MOUSE MODEL OF CRITICAL PERSISTENT INFLAMMATION, IMMUNOSUPPRESSION, AND CATABOLISM SYNDROME

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ABSTRACT—Persistent inflammation, immunosuppression, and catabolism syndrome (PIICS) is a growing challenge in intensive care units (ICUs). PIICS causes a severe illness with high mortality. Currently, treatment is expensive, and the outcomes are dismal. Herein, we established a PIICS model to study the disease pathophysiology and its potential treatment. Using a modified sublethal cecal ligation and puncture (CLP) to induce sepsis (day 1) and the injection of lipopolysaccharide (LPS) to induce an aggravated inflammation response (day 11), CLP + LPS mice recapitulating PIICS features were successfully generated (day 14). Adult male mice were divided into CLP + LPS, CLP + daily chronic stress (DCS), CLP, DCS, LPS, and sham control groups. A survival curve was generated, and phenotypes were analyzed using markers for catabolism, inflammation, and immunosuppression. The CLP + LPS model showed two mortality peaks (after CLP and after LPS), whereas the CLP + DCS and CLP groups showed one peak. Surviving CLP + LPS mice exhibited significantly increased catabolism and inflammatory cytokine levels and aggravated inflammation, including organ inflammation. CLP + LPS mice exhibited strong immune suppression as evidenced by decreased splenic cluster of differentiation (CD)8+ and interferon-γ+ CD8+ T cell counts and a concomitant and significant increase in the myeloid-derived suppressor cell population. This CLP + LPS-induced PIICS model differs from acute sepsis models, showing two mortality peaks and a protracted course of 14 days. Compared to previous PIICS models, ours shows a re-aggravated status and higher catabolism, inflammation, and immunosuppression levels. Our aim was to use the PIICS model to simulate PIICS pathophysiology and course in the ICU, enabling investigation of its mechanism and treatment.

KEYWORDS—Cecal ligation and puncture, chronic critical illness, immunoparalysis, lipopolysaccharide, muscle wasting, sepsis

INTRODUCTION

Sepsis affects more than 30 million people worldwide (1). A mortality distribution of sepsis shows three peaks, an early peak in the first few days, a mid-peak after several weeks, and a long-term peak several months after initial sepsis, all contributing to the final outcome (1, 2). Early mortality is well-controlled because of advances in treatments in the intensive care unit (ICU); however, mortality rates remain as high as 18% to 29% (3, 4). An important factor affecting this rate is persistent inflammation, immunosuppression, and catabolism syndrome (PIICS) (5). PIICS is a life-threatening illness accounting for 40% of indolent deaths from sepsis (3, 6). No effective treatments are available for PIICS (7).

The relapse state of PIICS mainly leads to mid-peak mortality of sepsis or severe trauma, both of which are dangerous and challenging conditions for clinicians to solve. To improve the treatment of PIICS, a robust preclinical model needs to be developed, in particular, for modeling the critical relapse PIICS state. Sepsis models such as cecal ligation and puncture (CLP), lipopolysaccharide (LPS) treatment, or the injection of a cecal slurry mainly focus on acute changes and early death, which is different from what occurs in PIICS (8–10). Two PIICS models are available based on CLP and exhibit a prolonged course compared with traditional sepsis models. One model manifests PIICS characteristics 8 days after CLP (11). Recently, a CLP + daily chronic stress (DCS) model in aged mice has been shown to recapitulate PIICS pathophysiology with respect to stress and a long-term disease course (12). While mice in these CLP or CLP + DCS models did show features of PIICS, the mice showed a gradual recovery after CLP because total inflammation continuously declined after CLP. The continuous improvement observed in these models is significantly different from the repeatedly aggravated course of PIICS. Additionally, a PIICS model with a clear aggravation point would be optimal to explore and assess potential treatments for aggravated PIICS.

