

Original Article

IL-22/IL-22R1 axis is involved in myocardial injury of a mouse cecal ligation and puncture model

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Abstract: Myocardial depression is a hallmark of severe sepsis, which may result from a complex interplay among several factors. However, the mechanisms are still unclear yet. In this study, we aimed to explore if IL-22/IL-22R1 axis plays a role in the myocardial injury during sepsis. A cecal ligation and puncture (CLP) mouse model was established to explore the histopathological changes and to analyze the role of IL-22/IL-22R1 axis in myocardial injury during the process of sepsis. Histopathologically, myocardial injury was apparently observed with the progress of sepsis but it was improved at 72 h after surgery. On the contrary, the heart tissue in the sham group revealed injury at a limited degree at the first 8 h after surgery and then restored to normal. Results from immunohistochemical study and real-time qPCR showed that IL-22, IL-22R1 and IL-22BP had different changing trends in the progress of sepsis at both protein and mRNA levels. The expression of IL-22R1 and IL-22BP was markedly induced after CLP modeling ($P < 0.01$), while that of IL-22 was sharply reduced in both groups ($P < 0.01$). The differences in the expression of IL-22, IL-22R1 and IL-22BP between the sham and CLP groups were significant only at 72 h after surgery ($P < 0.05$) but not at the other time points ($P > 0.05$). In conclusion, IL-22/IL-22R1 axis is involved and may have a potential immunoprotective role in the cardiac tissue repair, but the immunoprotection on the cardiac tissue of CLP mice was remarkably damaged in the progress of sepsis and even in the recovery phase.

Keywords: Cecal ligation and puncture, sepsis, mouse, IL-22, IL-22R1, IL-22BP

Introduction

Sepsis refers to severe systemic inflammation initiating a complex immune response that varies over time and accompany with both pro-inflammatory and anti-inflammatory mechanisms [1]. Life-threatening but potentially reversible organ dysfunction is its hallmark, and the organ dysfunction failed to be restored is the dominant cause of death in the patients with sepsis [2]. An overwhelming immune response mediated by the systemic release of inflammatory mediators leads to endothelial and microvascular dysfunction, edema, and vasodilatation that are mainly involved in sepsis-associated organ dysfunction and even death [3]. The overproduction of inflammatory mediators including cytokines, proteases, lipid mediators, vasoactive peptides, and cell stress markers amplify inflammation and tissue damage and thereby play pivotal roles in sepsis pathophysi-

ology. The high levels of the proinflammatory cytokines and anti-inflammatory cytokines in the blood stream induce an autodestructive systemic inflammatory reaction, which is termed "cytokine storm" [4]. Cytokine storm vividly reflects the disorder of immune system and the uncontrollable inflammatory reaction. Since previous clinical trials aiming to block or inhibit inflammation by biomodulators have achieved no improvement in the outcomes of patients with severe sepsis, septic shock and multiple organ dysfunction syndrome (MODS), researchers focus their attentions on balancing the pro-inflammatory and anti-inflammatory mechanisms for newer therapies [5]. However, the interplay between the proinflammatory and anti-inflammatory cytokines still needs further clarification.

In a single-center clinical study, serum interleukin 22 (IL-22) level has been found to be signifi-

cantly higher in patients with abdominal sepsis than in those undergoing abdominal surgery without sepsis [6]. Ziesche E *et al.* also reported that serum IL-22 is upregulated in a rat CLP model [7]. IL-22 is a pro- and anti-inflammatory cytokine that is mainly produced by lymphocytes and signals through IL-22R1/IL-10R2 heterodimer, and thereby activates a signal-transduction cascade [8]. Although IL-10R2 is widely expressed on various types of cells, the expression of IL-22R1 is normally restricted to certain tissues such as epithelial cells and stromal cells, but not on the immune cells. Thus, the organs but not the immune cells are the targets of IL-22, via the bridge role of IL-22/IL-22R1 axis in the crosstalk between immune cells and tissues. IL-22 has been reported to be tissue protective in wound healing, liver regeneration and epithelial barrier [9]. And Rendon JL *et al.* also suggested IL-22 may act as a therapeutic agent in the treatment of critically ill patients who sustain secondary organ damage, such as burn and trauma patients [10]. However, another study showed that IL-22 blockade can elevate the bacterial clearance in liver and kidney and reduce renal injury in polymicrobial peritonitis [11]. Regarding this, it could be helpful to find out a new target for the treatment of sepsis if we clarified how the organs become the targets of immune reaction in sepsis.

