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The Effect of Immunosuppression on Manifestations of Sepsis in an Animal Model of Cecal Ligation and Puncture

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ABSTRACT

Objective. The diagnosis of sepsis is difficult in immunocompromised patients owing to their modified response to infection. Our experiment in minipigs was designed to compare responses to sepsis between experimental groups of septic minipigs with and without immunosuppression.

Methods. Minipigs with identical baseline parameters were randomized into 3 groups: Sepsis (n = 10); immunosuppression (n = 11), including cyclosporine, methylprednisolone, and mycophenolate mofetil treatment before surgery, and a sham group (n = 6). Sepsis was induced by cecal ligation and puncture (CLP). We recorded selected clinical and laboratory parameters up to 24 hours postoperatively.

Results. All CLP animals developed septic shock with a febrile response, tachycardia, and hypotension requiring noradrenaline administration. The hemodynamic responses to sepsis in septic groups with and without immunosuppression were similar. Noradrenaline infusion was started on average later in the immunosuppression than in the group without immunosuppression; however, the difference was not significant. The kinetics of the plasma levels of most selected cytokines and C-reactive protein were similar in both septic groups. At 10 hours after surgery, the immunosuppression group showed significantly lower interleukin (IL)-6 levels compared with the sepsis group. At 19, 22, and 25 hours after surgery immunosuppressed animals displayed significantly greater increases in IL-10 levels compared with the cohort without immunosuppression.

Conclusions. CLP is a simple, reproducible model of sepsis in minipigs. All CLP animals developed sepsis within 24 hours on average. Significant differences in IL-6 and IL-10 plasma levels were recorded between septic animals with versus without immunosuppression.

SEPSIS, a systemic inflammatory response to infection, is characterized by interactions between the patient and the infectious agent. The diagnosis is based on an assessment of the patient's clinical status, identification of the infectious agent, and laboratory tests. Rapid performance of these examinations is a crucial prerequisite for the successful management.[1,2] Immunocompromised patients are at particular risk of developing sepsis.[3,4]

Despite impressive advances in the prevention, diagnosis, and treatment of infection, sepsis remains the main cause of

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morbidity and mortality among immunocompromised patients.[5] Therefore, understanding the pathophysiology of sepsis in these patients and the specific features of its diagnosis is of crucial importance. Although a serious issue, sepsis in immunosuppressed patients continues to be poorly understood. As in other populations, an early diagnosis and early institution of adequate treatment are crucial for patient survival. However, immunocompromised patients pose numerous pitfalls. The clinical picture of sepsis is highly variable, often not reflecting the severity of the condition. The symptoms are often nonspecific; unlike immunocompetent individuals, there are no typical features of a systemic inflammatory response.[6] Given the weaker response to infection in the presence of immunosuppressive therapy, the standard definition of sepsis or severe sepsis may not apply in this patient populations because host factors blunt or minimize objective or subjective signs of inflammation. Immunocompromised patients often present several simultaneous immunologic disorders, including neutropenia and lymphopenia as well as functional T-cell impairments, reduced antibody responses and tissue injuries. In fact, all critical determinants of immunocompetence may be impaired.[7,8]

Little is known about differences in the dynamics of sepsis markers between immunosuppressed and nonimmunosuppressed individuals. Experimental studies in small animals have reported some of these differences.[9,10]

The main goal of sepsis research of is to improve our understanding of the pathogenic mechanisms of sepsis allowing the development of modalities to effectively treat sepsis in man. Various animal models have been designed to study complex molecular mechanisms of sepsis. The most frequently used in small laboratory animals is cecal ligation and puncture (CLP),[11] a gold standard for sepsis research. This model provides a polymicrobial source of sepsis from infection in the abdominal cavity with subsequent bacterial translocation into the blood leading to systemic inflammatory response. The aim of our experiment was to compare selected clinical and laboratory parameters between a group of immunocompetent septic minipigs and a group of immunosuppressed septic minipigs.