To generate a PIICS model, sepsis, a high degree of severity, repeated inflammation, and a prolonged course are all required.
Therefore, we used a modified CLP approach to trigger sepsis, along with the sequential administration of low doses of LPS to induce repeated inflammation reactions to mimic the critical PIICS status on day 14. Compared to the two other PIICS models, our CLP + LPS model showed higher severity, controllability, and stability. This PIICS model displayed the characteristics of protracted critical PIICS in the ICU, which should enable the effective analysis of potential treatments.

**MATERIALS AND METHODS**

**Animals**

Adult (28-week-old, 30–35 g) male C57BL/6 mice were obtained from the Animal Core Facility of Nanjing Medical University. Mice were provided with a regular pellet diet and water under standard environmental conditions at the Animal Research Center of Nanjing Drum Tower Hospital in pressurized intraventilated cages. Mice were acclimatized for 1 week before starting experiments. All animal handling techniques were approved by the Animal Care and Use Committee of Nanjing Drum Tower Hospital (2020AE01018).

**Experimental design**

Male mice were randomly divided into six groups: sham-operated control, LPS control, DCS control, CLP + DCS, and CLP + LPS. The CLP + LPS group was subjected to CLP (on day 1), followed by LPS administration (1 mg/kg, on day 11). The sham and DCS groups each contained 10 mice, and the remaining groups contained 20 mice each. The evaluation included four aspects consistent with the main pathophysiology of PIICS, namely survival and phenotype, catabolic indicators (body weight, muscle morphology, and protein levels), inflammation-related indicators (inflammatory cytokines, chemokine, and C-reactive protein [CRP]), and immunosuppression indicators (populations of myeloid-derived suppressor cells (MDSCs), interferon [IFN]-γ CD8+ T cells, and soluble programmed death-ligand 1 [sPD-L1]). All mice in different groups were euthanized at day 14, and the samples were collected. The experiments were carried out in accordance with the Minimum Quality Threshold in Preclinical Sepsis Studies (MQTIPSS) guidelines for standardizing the animal model of sepsis (see Supplemental Digital Content 1, Supplemental Table 1, http://links.lww.com/SHK/B373, CLP + LPS model adherence to MQTIPSS) (13).

**CLP**

CLP surgeries were performed under 2.5% isoflurane anesthesia as described previously (14). Briefly, after shaving and disinfecting the abdominal area, a 1 cm midline-incision laparotomy was performed, and the cecum was exposed. The latter one-third of the cecum was ligated with a 4-0 silk suture, and a full-thickness puncture was made using a 20-gauge needle. Care was taken to ensure that the ligation was completed without damaging the mesenteric blood vessels. A small amount of cecal content was gently squeezed out to ensure full-thickness cecum perforation, being the source of abdominal infection. Thereafter, the cecum was returned to the abdomen, and the incision was closed using a 4-0 silk suture in two layers. The mice were resuscitated with 0.6 mL sterile saline and allowed to recover on a heating blanket at 37°C until they were completely awake. To ensure the desired post-experimental mortality, the mice were administered postoperative antibiotics (25 mg/kg imipenem monohydrate, every 12 h, intramuscular injection) and fluid resuscitation (0.6 mL 0.9% sodium chloride, every 12 h, subcutaneous injection) from 6 to 72 h post-CLP. Meloxicam (2 mg/mL, Dobio, China) at a dosage of 20 mg/kg daily was used for postoperative analgesia for 48 h.

**DCS**

A CLP + DCS mice model of PIICS was generated as described previously (12). On the next day following the CLP procedure, the mice were restrained in a special holder for 2 h in the morning. To avoid stress endurance and to simulate the type of stress typically experienced in the ICU, a modified, chronic, unpredictable stress was combined with restraint. Unpredictable stress was randomly presented from days 2 to 13 and included cage tilt at 45° for 2 h, wet litter in a squirrel-cage overnight, exposure to darkness for 3 h during the daytime phase, and exposure to light overnight (15). LPS (Escherichia coli O55:B5, Sigma, St. Louis, MO; L2880) was diluted in 0.9% sodium chloride and injected intraperitoneally at a dose of 1 mg/kg on day 11 in the CLP + LPS group and LPS group. Mortality and weights were recorded daily for all mice.