Turillazzi E *et al.* believed that the host's immune-inflammatory response plays a pivotal role in the pathophysiology of myocardial depression in sepsis [12]. Additionally, it has been reported that a complex interplay among several factors contributes to the myocardial dysfunction in sepsis [13]. Myocardial depression is a hallmark of severe sepsis, characterized by hypotension or shock [14]. It has been reported that plasma IL-22 levels are significantly increased in acute coronary syndrome (ACS) patients and have a pivotal role in the development of atherosclerosis and the onset of ACS [15] and increased IL-22 levels play an important role in the pathogenesis of CVB3-induced acute viral myocarditis [16]. Apparently, IL-22 is correlated with several cardiovascular diseases such as ACS and acute viral myocarditis. Therefore, we proposed that IL-22/IL-22R1 axis is involved in the myocardial dysfunction and may have some roles in sepsis. In this study, we used a CLP mouse model to explore if IL-22/IL-22R1 axis is involved in the myocardial

injury during sepsis and may play some roles in the progress of sepsis. This study would provide a new prospect for the researches of sepsis and add a potential new target for the clinical treatment of sepsis.

Materials and methods

Ethical statement

The protocol in this study was approved by the Institutional Animal Care and Use Committee of 306 Hospital of PLA, Beijing, China. All animals were cared in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (8th edition; Washington DC, National Academic Press, 2011).

Animals

A total of 191 female C57BL/6J mice, aged 6-8 weeks and weighing 18-22 g, were purchased from China Research Institute of Food and Drug Verification (SCXK [Jing] 2014-0013, Beijing, China) and housed at a room with controlled room temperature and natural day/night cycle. The animals were free access to food and water. The mice were housed for at least 1 week for acclimation before surgery.

Mouse cecal ligation and puncture (CLP) model of sepsis

The establishment of CLP model was performed by an experienced surgeon (Yang HM). CLP or sham surgery was carried out following the methods of Watanabe E *et al.* as previously described [17] with minor modifications. Before initiating surgical procedures, mice were anesthetized with ketamine (100 mg/kg) by intraperitoneal injection. A small midline abdominal incision was performed, and the cecum was exposed and ligated immediately with a sterile 3-0 silk suture at the middle of cecum. To prevent cecum from ischemic necrosis, cecal ligation should not be too tight. Then the cecum was punctured through once with a 22G needle at the cecum distal to the point of ligation, followed by extrusion of a small amount of fecal material into the peritoneal cavity. After that,

the cecum was returned to the abdominal cavity, and the abdominal wall was closed in two layers. Each animal received a subcutaneous injection of 100 μ L warm 0.9% NaCl immediately to compensate for fluid loss. Sham-operated mice underwent laparotomy and cecum exposure but without cecal ligation or puncture. Mice were food-deprived but had free access to water postoperatively.

Tissue sampling

Six of the living mice in both groups were sacrificed at postoperative 8 h, 32 h and 72 h respectively by CO₂ asphyxiation for collecting the heart tissues. Then, heart was cut into two parts, one of which was fixed in 10% neutral buffered formalin for histopathological observation, and the other was frozen in liquid nitrogen for mRNA extraction.

Histopathological analysis

Tissue samples collected for histopathological observation were fixed in 10% neutral buffered formalin for 48 hours and then subjected to dehydration and paraffin embedding. Serial sections of 4 μ m thickness were obtained to stain with hematoxylin and eosin (H&E) following the traditional procedures. The histopathological change of each sample was evaluated by an experienced pathologist (Zhang JZ).

Immunohistochemical (IHC) staining

Immunohistochemical staining for IL-22, IL-22R1 and IL-22BP was performed using PV-9001/9004 kits (Beijing ZSGB Biotech, Ltd, Co., China). Firstly, 4-mm sections from paraffin-embedded tissue blocks were conventionally dewaxed and rehydrated, followed by incubation with 3% hydrogen peroxide (H₂O₂) for 30 min in dark to eliminate endogenous peroxidase. Thereafter, the slides were subjected to high-pressure antigen retrieval in EDTA buffer (pH 9.0) in a pressure cooker for 2 min and cooled naturally to room temperature. After incubated with 5% BSA in PBS for 30 min, sections were incubated with primary antibodies (1:500 diluted rabbit anti-mouse IL-22 polyclonal antibody (ab18499, Abcam, USA); 1:250 diluted rat anti-mouse IL-22R1 polyclonal antibodies (MAB42941, R&D Systems Co., USA); 1:1000 diluted rabbit anti-mouse IL-22RA2 (IL-22BP, ab203211, Abcam, USA)) or 2% BSA in

PBS as negative control at 4°C overnight. After rinsed with 0.01 M PBS, the slides were incubated with the corresponding secondary antibodies (PV9001 kit for rabbit or PV9004 kit for rat) according to the manufacturer's instructions. After developed by DAB kit (Beijing ZSGB Biotech, Ltd, Co., China), the nuclei of the sections were counter-stained with hematoxylin. After dehydration and mount, the slides were read under a light microscope by an experienced pathologist and photographed at five fields of the positive areas at high magnification (400 $\times$).

