axis in an Endotoxemic Mouse Model. Anesthesiology. 2017; 127(2): 347-354.
42. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, Ofrecio JM, Wollam J, Hernandez-Carretero A, Fu W, Li P, Olefsky JM. Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity. Cell. 2017; 171(2): 372-384.e312.
43. Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, Liebler DC, Ping J, Liu Q, Evans R, Fissell WH, Patton JG, Rome LH, Burnette DT, Coffey RJ. Reassessment of Exosome Composition. Cell. 2019; 177(2): 428-445.e418.
44. Wang G, Jin S, Ling X, Li Y, Hu Y, Zhang Y, Huang Y, Chen T, Lin J, Ning Z, Meng Y, Li X. Proteomic Profiling of LPS-Induced Macrophage-Derived Exosomes Indicates Their Involvement in Acute Liver

Injury. Proteomics. 2019, 19(3): e1800274.

45. Mathison JC, Schreiber RD, La Forest AC, Ulevitch RJ. Suppression of ACTH-induced steroidogenesis by supernatants from LPS-treated peritoneal exudate macrophages. J Immunol. 1983; 130(6): 2757-2762.
46. Mathison JC, La Forest AC, Ulevitch RJ. Properties and requirements for production of a macrophage product which suppresses steroid production by adrenocortical cells. Infect Immun. 1984; 45(2): 360-366.
47. Liu D, Dong Y, Liu Z, Niu B, Wang Y, Gao X. Impact of TREM-2 gene silencing on inflammatory response of endotoxin-induced acute lung injury in mice. Mol Cell Biochem. 2014; 394(1-2): 155-161.

