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Sepsis Induced by Cecal Ligation and Puncture

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Summary

Despite advances in intensive care unit interventions, including the use of specific antibiotics and anti-inflammation treatment, sepsis with concomitant multiple organ failure is the most common cause of death in many acute care units. In order to understand the mechanisms of clinical sepsis and develop effective therapeutic modalities, there is a need to use effective experimental models that faithfully replicate what occurs in patients with sepsis. Several models are commonly used to study sepsis, including intravenous endotoxin challenge, injection of live organisms into the peritoneal cavity, establishing abscesses in the extremities, and the induction of polymicrobial peritonitis via cecal ligation and puncture (CLP). Here, we describe the surgery procedure of CLP in mice, which has been proposed to closely replicate the nature and course of clinical sepsis in humans.

Keywords

Sepsis; Cecal ligation and puncture; CLP; Cytokine; Macrophage

1. Introduction

Sepsis, caused by gram-negative (G^-) and gram-positive (G^+) bacteria, fungi, viruses, and parasites, has become increasingly significant over the past decades. This condition affects approximately 700,000 people annually and accounts for about 210,000 deaths per year in the US (1-5). Despite technical developments in intensive care units (ICUs) and advanced supportive treatment, the incidence of sepsis is rising at rates between 1.5% and 8% per year (4, 5). These rates may be caused by the increased usage of catheters and other invasive instruments, chemotherapy for cancer patients, and immunosuppression in patients with organ transplants or inflammatory diseases. Sepsis represents a major burden to the US health care system, with costs of approximately \$16.7 billion per year, due in part to the extended hospitalization of septic patients (4). Interestingly, the contribution of G^+ bacteria to sepsis has dramatically increased during the past thirty years (5), with *Staphylococcus aureus* and *S. epidermidis* being responsible for more than half of these cases of sepsis (6, 7). In addition, the rate of fungal infections is reported to have increased more than 200% (5). The clinical definition of sepsis has been continuously modified based on additional clinical symptoms and laboratory findings. Discussion at the most recent consensus conference led to the establishment of the extended definition of sepsis, which is outlined in table 1 (8-10).

During the initiation and progression of sepsis, two dynamic stages are present; a systemic inflammatory response syndrome (SIRS) in the acute phase and a compensatory anti-inflammatory response syndrome (CARS) in the later phase (9). The hallmark of SIRS/CARS is an exacerbated production of pro- and anti-inflammatory cytokines/chemokines, leading to the so-called “cytokine storm”. While these inflammatory mediators are essential in providing an effective host defense, their overzealous production can be deleterious, resulting in a “double-edge sword” property of reducing pathogens at the expense of organ injury (11, 12). In the host, toll-like receptors (TLRs) and intracellular pattern recognition receptors (PRRs), such as nucleotide-binding oligomerization domain (NOD)-leucine-rich repeat (LRR) family (NOD-LRR) proteins, act as sensors for invading pathogens or their components. Activation of these sensors results in the release of an array of proinflammatory mediators from both leukocytes and structural cells. These mediators include, but are not limited to, tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, CXCL8 (IL-8), IL-18, CCL2 (MCP-1), CCL3 (MIP-1 α), CXCL10 (IP-10) (10, 11, 13, 14), prostaglandins, lipid mediators and reactive oxygen species (7). The effects of these host-derived factors include vasodilatation and upregulation of adhesion molecules, resulting in the extravasation of leukocytes and the activation of these cells along with epithelial and endothelial cells. Coagulation is an additional vascular event that is dictated directly by pathogen components and indirectly by pathogen-induced cytokines. This physiological response can be more severe and result in disseminated intravascular coagulation (DIC), causing hypoperfusion, hypoxia and death. Collectively, the tissue damage caused by activated immune cells and pathological coagulation resulting in DIC can lead to multiple organ failure (MOF), involving the lungs (acute respiratory distress syndrome), liver, and kidneys (2, 15-17). Based on the understanding of molecular mechanisms underlying sepsis, new clinical therapies have been designed to neutralize several key molecules involved in the initiation and progression of sepsis, including lipopolysaccharide (LPS), a core component of G⁻ bacteria cell wall, IL-1 and TNF- α . Although experimental studies using animal models of sepsis have documented a beneficial effect of these therapies (18-22), subsequent clinical trials were disappointing since they showed no substantial benefits (23-27). So far, recombinant human activated protein C that targets coagulation remains the only effective new therapy for clinically severe sepsis (28). These observations support the concept that severe sepsis is a highly dynamic and complex disorder and efficient treatment strategies remain elusive.

In order to understand the mechanisms of clinical sepsis and develop effective therapeutic modalities, there is a need to use effective experimental models that faithfully replicate what occurs in patients with sepsis. Several models, including endotoxin challenge, live organism challenge, establishing abscesses in the extremities, and the induction of polymicrobial peritonitis via cecal ligation and puncture (CLP) have been used to study sepsis (29-31). The latter experimental model, CLP, has been proposed to more closely replicate the nature and course of clinical sepsis, as compared to other models (32, 33). The benefits of the CLP model is its reproducibility and the potential to alter the severity of sepsis by controlling needle size, number of cecal punctures and antibiotics utilization (34). Following the induction of CLP-induced peritonitis, inflammatory cytokines/chemokines such as IL-12p70, IL-10, TNF- α , CCL2, CCL3 and CXCL10 are rapidly induced in the peritoneal

cavity (local response), blood and peripheral organs (systemic response) within 4 hours and peak at 24 hours. Three days later, the local and systemic levels of inflammatory cytokines/chemokines mostly return to baseline levels, indicating the end of the acute phase of sepsis.