Interpretation of IHC results

IL-22 and IL-22BP localized in the cell cytoplasm, while IL-22R1 located in cell membrane or cytoplasm. The expression of the targets was evaluated by intensity and positive rate. The intensity of positive staining was scored as 0 for no positive staining, 1 for faint yellow, 2 for brown and 3 for dark brown; the positive rate was scored as 0 for no positive, 1 for 1%-25%, 2 for 25%-50% and 3 for 50% or more. The product of intensity score and positive rate score was regarded as the score of the field. Five fields of the positive areas of each sample were evaluated at high power magnification (400 $\times$) and then the overall IHC score of each sample was semi-quantitatively defined as mean score of the five fields.

Total RNA extraction and real-time PCR

Total RNA from frozen tissues were extracted using Trizol reagent (Invitrogen, Thermo Fisher, Shanghai, China) according to the manufacturer's instruction. After quantified by NanoDrop 1000 Microvolume Spectrophotometers (Thermo Fisher, Shanghai, China), 1 μ L total RNA was used to synthesize the first-strand cDNA using PrimeScript™ RT reagent Kit (RR037A, Takara, Japan) following the manufacturer's instructions. Subsequently, quantitative PCR was performed using SYBR Premix Ex Taq™ II (RR820A, Takara, Japan) on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA). The specific primer pairs for genes used in this study were listed in **Table 1**. The reaction mixtures were incubated at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 40 s. After amplification, melting curve analyses were performed in order to validate the specific generation of the expected PCR prod-

Table 1. The specific primer pairs for genes used in this study

Gene	Forwards	Reverse	Product size (bp)
IL-22	5'-ATGAGTTTTTCCCTTATGGGGAC-3'	5'-GCTGGAAGTTGGACACCTCAA-3'	124
IL-22R1	5'-ATGAAGACACTACTGACCATCCT-3'	5'-CAGCCACTTTCTCTCTCCGT-3'	198
IL-22BP	5'-GCTCTTCTGTGACCTGACCA-3'	5'-AGTCACGACCGGAGGATCTA-3'	154

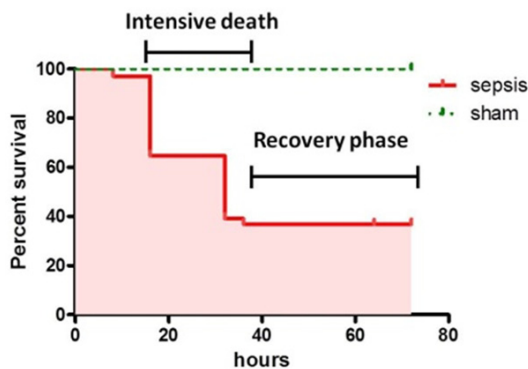


Figure 1. Survival curve of mice in CLP model group and sham group. One hundred female C57BL/6J mice were randomly divided into sham ($n = 30$) and CLP modeling ($n = 70$) groups and closely observed for more than 72 h after surgery. The survival analysis was performed using Kaplan-Meier method and compared using log-rank test. It was revealed that the CLP mice most died between 16 h and 32 h after modeling and then began to recover. Log-rank test showed significant difference between the survival rates of the two groups.

uct. All samples were run in triplicate. The expression levels of IL-22, IL-22R1 and IL-22R2 were determined using the $2^{-\Delta\Delta Ct}$ method [13] with the internal gene GAPDH (B661304, Sangon Biotech. Co. Ltd., China).

Statistical analysis

IBM SPSS v17.0 statistics software (Armonk, New York, USA) was used for data processing in this study. Data are shown as mean $\pm$ standard error if they were normally distributed, or they were expressed as median (range). Since this is a 2×3 factorial design, two-way ANOVA were used to analyze the interaction between treatment and time. For the comparisons among the time points in a group, Mann-Whitney U test of non-parametric analysis was used, while for the group-group comparisons in a time point, rank sum test was used. Graphpad prism 6 (La Jolla, CA, USA) was used for establishing the graphs. The survival analysis was performed using Kaplan-Meier method, while the comparison of the survival rates between different treatments

was carried out using log-rank test. $P < 0.05$ was considered as significant difference.

Results

The survival of CLP model is approximate to that of patients with sepsis

At first, 100 mice were used to observe the survival rates of mice in the CLP model ($n = 70$) and sham group ($n = 30$) established in our laboratory, so as to confirm how many mice should be used in the following experiment. As shown in **Figure 1**, all mice in the sham group survived for more than 72 h, while the mice in CLP group began to die from 8 h after the surgery, and died intensively during 16 h and 32 h after the surgery. Then the living mice began to get better and survived for more than 72 h. In this study, the survival rate of CLP group was 36.8% and that of the sham group was 100%, with a significant difference ($P < 0.01$). The survival rate of CLP model mice was approximate to that of patients with severe sepsis. Thus, the CLP model was successfully established in our laboratory and suitable for the following experiments.

