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Peritoneal wash contents used to predict mortality in a murine sepsis model

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ABSTRACT

Background: Cecal ligation and puncture (CLP) is considered the gold standard for inducing abdominal sepsis in mice. However, the model lacks source control, a component of sepsis management in humans. Using a CLP-excision model, we characterized peritoneal cytokines and cells and hypothesized these analyses would allow us to predict survival.

Methods: Fifty-eight mice were first subjected to CLP. Twenty hours later, the necrotic cecums were debrided, abdominal cavity lavaged, and intraperitoneal antibiotics administered. Peritoneal cytokines and leukocytes collected from the peritoneal lavage were analyzed. These immune parameters were used to generate receiver operator characteristic curves. In separate experiments, the accuracy of the model was verified with a survival cohort. Finally, we collected the peritoneal lavage and analyzed both serum and peritoneal cytokines, bacterial load, and leukocyte functionality.

Results: Peritoneal interleukin (IL)-6 levels and neutrophil CD11b intensity were observed to be significantly different in mice that lived *versus* those who died. In separate experiments, mice predicted to live (P-LIVE) had decreased bacterial loads, systemic IL-10, and neutrophil oxidative burst and increased peritoneal inflammatory monocyte numbers and phagocytosis.

Conclusions: This study couples a clinically relevant sepsis model with methodology to limit pathogen spread. Using surgical waste, stratification of the mice into groups P-LIVE and predicted to die was possible with a high degree of accuracy and specificity. In mice P-LIVE, increased inflammatory monocyte recruitment and phagocytosis were associated with decreased systemic IL-10 and bacterial loads.

1. Introduction

Despite advances in technology and medical care, mortality from sepsis remains relatively constant at 18%–30% [1–3]. Severe sepsis and septic shock are leading causes of death in the United States, accounting for 215,000 deaths annually [1,4]. Over the past decade, the Surviving Sepsis Campaign, with its of hallmarks of early broad-spectrum antibiotics, early source control, and administration and maintenance of tissue perfusion, has demonstrated modest improvements in survival with strict adherence to the guidelines [5,6]. Recent randomized controlled trials, however, have illustrated that these hallmarks of sepsis management have likely reached an asymptote for improving survival [2,3]. In general, these treatments largely lack disease and patient specificity.

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The pathophysiology of sepsis revolves around a complex immunological balance between an initial phase dominated by hyperinflammation followed by a prolonged period of profound immunologic compromise or paralysis [7–9]. Countless attempts to improve survival through the modulation of this ever-changing immune response have largely been unsuccessful [10,11]. Improvements in expeditious diagnosis, determination of the immune status and tailoring immunomodulation therapies to a specific patient, and disease populations appear to be crucial in advancing the management of this complex disease [12,13].

Previous research in mice subjected to cecal ligation and puncture (CLP) has demonstrated that interleukin (IL)-6 levels in the blood could be used to predict survival in mice and guide immunomodulation therapies [14]. Although CLP is the gold standard for inducing sepsis in experimental murine models, the lack of source control is a limitation when extrapolating to sepsis management in humans [15]. Therefore, we used a CLP-excision (CLP-E) model in which cecal excision, peritoneal wash, and antibiotic treatment were performed after CLP [16]. Using this model, we hypothesized that we could use the surgical waste from the peritoneal washes to predict survival, and in mice predicted to live (P-LIVE), we would observe an initial increased inflammatory response and decreased bacterial loads.

2. Materials and methods

2.1. Mice

Outbred CF-1 male mice were purchased from Charles River Laboratory (Spencerville, OH). All mice were 6-wk-old when purchased from Charles River Laboratory and allowed to acclimate for 1–3 wk. All mice were housed in standard environmental conditions and were fed with a commercial pellet diet and water *ad libitum*.

2.2. Cecal ligation and puncture

Male mice aged between 6 and 10 wk (28–35 g) were used. All experiments involving animals were performed under protocols approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. Polymicrobial sepsis was induced similarly as described [17]. Briefly, the CLP operations were always performed between 8 AM and 1 PM. Mice were anesthetized to effect by 2% isoflurane in oxygen via a face mask. The skin was shaved and disinfected. After a 1–2-cm laparotomy, the latter 50% of the cecum was ligated with a 3-0 silk tie (Ethicon, Cincinnati, OH) and a full thickness through and through puncture was made on the antimesenteric side with a 20-gauge needle. A small amount of the bowel contents was extruded through the puncture holes to assure a full thickness perforation. The cecum was replaced in its original location, and the midline incision was closed by two-layer suture with 4-0 silk (Henry Schein, Melville, NY). The animals were resuscitated with 1 mL of sterile saline administered subcutaneously and kept on a heating blanket for 1 h [17]. Survival studies were carried out for 10 d.

2.3. Pathogen source control

At 20 h after CLP, the mice were anesthetized identically to the procedure mentioned previously. The laparotomy incisions were opened, and their abdomens were explored. All filmy adhesions were lysed, and the latter 50% of the cecum, now 100% necrotic, was debrided at the previous ligation site. The peritoneal cavity was washed with 35 mL of warm sterile phosphate-buffered saline and collected [18,19]. Finally, a single intraperitoneal dose of imipenem (Merck, Whitehouse Station, NJ) was administered at a dose of 2.5 mg/kg [20]. The abdomens are then closed, and the mice resuscitated in manner consistent with the CLP procedure.

2.4. Enzyme-linked immunosorbent assay

Peritoneal fluid was harvested from mice by peritoneal wash and centrifuged at 450*g* for 10 min. The supernatant fluid was used for cytokine analysis, and pelleted cells were analyzed by flow cytometry. For serum collection, blood collected by cardiac puncture was placed in serum separator tubes (BD Biosciences, San Jose, CA), centrifuged at 1000*g* for 10 min with isolated serum subsequently transferred to a new sterile tube. The IL-6 and IL-10 concentrations in the peritoneal fluid and serum were analyzed by enzyme-linked immunosorbent assay (BD Biosciences) as previously described [17].

2.5. Bacterial counts

Bacterial counts were performed on aseptically harvested peritoneal wash. Aerobic samples were serially diluted in sterile saline and cultured on Tryptic Soy Agar plates (BD Biosciences). Aerobic plates were incubated at 37°C for 24 h, and colony counts were performed [21]. Anaerobic samples were serially diluted in sterile saline and cultured on PRAS Brucella plates (Anaerobe Systems, Morgan Hill, CA). Anaerobic plates were incubated in BD GasPak Anaerobic Containers with three GasPak sachets (BD Biosciences) incubated at 37°C for 48 h, and colony counts were performed [22]. All septic mice had positive cultures resulting from fecal spillage into the peritoneum.

2.6. Flow cytometry

Analyses of cell surface antigen expression and of cytokine expression *in situ* were performed. The cells were labeled with Phycoerythrin–anti-mouse Ly-6G (clone: 1A8) and Allophycocyanin–anti-mouse CD11b (clone M1/70), and FITC anti-mouse CD54 (clone: 3E2). Using flow analysis, Ly-6G/CD11b positive cells were identified as neutrophils. CD11b and CD54 were used as markers of neutrophil activation [23–25]. All antibodies used for flow cytometry were obtained from BD Pharmingen [26]. Flow cytometry acquisition and analysis were performed on an Attune Flow Cytometer (Life Technologies, Foster City, CA).

2.7. Oxidative burst measurement

Measurement of spontaneous hydrogen peroxide was determined by measuring the oxidation of dihydrorhodamine