**Blood sample collection and laboratory analysis**

Mice were euthanized following isoflurane inhalation. Their blood was collected in heparin tubes (BD Biosciences, Franklin Lakes, NJ) and centrifuged at 4°C for 5 min at 300 × g, from which the plasma was collected and stored at -80°C. Aspartate aminotransferase (U/L) was evaluated at the Department of Laboratory Medicine, Nanjing Drum Tower Hospital.

**Real-time quantitative polymerease chain reaction (qPCR)**

Total RNA was extracted from the mouse gastrocnemius extensor muscles using TRIzol reagent (Servizio, China, Wuhan; G3013) and reverse transcribed into cDNA. qPCR was performed with a 2 × SYBR Green qPCR Master Mix (Low ROX) (Servicebio, G3321) on a qPCR machine (CFX, Bio-Rad, Hercules, CA) for 40 cycles using the following primers: muscle ring-finger protein-1 (Murf-1): 5’-GATGTGCCAAGGAACGAA-3’ (forward) and 5’-CCTTCACTCTTTGCTATTCT-3’ (reverse); Forkhead box O-1 (Fox-O1): 5’-CGTGGATCCTCCGTTGTATCC-3’ (forward) and 5’-GGCGGTGCAAGAATGC-3’ (reverse); Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5’-CCTTGGCTCCGTGAGAACAT-3’ (forward) and 5’-TGAGCTCAATGAAGGGTTGCTG-3’ (reverse). The expression of each gene was normalized to that of an internal control gene (GAPDH mRNA) and calculated using the 2–ΔΔCT method (where ΔΔCT = ΔCTtarget - ΔCTGAPDH).

**Western blotting**

Proteins extracted from the extensor muscles were dissolved in lysis buffer, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto polyvinylidene difluoride membranes. Total protein levels were determined using the Pierce™ BCA Protein Assay Kit (Thermo Scientific). The following primary antibodies were used to detect proteins on the membrane: anti-Murf-1 (1: 10,000, Protein-tech, Rosemont, IL; 55:5465-1-AP), anti-Fox-O1 (1: 200,000, Servicebio, Wuhan, China; GB11286), and anti-GAPDH (1: 1000, Servicebio; GB12002). The membranes were then incubated with secondary antibodies diluted at 1:3000 in Tris-buffered saline for 30 min, and the blots were developed with a chemiluminescent reagent (Pierce Biotechnology, Rockford, IL.). Densitometric analyses of the western blot images were performed using AlphaEase FC software (Alpha Innotech, San Jose, CA).

**Plasma cytokine analyses**

The levels of tumor necrosis factor (TNF)-α, chemokine (C-C motif) ligand (CCL) 2, CCL 4, CCL 7, chemokine (C-X-C motif) ligand (CXCL) 2, CXCL 10, interleukin (IL)-6, and IL-10 in the plasma were measured using commercially available Luminex Mouse Magnetic Assay kits (R&D Systems, Minneapolis, MN). All assays were performed according to the manufacturer’s protocols. Cytokine levels were then determined using MILLIPLEX Analyst (V5.1) software (Millipore, Billerica, MA). Commercial ELISA kits were used to evaluate the levels of CRP (USCN Life Science, Wuhan, China; SEA821Mu), sPD-L1 (USCN Life Science; SEA788Mu), albumin (USCN Life Science; CEB028Mu), and prealbumin (USCN Life Science; SEA726Mu) in the plasma according to the manufacturer’s instructions.

**Histology**

The liver, lungs, and spleen of mice were stored frozen at −80°C and processed for hepatocytoma and eosin staining (Department of Pathology, Nanjing Drum Tower Hospital). The stained sections were histologically evaluated by two independent observers blinded to the experimental data to assess the degree of organ injury (16, 17). The degree of lung inflammation was quantified based on a histologic score from 0 to 4, varying equally from no inflammation to mild, moderate, and severe inflammation. Histological evaluation of liver damage scores was as follows:

1. normal rectangular structure;
2. rounded hepatocytes with an increase in sinusoidal spaces;
3. vacuolization;
4. nuclear pyknosis; and
5. necrosis.