From the survival curve, we knew that postoperative 16 h to 32 h was the death phase of CLP mice. After that, the CLP mice began to recover. Thus, the end point of our observation was set at 72 h. Considering the survival rate of CLP, another 91 mice were used for the following experiment, among which 18 mice were used for sham group and 67 were used for CLP group, and the remaining 6 healthy mice were sacrificed directly at the last time point to serve as control group (0 h) for both sham and CLP groups.

Degeneration of cardiomyocytes is histopathologically observed in CLP mouse

Histopathologically, compared with the control group (0 h), the acidophil of cardiomyocytes in both sham and CLP group was attenuated obviously (**Figure 2**). However, the acidophil of car-

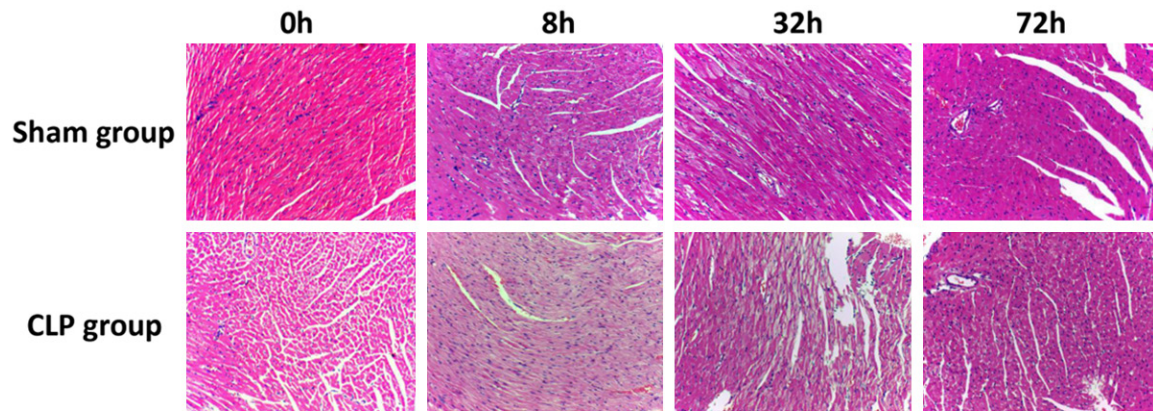


Figure 2. The histopathological features of heart in both groups at different time points (H&E staining, 200 ×). Heart tissues collected from the mice of both groups at different time points were fixed with neutral buffered formalin and paraffin-embedded. Then the 4 μm sections were stained with hematoxylin and eosin. The normal control group served as 0 h subgroup for both sham and sepsis groups (n = 6 for each time point). The cardiac tissue of CLP mice was seriously destructed at 8 h and 32 h after modeling and had a marked improvement at 72 h after modeling, while there was no significant histopathological change found in the sham group.

diac myocytes restored gradually with time, especially in the sham group, which was almost restored to the normal level. Degeneration of cardiomyocytes was obviously observed in the 8 h and 32 h subgroups of CLP group, especially in the 32 h subgroup, and the eosin staining decreased significantly. However, if the mice survived to the 72 h after surgery, the histopathological appearance was significantly improved as compared with that in the 8 h and 32 h subgroups of CLP group, although a small amount of myocardial fiber dissolution was still found. Moreover, cardiomyocyte vacuolization was also observed in CLP group at 32 h and 72 h subgroups, which was more serious in 32 h subgroup. No obvious inflammatory cell infiltration was found in both groups at all the time points.

Expression of IL-22, IL-22R1 and IL-22BP mRNA in heart tissues in CLP group is remarkably different from that in sham group at post-operative 72 h

In view of the histopathological changes in heart tissues, we expected if the expression of IL-22/IL-22R1 axis, a bridge between immune cells and organs, is also affected by CLP in heart tissue. Then we detected the mRNA expression of IL-22 and IL-22R1 in heart tissues of both groups using real-time PCR. As shown in **Figure 3**, the mRNA expression of IL-22 was obviously high in normal heart tissues (0 h), but it was sharply reduced by surgery in both sham

and CLP groups at 8 h after surgery with significant differences ($P = 0.001$ and $P < 0.0001$). Then the mRNA expression of IL-22 began to rise gradually and almost reached the normal at 72 h after surgery in the sham group. However, it kept to decrease in CLP group until 32 h after surgery and then had a weak increase at 72 h after surgery. Two-way ANOVA showed that there was an interaction between treatment and time. There were significant differences in the mRNA expression of IL-22 between sham and CLP groups at 32 h and 72 h after surgery ($P = 0.02$ and $P = 0.005$, Kruskal-Wallis test). On the contrary, IL-22R1 mRNA almost did not express in normal cardiac tissue, but its expression rose obviously after trauma in both sham and CLP groups within 8 h after operation, especially in CLP group ($P = 0.017$ and $P < 0.001$, **Figure 3**). Then, it kept rising in CLP group as time went on and reached a peak at 72 h after surgery, but it turned to decrease and almost returned to normal value at 72 h in sham group. In addition, the expression of IL-22R1 mRNA in CLP group was significantly higher than that in the sham group at 72 h after surgery ($P = 0.005$). The results showed that the expression of IL-22R1 mRNA had opposite change trend to that of IL-22 mRNA.