2. Materials

2-1. Animals

1. Female C57BL/6 mice (6-8 wks; The Jackson Laboratory) housed under specific pathogen-free conditions.

2-2. Surgical Supplies and Equipment

1. 3-0 silk suture
2. Autoclip wound closing system, including applier
3. Wound clips and remover
4. Scissors and forceps
5. Heating pad
6. 5 ml syringe
7. 21-gauge needles
8. Instant sealing sterilization pouch

2-3. Reagents

1. Ketamine HCL (Abbott Laboratories)
2. Xylazine (Lloyd Laboratories)
3. Antibiotic INVANZ (Ertapenem) (Merck)
4. Sterile saline (Hospira)
5. Veterinary ointment (Pharmaderm)

3. Methods

1. Autoclave all of the surgical equipment, including the scissors, forceps, and wound clips, with the Instant Sealing Sterilization Pouch.
2. Prior to CLP surgery, anesthetize the mice with a combination of 2.25 mg of ketamine and 150 µg of xylazine administered intraperitoneally. Once the mice are anesthetized, apply moisturizing vet ointment to the eyes to prevent drying.
3. Under sterile surgical conditions, a 1-cm midline incision is made to the ventral surface of the abdomen, and the cecum is exposed (Figure 1A).
4. The cecum is partially ligated at its base with a 3-0 silk suture (Figure 1B).

5. The ligated cecum is punctured up to nine times with a 21-gauge needle (Figure 1C). Sham-operated mice will undergo an identical operation except for the actual cecal ligation and puncture. These sham-operated mice will serve as controls.
6. The cecum is returned to the peritoneal cavity and the abdominal incision is closed using two or three surgical wound clips (Figure 1D).
7. Immediately administer 1 ml of sterile saline subcutaneously to each mouse for fluid resuscitation.
8. Allow the mice to recover from anesthesia in a face-down position in a bedding-free, pre-warmed fresh cage placed over a heating pad. Monitor the cage temperature to prevent over-heating. It usually takes about 30 minutes for mice to recover from anesthesia.
9. Treat both CLP and sham mice with the antibiotic INVANZ (Ertapenem). INVANZ should be administered intraperitoneally at 75 mg/kg beginning at 6 h after surgery and re-injected every 24 h until day 3 post-surgery.
10. Disease progression should be monitored three times per day by evaluating weight loss, temperature decrease and behavioral changes. Monitor the animal survival for 6 days.
11. Peritoneal lavage, serum and various tissues such as lung, liver and kidney are collected at day 1 or day 3 for cytokine measurement.

4. Notes

1. The genetic background of the animals is important. In contrast to T helper (Th) 2-dominant BALB/c mice, C57BL/6 mice are Th1-dominant and more resistant to trauma-induced sepsis (35) and Th1-dependent pathological infections, such as *Leishmania major* (36, 37). Thus, female C57BL/6 mice were used extensively as an animal of choice for inducing experimental sepsis. On average, this nine-puncture CLP procedure results in approximately 40% mortality in the acute phase of sepsis (38-40).
2. The animal ethical committee approved the experiments.

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Figure 1. Cecal ligation and puncture procedure

(A) A 1-cm midline incision is made by scissor to the ventral surface of the abdomen, and the cecum is exposed. (B) The cecum is partially ligated at its base with a 3-0 silk suture. (C) The ligated cecum is punctured up to nine times with 21-gauge needle. (D) The cecum is returned to the peritoneal cavity and the abdominal incision was closed using two or three surgical wound clips.

Table 1.1
Sepsis diagnostic criteria

General changes	Hyperthermia ($> 38.3^{\circ}\text{C}$) or hypothermia ($< 36^{\circ}\text{C}$) Heart rate $> 90/\text{min}$ or > 2 SD above normal value for age) Breath rate $> 30/\text{min}$ Changes in mental state Edema or positive fluid balance ($> 20\text{ml/kg}$ over 24 h) Plasma glucose $> 120\text{mg/dl}$ or > 7.7 mM
Inflammatory changes	WBC $> 12 \times 10^9/\text{L}$ or $< 4 \times 10^9/\text{L}$ Normal WBC count with $> 10\%$ immature band forms Plasma C reactive protein > 2 SD above normal Plasma procalcitonin > 2 SD above normal
Hemodynamic changes	Arterial hypotension (SBP < 90 mmHg, MAP < 70 , or a SBP decrease > 40 in adults or < 2 SD below the normal for age) SvO ₂ $> 70\%$ Cardiac index > 3.5 L/min/M ^{-2.3}
Organ dysfunction	Arterial hypoxemia (PaO ₂ /FiO ₂ < 300) Acute oliguria (urine output < 0.5 ml/kg/h or < 45 mM for at least 2 h) Creatinine increase > 0.5 mg/dl Coagulation abnormalities (INR > 1.5 or aPPT > 60 s) Ileus (absent bowel sounds) Platelet count $< 100,000/\mu\text{L}$ Plasma total bilirubin > 4 mg/dl or > 70 mM
Tissue perfusion	Serum lactate level $> 1\text{mM}$ Decreased capillary refill

WBC, white blood cell; SBP, systolic blood pressure; MAP, mean arterial pressure; SvO₂, mixed venous oxygen saturation; PaO₂, arterial partial pressure of oxygen; FiO₂, fraction of inspired oxygen; INR, international normalized ratio; aPPT, activated partial thromboplastin time.