Figures

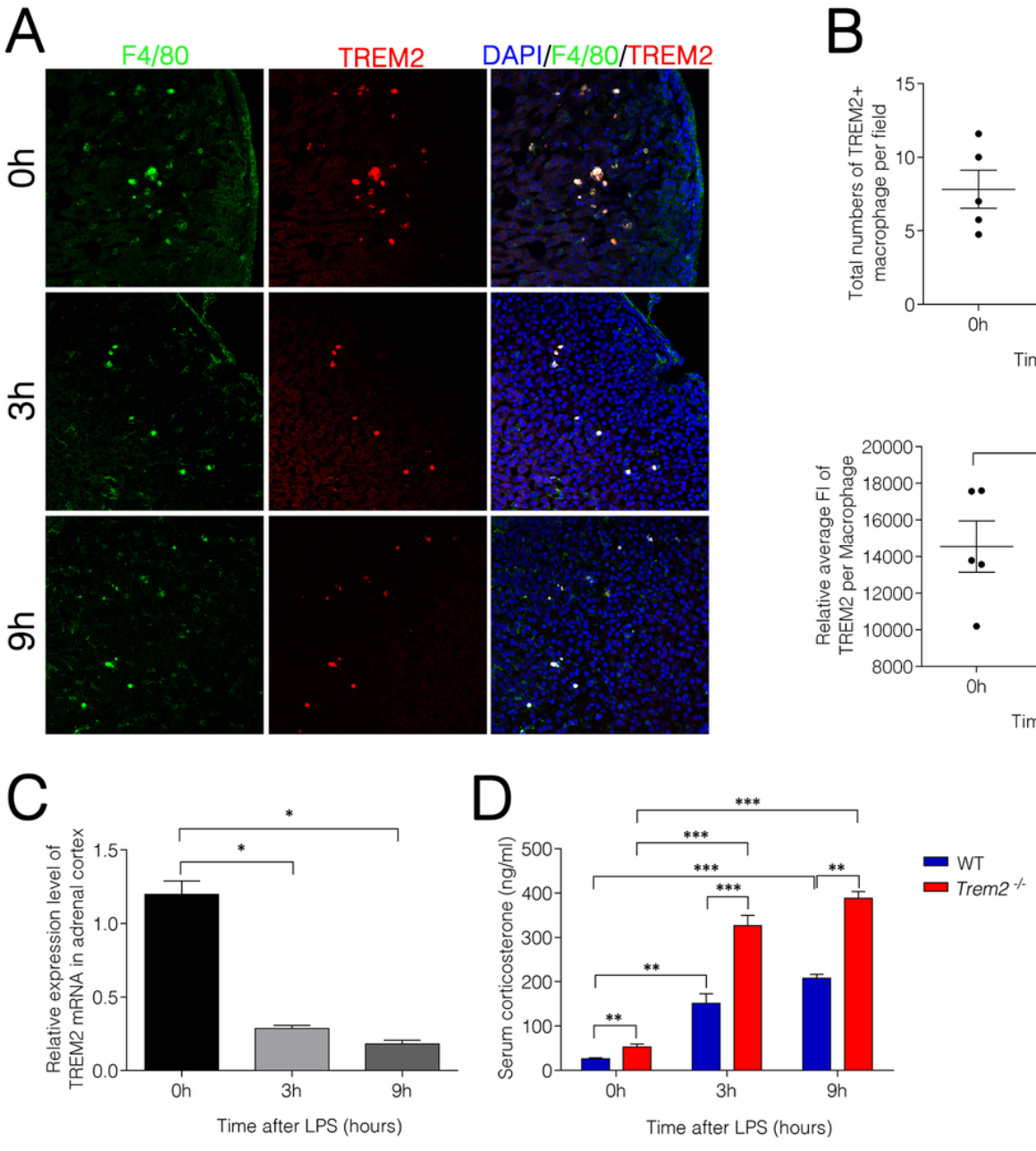

Figure 1

Deficiency of TREM2 expressed on macrophage significantly increased steroidogenesis in adrenocortical tissue during septic shock. WT mice were intraperitoneally stimulated with LPS (30 mg/kg) for 0 h, 3 h and 9 h. (A) Representative images of cellular localization of F4/80 (green) and TREM2 (red), then counterstained for nuclei with DAPI (blue) in adrenal gland were shown by double immunofluorescence (n = 5 at 0 h and 3 h; n = 3 at 9 h). (B) The numbers and fluorescent intensity (FI) of TREM2-expressing macrophages were evaluated at the indicated times (0 h, 3 h and 9 h). Three fields per sample were analyzed (n = 5 at 0 h and 3 h; n = 3 at 9 h). (C) Adrenal gland after LPS-induced septic shock were collected and RNA was extracted for quantitative real-time polymerase chain reaction (qRT-PCR) analysis. mRNA expression level of TREM2 was determined (n = 5 per group). (D) WT and Trem2-/- mice were intraperitoneally treated with LPS (30 mg/kg) and sacrificed after 0, 3 and 9 h. Serum corticosterone levels were determined using specific ELISA kits (n = 5 per group). Data are expressed as the mean ± SEM and analyzed by Student's t test. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus respective controls.