We also determined the mRNA expression of the soluble IL-22 receptor, a natural antagonist of IL-22, in heart tissues of both groups. As shown in **Figure 3**, IL-22BP mRNA was weakly expressed in normal cardiac tissue either, but

IL-22/IL-22R1 axis in myocardial injury of CLP mouse

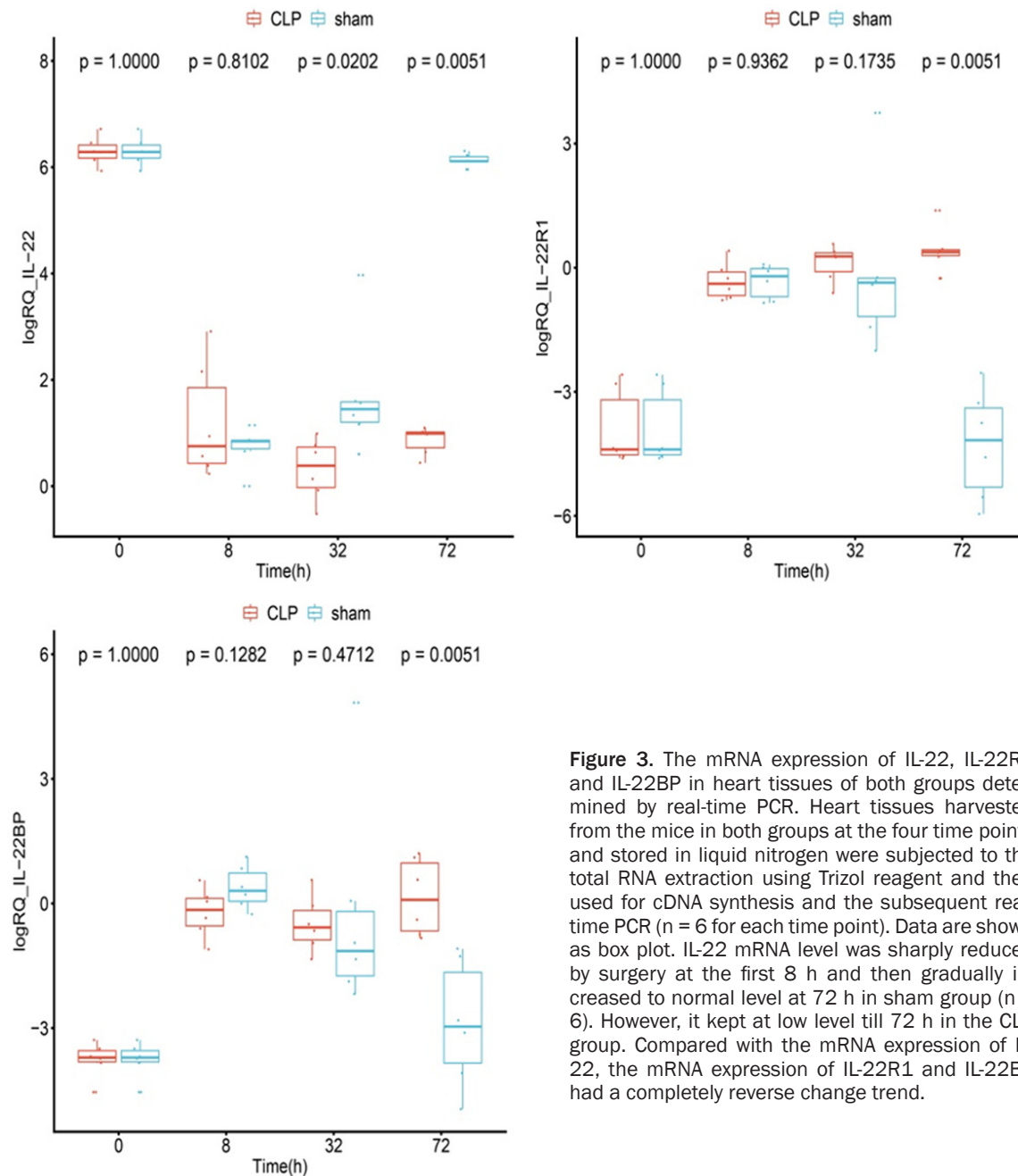


Figure 3. The mRNA expression of IL-22, IL-22R1 and IL-22BP in heart tissues of both groups determined by real-time PCR. Heart tissues harvested from the mice in both groups at the four time points and stored in liquid nitrogen were subjected to the total RNA extraction using Trizol reagent and then used for cDNA synthesis and the subsequent real-time PCR (n = 6 for each time point). Data are shown as box plot. IL-22 mRNA level was sharply reduced by surgery at the first 8 h and then gradually increased to normal level at 72 h in sham group (n = 6). However, it kept at low level till 72 h in the CLP group. Compared with the mRNA expression of IL-22, the mRNA expression of IL-22R1 and IL-22BP had a completely reverse change trend.