Extensor digitorum longus sections were incubated in 2.5% glutaraldehyde buffer solution in 0.1 M cacodylate (pH 7.4) and then fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer. The samples were dehydrated and embedded in epoxy resin overnight at 60°C and then observed and imaged using electron microscopy (HT7700, Hitachi, Tokyo, Japan).

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Flow cytometry

Spleen single-cell suspensions were prepared by passing the cells through a 70 μm pore-size cell strainer (BD Biosciences). To detect MDSCs, splenocytes were stained with fluorescein isothiocyanate labeled anti-CD11b (eBioscience, San Diego, CA) and PerCP-Cy5.5 anti-Gr-1 (Biolegend, San Diego, CA). MDSCs and PMN-MDSCs were detected using FITC anti-mouse CD11b (clone M1/70, eBioscience, San Diego, CA), BV786 anti-mouse Ly-6G (clone 1A8, BD Biosciences, San Diego, CA), and PerCp-cy5.5 anti-mouse Ly-6c (clone HK1.4, BioLegend, San Diego, CA) antibodies. To examine the IFN-γ protein level in CD8+ T cells, splenocytes were stained with Brilliant Violet 510 labeled anti-CD3 (Biolegend) and Alexa Fluor 488 labeled anti-CD8a (Biolegend), followed by intracellular staining with APC-labeled anti-IFN-γ (XMG1.2) (eBioscience). The cells were analyzed using an Attune Acoustic Focusing Flow Cytometer and the accompanying software (Life Technologies, Carlsbad, CA).

T cell suppression assays

Normal T cells were isolated from mice spleen using an EasySep™ Mouse T Cell Isolation Kit (Stemcell Technologies, Canada). Splenic T cells were stimulated with 2 μg/mL anti-CD3/CD28 antibodies (eBioscience, Waltham, MA) in RPMI 1640 medium. CD11b+Gr-1+ cells sorted by FACS were co-cultured with T cells (1:1, 2:1, 3:1) in a 96-well plate for 72 h. T cell proliferation was analyzed by 3H-thymidine incorporation and expressed as counts per minute (cpm).

Statistical analyses

Data are reported as means ± SD values. All analyses were performed using the IBM SPSS statistics software (version 23.0). The Shapiro–Wilk test was performed to test the normality of the data. When the assumption of homogeneity of variance was not met, logarithmic transformation was performed for the following indicators: IL-10, CCL 4, TNF-α, CXCL 10, IL-6, proportion of CD8+ T cells, and IFN-γ CD8+ T cells. A one-way ANOVA with post hoc analysis with the least significant difference test was used to compare the means among the six groups. Results with P < 0.05 were considered statistically significant.

RESULTS

Survival curve as well as inflammation and catabolism phenotype of CLP + LPS mice are consistent with those seen during PIICS

To induce sepsis and ensure a prolonged disease course, the classical CLP operation and postoperative treatment were modified, including the ligation of a limited percentage of the cecum and the single application of a large needle puncture. Effective rescue treatments including postoperative antibiotics and fluid resuscitation could protect CLP + LPS mice from acute death. The dose of LPS used to induce inflammation in the model mice was low. Schematic timelines for the different models are shown in Figure 1A. No animal died in the sham and DCS groups; the mortality rate in the CLP group was 45%, whereas the mortality rates in the CLP + DCS and CLP groups were 35% and 30%, respectively (Fig. 1B). The CLP + LPS group showed two severity peaks: CLP surgery leading to 30% mortality due to an infection episode, starting a sustained cycle of immunosuppression, catabolism, and inflammation and LPS leading to 15% mortality caused by recurrent inflammation. Weight loss in the CLP + LPS group was more severe than in the CLP + DCS and CLP groups. In comparison, the weight loss in the CLP + DCS and CLP mice generally showed slow weight gain, with good survival from 7 days after CLP (Fig. 1C). CLP + LPS mice had visible abdominal abscesses and even multiple abscesses on the visceral surface (see Supplemental Digital Content 2, supplemental figure 1). CLP + LPS mice showing typical more severe intra-abdominal abscesses. CLP + LPS mice also had obviously swollen spleens and markedly reduced skeletal muscles (see Supplemental Digital Content 3, supplemental figure 2). More severe consumptive catabolism is observed in the CLP + LPS mice than in the CLP + DCS and CLP mice