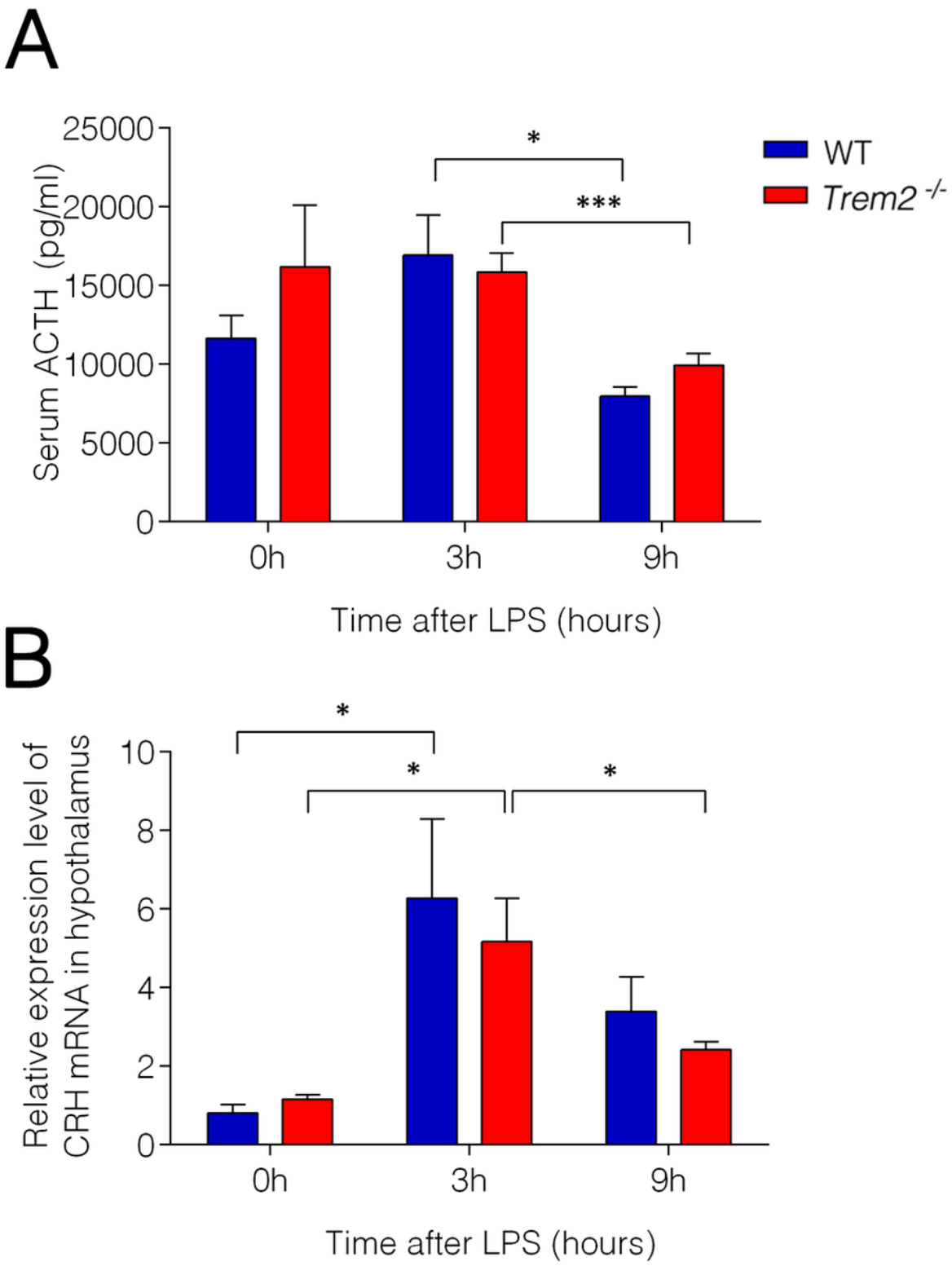

Figure 2

Deficiency of TREM2 did not increase CRH/ACTH synthesis during septic shock. The hypothalamus and serum were collected (0 h, 3 h and 9 h) for determination of CRH/ACTH synthesis after LPS (30 mg/kg) injection in WT and Trem2-/- mice (n = 5 per group). (A) Serum ACTH level was determined using ELISA kits. (B) mRNA expression level of CRH was analyzed by qRT-PCR. Data are expressed as the mean ± SEM and were analyzed by Student's t test.