it was induced rapidly in both sham and CLP groups at 8 h after operation ($P = 0.001$ and $P < 0.0001$). Then the expression of IL-22BP mRNA was declined rapidly in sham group and almost back to the normal at 72 h after operation. However, in the CLP group, after a bit decrease at 32 h after operation, the expression of IL-22BP mRNA was increased again. There was a significant difference between sham and CLP groups at 72 h after operation ($P = 0.005$, Kruskal-Wallis test).

Protein expression of IL-22, IL-22R1 and IL-22BP in heart tissues has a similar change trend to the mRNA expression in both groups

Regarding the changes in the mRNA expression, we expected if there is a similar change in the protein expression of IL-22/IL-22R1 axis. Thus, we detected the protein expression and location of IL-22/IL-22R1 axis and its antagonist IL-22BP using IHC methods. IL-22 is only produced by immune cells but not by any other

IL-22/IL-22R1 axis in myocardial injury of CLP mouse

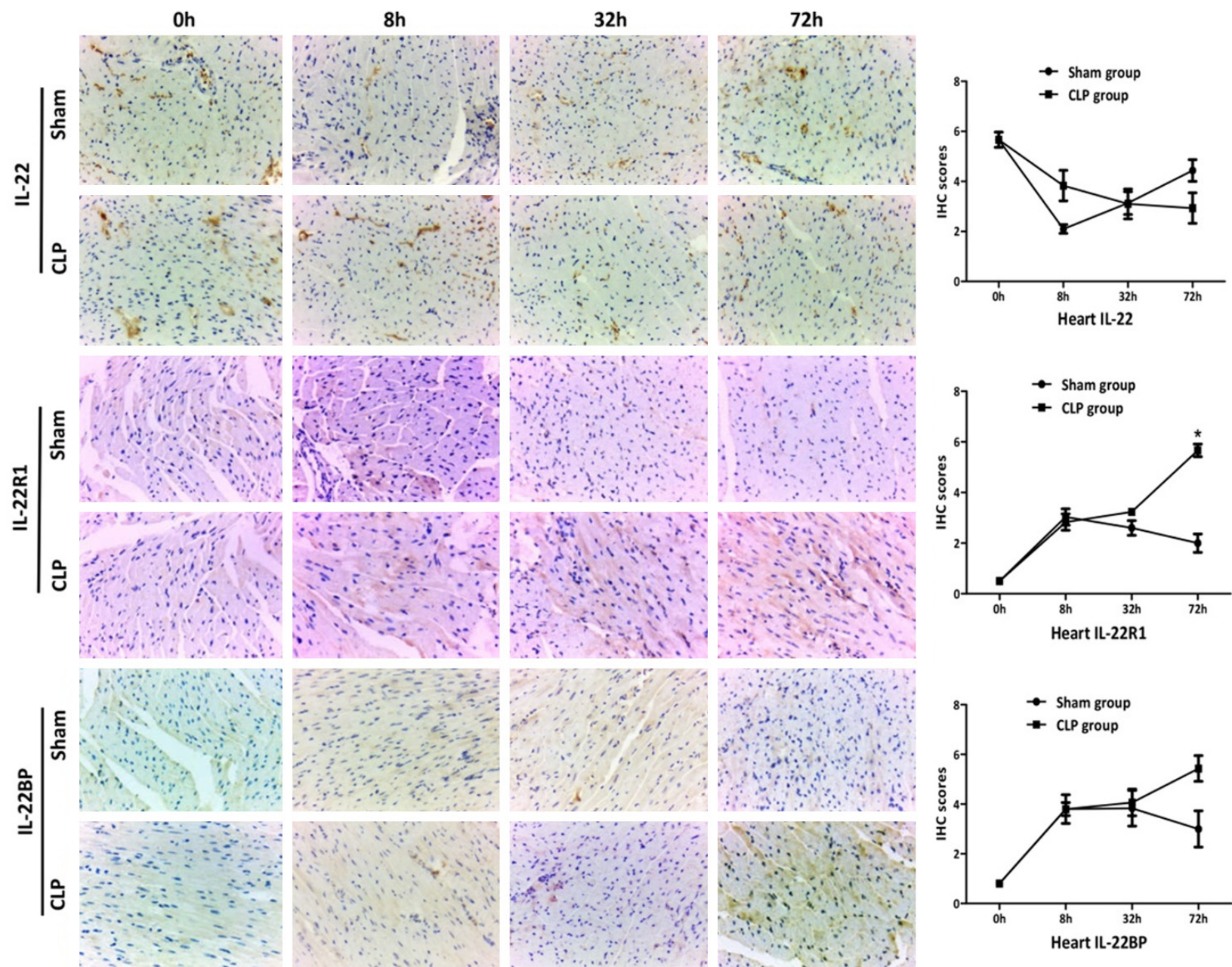


Figure 4. Protein expression of IL-22, IL-22R1 and IL-22BP in heart tissues in both groups determined by IHC (PV9001/9004 method, 400 ×). The 4 μm sections cut from paraffin-embedded heart tissues were subjected to IHC assay with primary antibodies IL-22, IL-22R1 and IL-22BP, respectively. After that, the sections were incubated with the corresponding secondary IgG (PV9001/PV9004 kit) and then developed by DAB kit. The immunoreactivity of the cells were semi-quantitatively transferred into scores and statistically analyzed at the right column. Data are shown as means ± S.E. (n = 6).

non-immune cells. In this study, no infiltrating lymphocytes were found in heart tissue, thus, IL-22-producing cells were only found in the blood vessels. However, the positive staining for IL-22 was also obviously seen in the intercellular space of cardiomyocytes. As shown in **Figure 4**, high expression level of IL-22 was found in the intercellular space of cardiomyocytes in the control group (0 h), but it was prominently reduced in both sham and CLP groups at 8 h after surgery, and continued to decrease at 32 h and 72 h after surgery in the CLP group. However, in the sham group, IL-22 expression was then elevated gradually at 32 h and 72 h after surgery. After scoring, the expression of IL-22 in both groups was shown in a line chart (**Figure 4**). The IHC scores of IL-22 in the two groups were compared at each time point, and the results showed that there was no significant difference at any time point. However, the comparisons of IHC scores among the time points in both groups showed significant differences ($P = 0.0002$), suggesting that time has a more obviously influence on the protein expression of IL-22 in heart tissue than the CLP modeling, which is similar to that on the mRNA expression of IL-22.