We focused on catabolism in PIICS, including both weight loss and the nutritional indices, such as albumin and prealbumin levels. Prealbumin is a conserved protein that transports holo-retinol-binding protein and thyroxine and is widely used to assess the clinical nutritional status, along with the use of albumin (18). Plasma prealbumin levels in mice in the CLP + LPS group were lower than those in mice in the sham group; furthermore, the CLP + LPS group showed significantly lower prealbumin levels than the CLP and CLP + DCS groups (Fig. 2A). Albumin levels in the CLP + LPS group were lower than those in the CLP and CLP + DCS groups, indicating a poor protein synthesis status in the former group (Fig. 2B). Significant muscle wastage was observed in the CLP + LPS mice, consistent with the clinical course of sepsis or severe
Trauma (10). Muscle atrophy and mitochondrial dysfunction contributed to muscle wastage. MuRF-1 and FoxO-1 were involved in muscle atrophy and showed higher expression in CLP + LPS mice than that in the other groups (Fig. 2C and D). The protein expression levels of FoxO-1 and MuRF-1 were verified by western blotting (Fig. 2E–G). Using transmission electron microscopy, myofibrillar fragmentation and striking morphological alterations of the mitochondria were clearly observed in the skeletal muscles of CLP + LPS mice (Fig. 2H).

**Inflammation severity is higher in CLP + LPS mice than in CLP + DCS and CLP mice**

One of the most important characteristics of PIICS is persistent inflammation, manifested as organ inflammation and the presence of inflammatory cytokines and chemokines. The degree of lung and liver damage is estimated based on inflammatory cell infiltration and organ damage, which in turn are quantified by measuring lung injury and liver damage scores, respectively. CLP + LPS mice exhibited more severe lung and liver injury and distinct hyperplasia of the germinal center and lymphocytes in the spleen than the other groups (Fig. 3A). CLP + LPS mice showed increased lung injury scores (Fig. 3B) and liver damage scores (Fig. 3C), indicating higher inflammation injury because of the combined effects of CLP and LPS. Moreover, aspartate aminotransferase levels were higher in CLP + LPS mice than in the other mouse groups (Fig. 3D).
inflammatory status. CLP + LPS mice had higher levels of CRP than the CLP + DCS and CLP groups (Fig. 4A). CLP + LPS mice showed significantly higher levels of TNF-α; IL-6; chemokine (C-C motif) ligands 2, 4, and 7; and chemokine (C-X-C motif) ligands 2 and 10 than CLP + DCS and CLP mice, indicating that CLP + LPS mice had an increased level of persistent inflammation on day 14 (Fig. 4B–H). IL-10 has been evaluated as a marker of sepsis outcomes and is highly correlated with inflammatory responses (19). CLP + LPS mice had higher levels of IL-10 than mice in the CLP + DCS, CLP, and sham groups (Fig. 4I).

Immunosuppression is more severe in CLP + LPS mice than in the CLP + DCS and CLP mice

Immune suppression was analyzed by typical indicators of immunosuppression and flow cytometry. The proportion of splenic CD8+ T cells was lower in the CLP + LPS group than in the CLP and CLP + DCS group (Fig. 5A and B). CLP + LPS mice showed significantly reduced numbers of IFN-γ CD8+ T cells (Fig. 5C and D), indicating the impairment of CD8+ T cells, which respond to infection. The proportion of MDSCs was significantly higher in the CLP + LPS group than in the other groups (Fig. 5E and F). Next, the inhibitory ability of MDSCs on T cells was evaluated, splenic MDSCs in CLP + LPS mice had a higher inhibitory activity than those in the other groups (Fig. 5G). Splenic PMN-MDSCs accounted for a more dominant proportion in all CD11b+ cells in CLP + LPS mice and had a higher proportion in all CD11b+ cells than other groups (Fig. 5H and I). The gating strategy and isotype controls of flow cytometry are described in Supplemental Digital Content 4.tif. Figure 3. The levels of sPD-L1, an important indicator of immunosuppression, were also significantly increased in the CLP + LPS group than in the other groups (Fig. 5J).