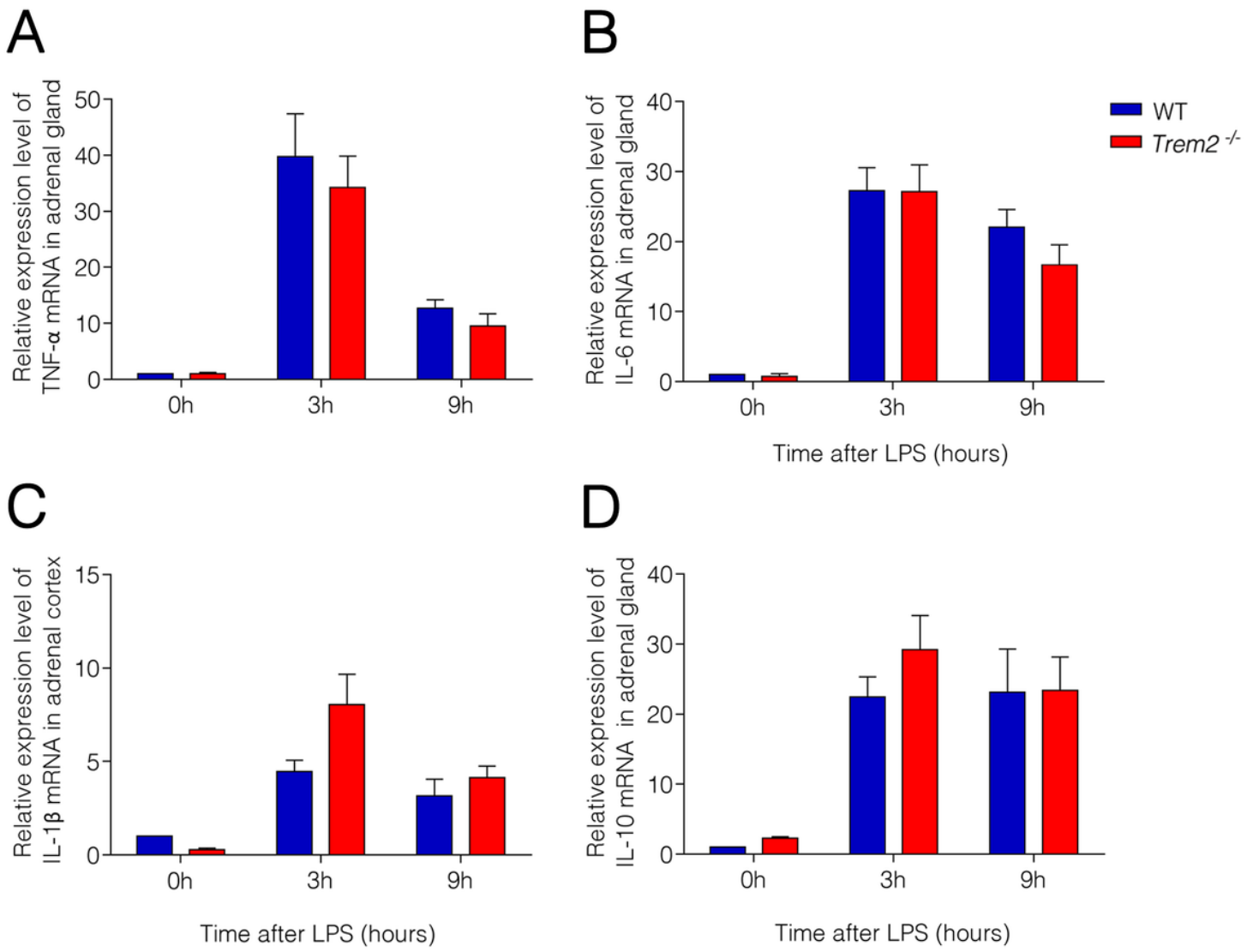

Figure 3

Deficiency of TREM2 did not increase inflammation in adrenocortical tissue during septic shock. WT and Trem2-/- mice were exposed to intraperitoneal LPS (30 mg/kg) for 0 h, 3 h and 9 h. The adrenal cortex was extracted for mRNA expression measurement (n = 5 per group). Relative mRNA expression levels of TNF-α (A), IL-6 (B), IL-1β (C) and IL-10 (D) in adrenal cortex were analyzed by qRT-PCR. Values are expressed as the mean ± SEM and were analyzed by Student's t test.