IL-22R1 is normally expressed by nonimmune cells in several organs including heart. In this study, IL-22R1 was less expressed in the heart tissues of normal group, but it was highly expressed by cardiomyocytes in CLP group after surgery and the expression was gradually elevated as time went on (**Figure 4**). On the contrary, IL-22R1 expression was increased at a limited degree in sham group at 8 h after surgery and then gradually decreased. The comparisons of IL-22R1 scores between the two groups at each time point revealed a significant difference at 72 h after surgery ($P < 0.05$) but not at other time points. However, the comparisons among the time points in both groups showed that there was significant difference in the CLP group ($P < 0.0001$) but not in the sham group, suggesting that the protein expression of IL-22R1 in cardiomyocytes was influenced by CLP modeling and might have a role in the process of sepsis.

IL-22BP is mainly produced by the mononuclear cells of inflammatory infiltration sites, plasma cells, and a subset of epithelial cells [18]. In this study, IL-22BP was weakly expressed in the heart tissues of control group but highly expressed after surgery in both sham and CLP groups at 8 h and 32 h after surgery, and then its expression remained at a high level in CLP group but began to decrease in the sham group at 72 h after surgery (**Figure 4**). The comparisons of IL-22BP scores between the two groups showed no significant difference at each time point, but the comparisons among the time points showed only a significant difference between 0 h and 72 h in the CLP group ($P < 0.001$, **Figure 4**), suggesting that the expression of IL-22BP was enhanced by surgical injury and keeping at the over-activated state during the progress of sepsis even at the recovery phase of sepsis.

Discussion

It has been confirmed that CLP mouse model has similar clinical features to human patients with sepsis [19], and thus we used CLP mouse model to monitor the histopathological changes and the IL-22/IL-22R1 axis expression in heart tissues during the progress of sepsis. The CLP mouse model established in our laboratory had a survival rate of 36.8%, which is almost consistent with the survival of severe sepsis in human. As shown in the survival curve (**Figure 1**), the CLP mice had an intensive death between 16 h and 32 h and then survived for more than 72 h. Therefore, we believed that the mice entered into the recovery phase after 32 h if they ran through the intensive death period. Based on the survival of the CLP mice and the changes in serum proteomic profiles (not shown), we selected 0 h, 8 h, 32 h and 72 h as our observation time points.