DISCUSSION

To mimic critical and protracted PIICS, a mouse PIICS model was created using a modified sublethal CLP combined with a low dose injection of LPS. CLP + LPS mice aimed to recapitulate the pathophysiology and severe critical course of PIICS, enabling mechanistic studies as well as an assessment of potential treatments.

PIICS is typically triggered by sepsis or devastating injury and is characterized by persistent high inflammation along with catabolism and immune suppression (20). The latter contributes to an increased risk of nosocomial infection, which in turn, leads to inflammation relapse (5). Prolonged inflammation, insufficient nutrient supply, and malabsorption accentuate muscle wasting (21, 22). There are no targeted treatments that can restore the normal state in patients with PIICS. In addition, PIICS notably differs from post-ICU symptoms (PICS), which
cause mental impairment and incomplete recovery after treatment in the ICU (23).

Based on the physiopathology and clinical characteristics of PIICS in the ICU, three main aspects should be indispensable in a PIICS model, that is, a sufficient degree of severity, a protracted course, and the presence of repeated inflammation. The composite multiple hits model may conform well to the features and common dynamic pathogenesis of critical PIICS. We refined the PIICS model to show the appropriate pathogenesis by modifying the CLP, effective rescue treatments, and repeated aggravated inflammation. The restrictive damage caused by modified CLP forms the basis for the formation of PIICS and ensures a relatively fixed degree of severity. Similar to clinical sepsis treatments, effective rescue treatments ensure a long survival time and delays the mortality peak following CLP (24).

Many cases of PIICS relapse without identification of the pathogenic bacteria or source and are typically difficult to identify, but the significant manifestations are recurrent fever and a severe inflammatory response (25). The runaway inflammation and ongoing severe organ dysfunction follow a shared terminal pathway (26). LPS, a constituent of gram-negative bacteria, has certain advantages in inducing an acute inflammatory response as well as endotoxic shock and has been used to elucidate numerous aspects of the pathophysiology of inflammation and immune function. Another advantage of using LPS is that the severity of inflammation and mortality can be easily controlled by adjusting its dose (27). Therefore, the second phase of aggravated inflammation was induced using LPS to mimic PIICS relapse. The low dose of LPS used induced a slight and temporal endotoxemia in normal mice but severe inflammation in the CLP + LPS mice. Three days after LPS injection, the acute inflammatory response began to weaken, whereas the anti-inflammatory response and catabolism increased in CLP + LPS mice. The aggravation after LPS injection is a clear recurrence point at which timely interventions and treatment may help rescue this state. Hence, by using this model, we can evaluate the effectiveness of the treatments.

For in-depth studies of the precise mechanisms of PIICS, there is no “one-fits-all” murine model. The various pathogenic and pathophysiological mechanisms make the heterogeneity of sepsis very obvious. One murine sepsis model can be used to recapitulate infection and inflammatory responses with definite sepsis etiologies but cannot be extrapolated to all types