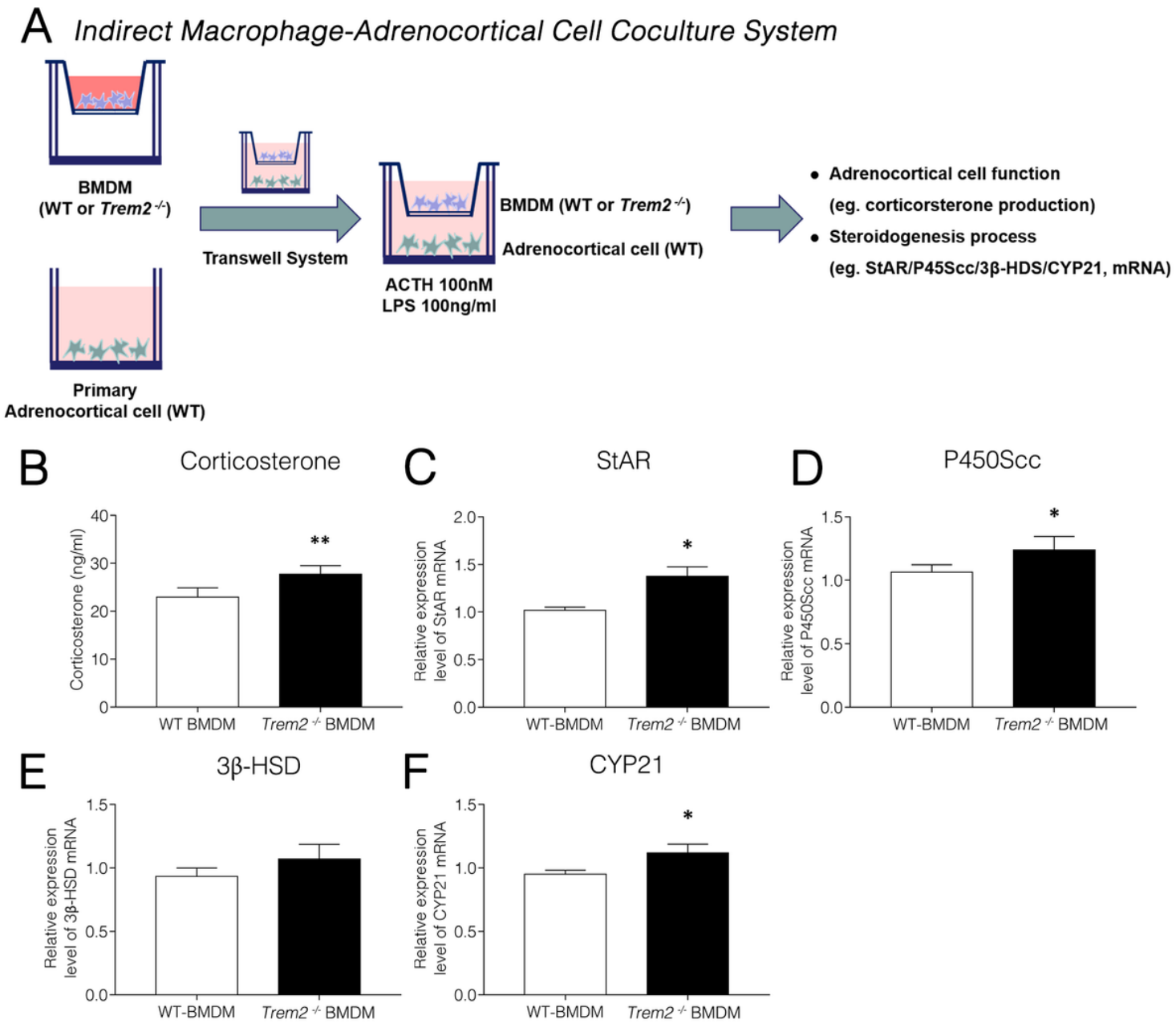

Figure 4

In ex vivo TREM2-deficient macrophages significantly increase adrenocortical steroidogenesis in primary adrenocortical cells. WT and Trem2-/- bone marrow derived macrophage (BMDM) on primary adrenocortical cells were incubated with primary adrenocortical cells for 6 h under the stimulation of LPS (100 ng/ml) and ACTH (100 nM). (A) Schematic diagram of the Tran-swell coculture system. (B) Corticosterone level was measured in cell culture supernatant by ELISA kit. Attached adrenocortical cells on the lower chamber were collected for RNA extraction. Relative mRNA expression levels of StAR (C), P450Scc (D), 3β-HSD (E) and CYP21 (F) in cultured adrenocortical cells were analyzed by qRT-PCR. Values are presented as mean ± SEM from four independent experiments and are analyzed by Student's t test. *$p < 0.05$, **$p < 0.01$ versus WT groups.