It has been reported that decreased body temperature, blood pressure and heart rate (HR) are important clinical features in the early stage of sepsis [19], and these features were also found in the CLP mice established in our labo-

ratory. To clarify the pathological basis of cardiac dysfunction in CLP mice, we carried out histopathological examination on the CLP mice at different time points and found that the eosin staining of cardiac myocytes was weakened in the heart tissues of both groups, especially in the CLP group. Moreover, myocardial degeneration and vacuolization was also observed in the CLP mice during the intensive death period, but had remarkable improvement in the recovery phase. On the contrary, the heart tissues in sham group began to recover gradually after postoperative 8 h and almost restored to the normal at 72 h after surgery. Our histopathological results provided a strong pathological basis for the cardiac dysfunction involved in the progress of sepsis.

It is reported that mediators release induced by infection in peritonitis causes direct myocardial effects and subsequently leads to cardiovascular insufficiency in severe multiple organ system dysfunction [20]. To date, there are only a few studies considering the expression of IL-22 or its receptor in sepsis [6, 7, 11]. In this study, the expression of IL-22 was sharply downregulated in heart tissue of both sham and CLP mice at both the mRNA and protein levels within the first 8 h after surgery. But it was then gradually returned to the normal at 72 h after surgery in the sham group although it kept decreasing in the CLP group. Weber GF *et al.* have reported that the mRNA expression of IL-22 was markedly induced in spleen and kidney but not in liver in the course of sepsis [11], prompting that the regulation of IL-22 expression was different in organs. The comparisons of IL-22 expression among the time points and between the groups indicated that time has a prominent influence on IL-22 expression but the CLP modeling only has influence at 72 h after operation.

In this study, the expression of IL-22R1 and IL-22BP was remarkably induced in the heart tissue of both sham and CLP groups at both protein and mRNA levels, although it was then gradually returned to the normal in the sham group, indicating CLP modeling enhanced the expression of IL-22 receptors both in the intensive period and in the recovery phase of sepsis. The expression of IL-22BP had a similar changing trend to that of IL-22R1 and were both opposite to the change of IL-22 expression in the progress and recovery of sepsis, implying that

the reduced IL-22 expression in heart tissue of CLP mice might be attributed to the increased expression of IL-22R1 and IL-22BP in heart tissue. The differences in the expression of IL-22R1 and IL-22BP in cardiac tissue of mice among the 4 time points in the CLP group demonstrated that the increases in the expression of IL-22 receptors in heart tissue of mice were induced by CLP modeling, but the change in the expression of IL-22 in heart tissue of mice suggested that it was triggered by trauma but not by the CLP modeling. The differences in the expression of IL-22, IL-22R1 and IL-22BP between sham and CLP groups at 72 h after operation also implied that the role of IL-22/IL-22R1 axis was effectively suppressed by the increased release of IL-22BP in the recovery phase of sepsis, while the elevated IL-22R1 expression might be consumed by other cytokines such as IL-20 and IL-24 which shared IL-22R1 as their subunit receptor [21, 22]. Combined with the changes in the expression of IL-22 in cardiac tissue, we speculated that only sufficient IL-22 for IL-22R1 and IL-22BP can guarantee the integrity of heart tissue, while the insufficient IL-22 for IL-22R1 and IL-22BP would lead to further damage in heart, and thus the immunoprotection on the heart tissue of CLP mice was still not entirely restored in the recovery phase although the CLP mice passed through the critical phase. This should be confirmed by in-depth studies.

Our observation covered the intensive death period and the recovery phase of the CLP, so as to dynamically explore the impact of CLP modeling on the IL-22/IL-22R1 axis in the heart tissue. However, considering that the systemic tissue degeneration and necrosis in the dead mice may have profound interference on the results of our experiment, we only selected the living mice at each time point for the following experiments but excluded the dead ones, which may cause a selection bias to the results. In our pre-experiment for survival analysis, we had carried out a proteomic profiling on the serum from the CLP mice at 4 h, 8 h, 12 h, 16 h, 32 h, 64 h and 72 h time points, and found that time points of 8 h, 32 h and 72 h were most representative (not shown). Thus, 8 h, 32 h and 72 h were selected as our observation time points. Currently, this study is only focused on the observational index, and the functional study would be carried out in the future to confirm our findings.

In summary, we demonstrated that the expression of IL-22 receptors in the cardiac tissue were prominently induced by CLP modeling, while that of IL-22 was sharply suppressed by trauma in both groups at the first 8 h after surgery. Our findings revealed that IL-22/IL-22R1 axis has an immunoprotective role in the cardiac tissue repair and only sufficient IL-22 level for IL-22R1 and IL-22BP can keep the integrity of cardiac tissue, but the immunoprotection on the cardiac tissue of CLP mice was remarkably damaged in the progress of sepsis and even in the recovery phase. Therefore, this study provides an immunopathological basis for the researches on the cardiac function impairment in the process of sepsis and may have some meaningful reference for the clinical treatment of sepsis. It also opens a new window for studying the mechanisms of myocardial dysfunction of sepsis.

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Disclosure of conflict of interest

None.

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