METHODS

Animals

The experiment included 30 adult minipigs of mean weight 37.6 kg (median, 36 kg). The animals were randomized into 3 groups: sham controls ($n = 6$), sepsis ($n = 10$), induced using a modified CLP mode or immunosuppression ($n = 11$), using 5 days treatment with cyclosporine (Sandimun Neoral, Novartis, Switzerland); 10 mg/kg in 2 daily doses, and methylprednisolone (Prednison, Zentiva, Czech); 20 mg in a single daily dose, and mycophenolate mofetil (CellCept, Roche Pharma, Switzerland); 2 g in 2 daily doses). On the day of surgery, the cyclosporine levels were determined. Three animals were excluded from the analysis. The study was approved by our Committee for Protection of Animals Against Abuse. We compared selected clinical and laboratory parameters.

Surgery

Under general anesthesia, peritonitis was induced using the CLP model. After a midline laparotomy, cecal ligation was performed at approximately one fourth of its length (5 cm from Bauhin's valve). The blood supply was interrupted along the length of cecal ligation. Before cecal ligation, the intestinal content was rubbed into the cecum from the ascending colon. Cecal perforation was performed on the antimesenteric aspect at the area of the taenie using a trocar; the incision was extended 3 cm (Fig 1). The abdominal cavity was contaminated with intestinal content. A gastrostomy and cystostomy were performed. The sham group only underwent laparotomy, gastrostomy, and cystostomy.

Anesthesia

Before surgery, animals were fasted for 12 hours with fluid intake ad libitum. On the day of surgery, the minipigs were weighed and premedicated intramuscularly with ketamine (10 mg/kg; Narketan, Vetoquinol, Switzerland), azaperone (5 mg/kg; Stresnil, Janssen, Belgium), and atropine (0.1 mg/kg; Atropin, Biotica, Czech). Upon cannulation of the ear vein, anesthesia was induced with propofol (2 mg/kg; Propofol 1%, Fresenius, Germany) and fentanyl (100 μg; Fentanil, Torrex, Czech). After endotracheal intubation, the hosts were connected to a ventilator (Servoventilator, Siemens, Sweden) delivering controlled ventilation using an oxygen/air mixture (Fio_2, 0.4; positive end-expiratory pressure, 5 cm H_2O; respiratory volume, 10 mL/kg). The respiratory rate was set to achieve a target arterial Pco_2 of 4.0–5.0 kPa. Relaxation was induced using vecuronium (Norcuron, Organon, Netherlands) initially at 0.1 mg/kg followed by 0.8 μg/kg per minute until the end of the surgery. During the procedure, the animals received fentanyl (10–15 μg/kg per hour) and propofol (6–10 mg/kg per hour).

After anesthetic induction, an arterial catheter (20 G, Arrow) was inserted into the femoral artery for blood pressure monitoring, a 3-lumen central venous catheter (7.0 Fr, Arrow) into the left jugular vein for infusion and drug delivery, and a flow-directed thermodilution catheter (7.0 Fr, Arrow) into the pulmonary artery via the right jugular vein for hemodynamic parameter measurements. Crystalloid solution (Plasmalyte, Baxter, USA); 10–15 mL/kg per hour) was infused seeking to compensate for fluid loss.

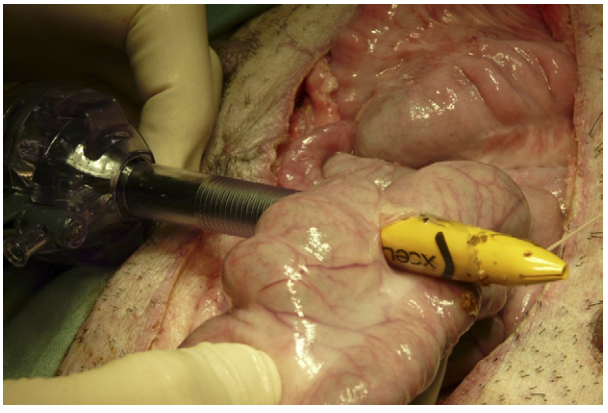

Fig 1. Cecal point perforation in minipig using a trocar.