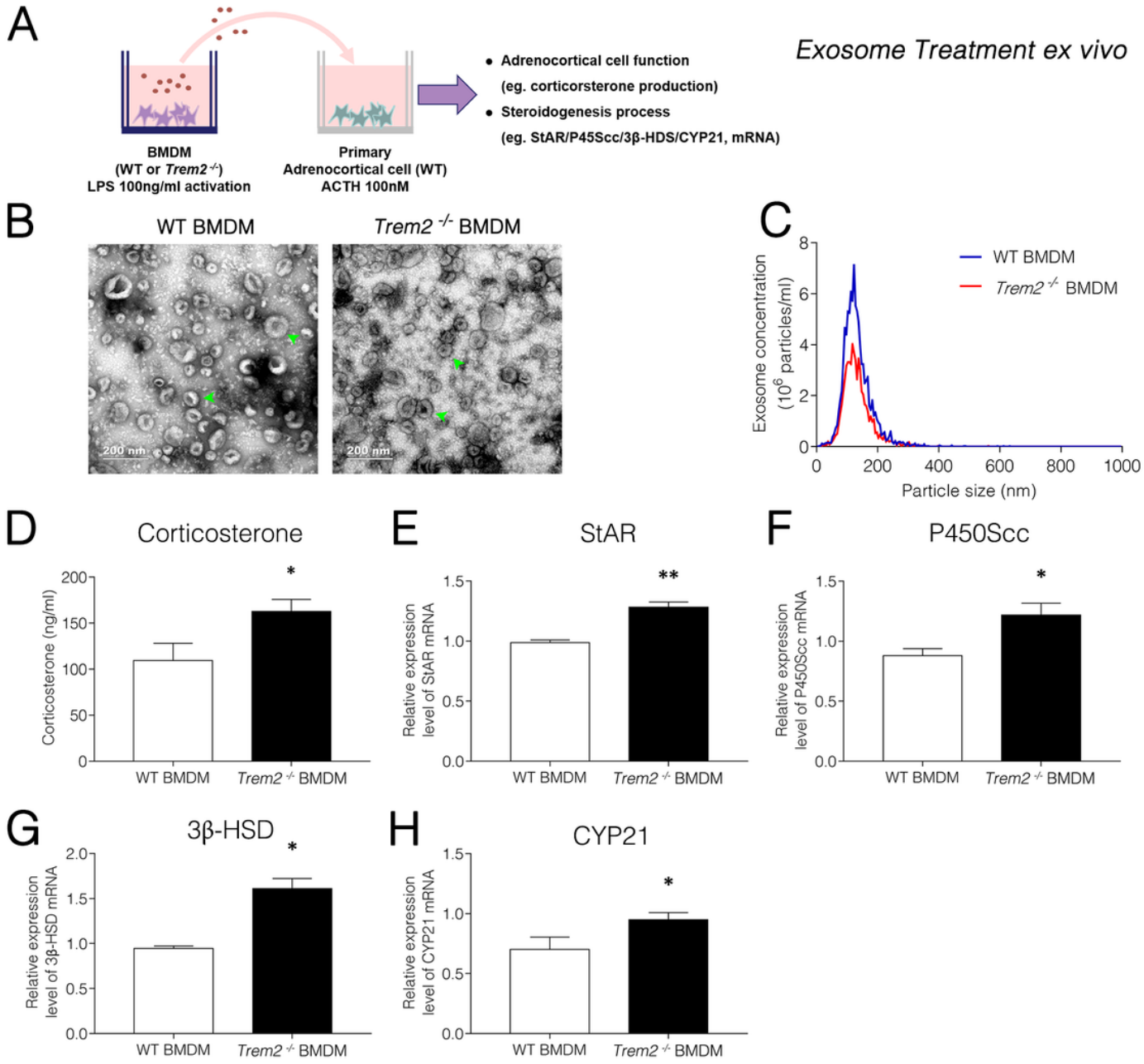

Figure 5

Exosomes derived from TREM2-deficient macrophage significantly enhanced adrenocortical steroidogenesis compared with those from WT macrophages. Exosomes were isolated from LPS-activated WT and Trem2-/- BMDM and were added to the supernatant of cultured adrenocortical cells for 6 h under the stimulation of ACTH (100 nM). (A) Schematic diagram of the exosome treatment ex vivo. (B) Representative images of morphology of WT and Trem2-/- BMDM-derived exosomes by transmission electron microscopy. Exosome are highlighted by green arrows. Scale bar = 200 nm. (C) Size distribution of WT and Trem2-/- BMDM-derived exosomes was measured via nanoparticle tracking analysis. (D) Corticosterone level was determined in cell culture supernatant using specific ELISA kit. Relative mRNA expression of StAR (E), P450Scc (F), 3β-HSD (G) and CYP21 (H) in cultured adrenocortical cells were

analyzed by qRT-PCR in adrenocortical cells. Values are presented as mean ± SEM from four independent experiments and are analyzed by Student's t test. *p < 0.05, **p < 0.01 versus WT groups.