Fig. 4. (A–H) Main inflammatory indices in the different models, including the plasma levels of CRP, TNF-α, IL-6, chemokine (C-C motif) ligand (CCL) 2, CCL 4, CCL 7, chemokine (C-X-C motif) ligand (CXCL) 2, and CXCL 10. Mice in the CLP + LPS group had higher cytokine, chemokine, and CRP levels than mice in the sham, CLP + DCS, or CLP groups. (I) The levels of IL-10, an important cytokine in the later stages of the inflammatory cascade, were significantly increased in the CLP + LPS group compared with the rest of the groups. Data are presented as the means ± SD, n = 5. * P < 0.05, ** P < 0.01, *** P < 0.005 vs. CLP + LPS; " P < 0.05, "" P < 0.01, """" P < 0.005 vs. sham. CLP indicates cecal ligation and puncture; CRP, C-reactive protein; IL, interleukin; LPS, lipopolysaccharide; TNF, tumor necrosis factor.
of sepsis. Philip and his colleagues clearly proved that CLP + DCS model mice can replicate surgical sepsis rather than community-acquired human sepsis in transcriptomic responses (28). CLP model mice do show PIICS characteristics and tend to recover (11, 29). Thus, this model may be suitable for studying the recovery phases of PIICS. The CLP + DCS model maintains moderate PIICS, along with stress in old mice, and may be more suitable for psychological research into PIICS, including delirium, depression, and cognitive impairment. Our CLP + LPS model is designed to mimic the aggravated stage of PIICS and to be used for studies on the pathophysiology of PIICS and for the development of effective treatments.

Consistency, repeatability, and ease of use of animal models are important for examining the pathophysiology and course of a disease. The MQTiPSS guidelines were the first to standardize preclinical sepsis models and improve the translation of preclinical findings (30). In our study, we followed the MQTiPSS guidelines to enhance the reproducibility and translational consistency of the PIICS model.

There are several limitations to our study. First, only mature male mice were used, which somewhat narrows the scope. The precise mechanism by which PIICS arises is only beginning to be understood, and further research is much required. Our study focused on immunity, catabolism, and inflammation in PIICS, and numerous factors are likely to affect these aspects of PIICS. To prevent too many factors from being intertwined, we actively avoided the influence of age and estrogen, which can clearly affect PIICS outcomes. It will therefore be meaningful to explore PIICS changes in female mice, older mice, or in mice with comorbidities. Second, the endpoints of all models used in this study were assayed on day 14, which slightly differs from the endpoint assessment of the CLP model reported by

Fig. 5. Assessment of the suppression of cluster of differentiation (CD)8⁺ T cells and the expansion of MDSCs in the spleen, as well as the level of immunosuppression indicators in the different groups. (A and B) CD8⁺ T cell suppression was significantly lower than that seen in other PIICS models. (C and D) IFN expression in CD8⁺ T cells, induced by soluble CD3 and CD28, reflecting the functional reserve of CD8⁺ T cells. (E and F) Compared to controls, both PIICS model mice had increased proportions of MDSCs. (G and I) The inhibitory ability of MDSCs on T cells in CLP + LPS mice was higher than that in CLP and CLP + DCS groups. PMN-MDSCs accounted for a more dominant proportion in all CD11b⁺ cells in CLP + LPS mice. (J) The level of soluble programmed cell death-ligand 1 (sPD-L1), which represents an important indicator of immunosuppression, was significantly increased in the CLP + LPS group compared with the CLP and CLP + DCS groups. Data are presented as the means ± SD, n = 5. In (A, C, E, H, I, J), *P < 0.05, **P < 0.01, ***P < 0.005 vs. CLP + LPS; †P < 0.05, ‡P < 0.01, §§P < 0.005 vs. CLP + LPS; ††P < 0.05, ‡‡P < 0.01, §§§P < 0.005 vs. sham. In (G), *P < 0.05, **P < 0.01, ***P < 0.005 vs. CLP + LPS; †P < 0.05, ‡P < 0.01, §§P < 0.005 vs. no-MDSC. CLP indicates cecal ligation and puncture; IFN, interferon; LPS, lipopolysaccharide; MDSCs, myeloid-derived suppressor cells.
Based on CLP-triggered sepsis, effective rescue treatments, coupled with LPS-mediated induced repeat inflammation, a novel model of PIICS was developed. This murine CLP + LPS model exhibited the principal features of PIICS, recapitulated a prolonged clinical PIICS course, and enables studies on identifying interventions and treatments.

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