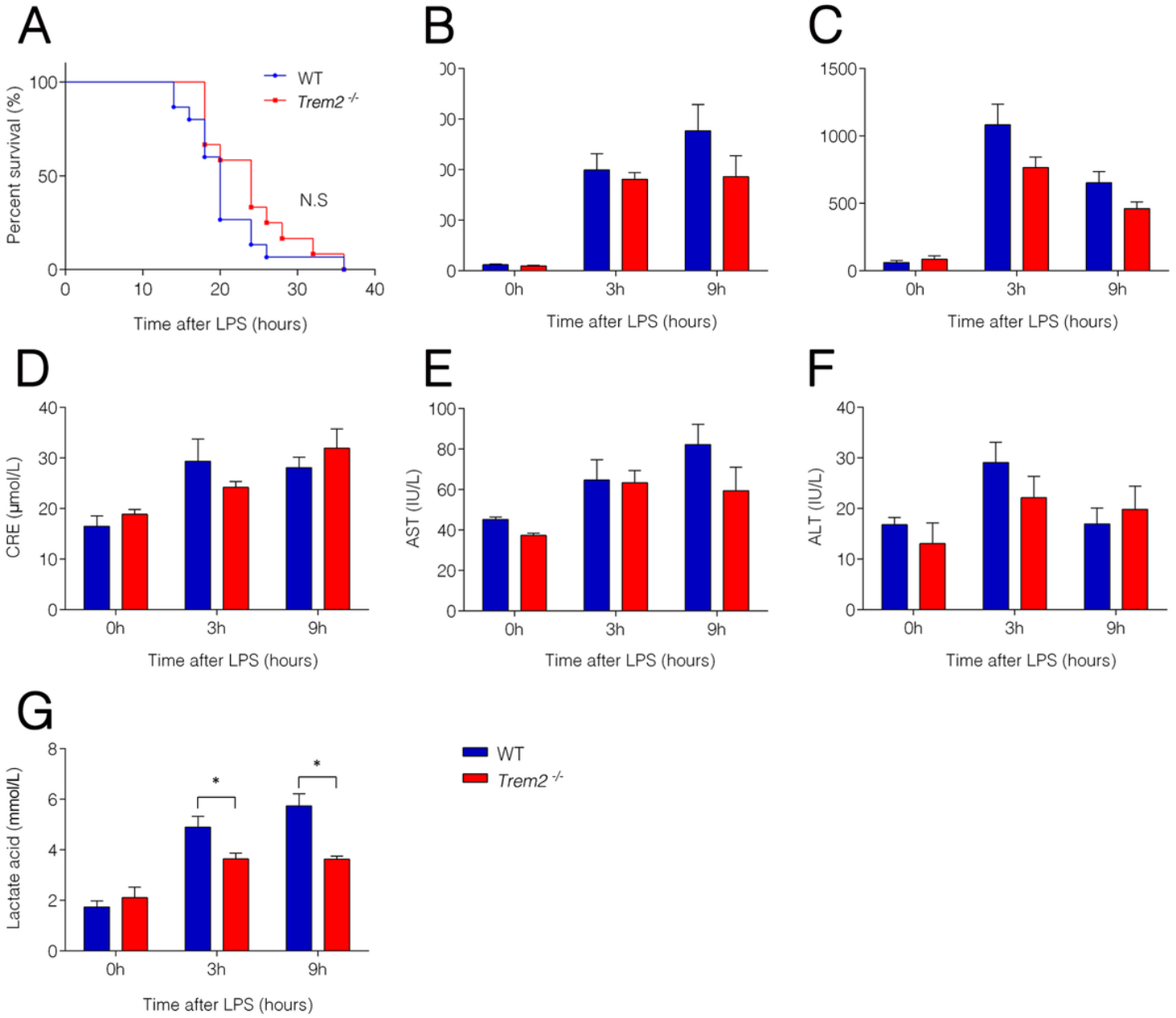

Figure 6

The impact of increased corticosterone on survival, systemic inflammation, organ functions and tissue perfusion. WT and Trem2-/- mice were exposed to intraperitoneal LPS (30 mg/kg) for 0 h, 3 h and 9 h. (A) Survival rate analysis using a log-rank (Mantel-Cox) test (n = 15 for WT versus n = 12 for Trem2-/- mice). Serum TNF-α (B), IL-10 (C), CRE (D), AST (E) and ALT (F) were measured by specific kits (n = 5 per group). (G) Plasma lactic acid was detected (n = 4 per group). Values are expressed as the mean ± SEM and were analyzed by Student's t test. N.S: no statistical significant, *p < 0.05 versus respective controls.

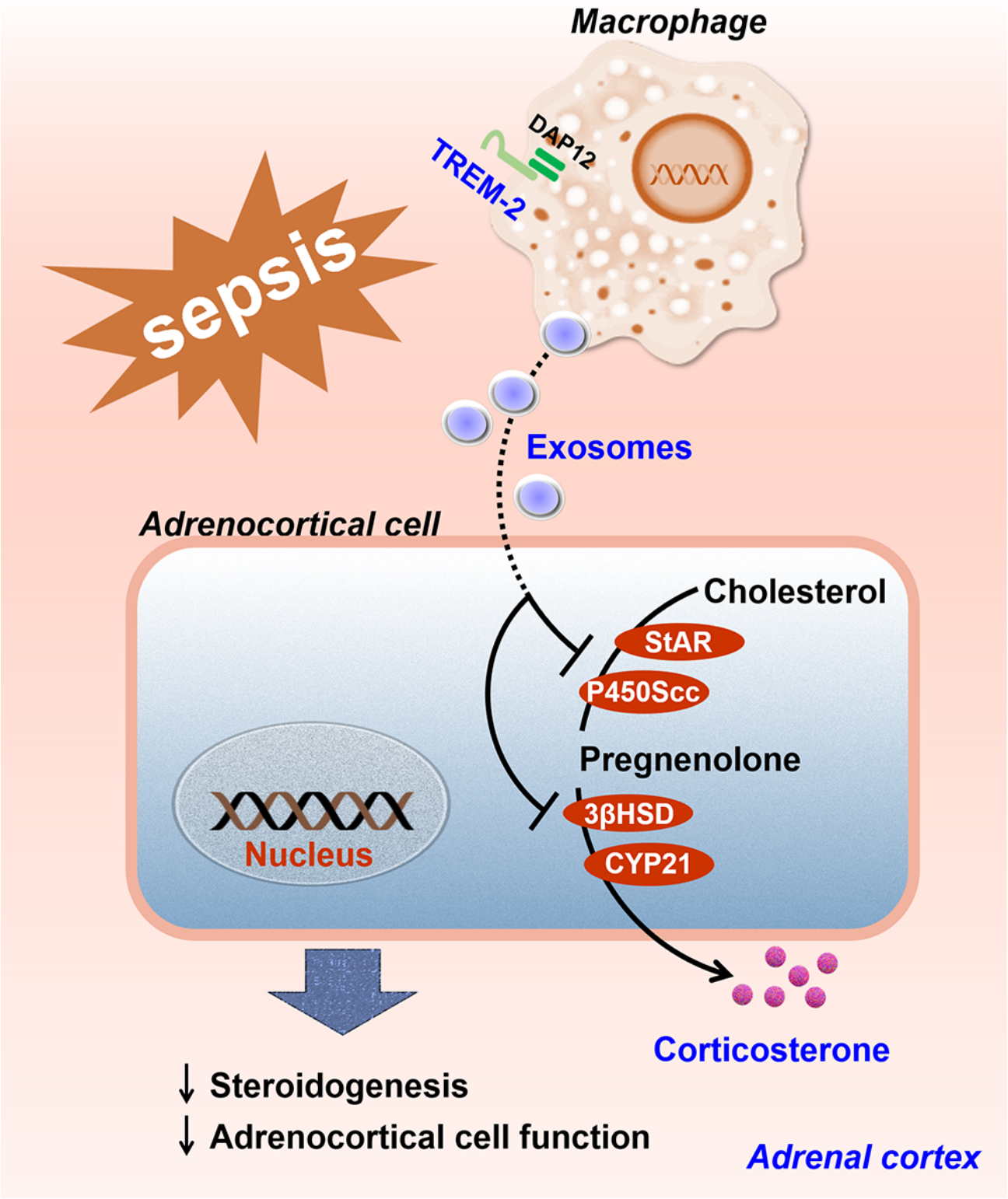

Figure 7

Schematic illustration of proposed mechanism of macrophage-derived exosomes on adrenocortical steroidogenesis during septic shock. At the early stage of LPS-induced septic shock, HPA axis is rapidly activated which is reflected by increased corticosterone into circulation. TREM2 is richly expressed on macrophages and genetic disruption of TREM2 elicits overproduction of corticosterone in adrenal gland, independent of CRH/ACTH pathway or intra-adrenal inflammation during sepsis. Cell-cell communication

between macrophages and adrenocortical cells by transfer information via macrophage-derived exosomes, at least in part, provide the potential mechanism at the early stage of septic shock.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.doc