

1 Experimental CLP setup © Goethe University Frankfurt, Fraunhofer IME / Katharina Schwarzkopf, Urs Christen, Tilo Knape.

2 Survival of CLP-operated mice © Fraunhofer IME / Tilo Knape.

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## CECAL LIGATION AND PUNCTURE AND CECAL SLURRY ANIMAL MODELS OF SEPSIS

Systemic inflammation or sepsis is a reaction of the immune system to intensive and massive infections. To study the pathophysiology of human sepsis, Fraunhofer IME uses two animal models of polymicrobial septic peritonitis: cecal ligation and puncture (CLP) and the cecal slurry (CS) model. Today, both tests are the most widely used animal models of the systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis and septic shock because they closely mimic the progression and characteristics of human sepsis.

#### Species

- Rodents (mice)

#### Field of application

- Basic sepsis research
- Modelling of pathophysiological processes within the human immune system during SIRS, sepsis, severe sepsis and septic shock

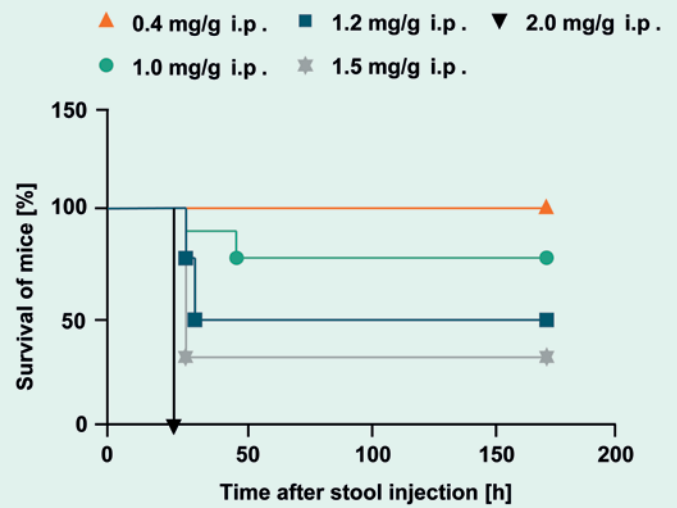
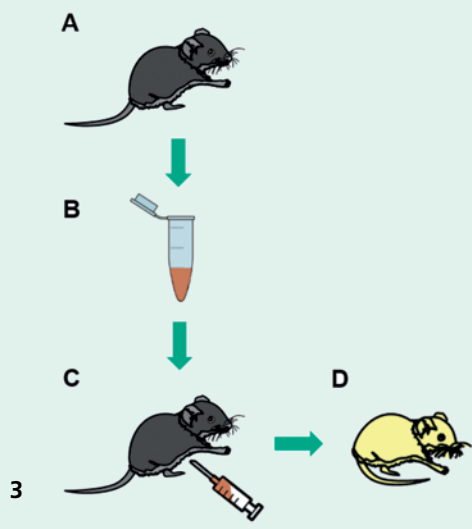
- Testing novel therapeutic agents, drugs and approaches

#### Endpoints

- Organ damage
- Defined time
- Survival
- Physiological parameters

#### Readout parameters

- Post mortem blood and organs are recovered for further analysis by various methods such as flow cytometry, cell sorting, western blot, immunohistochemistry, quantitative real-time polymerase chain reaction, cytometric bead array or enzyme-linked immunosorbent assay and high-performance liquid chromatography
- In vivo measurement of blood pressure, electrocardiography, heart rate, temperature and physical activity



### Quality management and validation

Thiazolidinediones like ciglitazone, pioglitazone and rosiglitazone are effective in reducing inflammation when administered in the hyper-inflammatory phase of sepsis. In contrast, selective peroxisome proliferator-activated receptor gamma modulators (SPPAR $\gamma$ M) and/or PPAR $\gamma$  antagonists (Fig. 2), for example, GW9662, are effective in reducing T cell apoptosis when administered in the hypo-inflammatory phase of sepsis. Such compounds are used as reference compounds for the development of new compounds for the treatment of sepsis or studying the pathophysiology of sepsis.

### Experimental setups

All animal experiments at Fraunhofer IME in Frankfurt follow the guidelines of the animal care and use committee of the State of Hesse.

### Cecal ligation and puncture (CLP) model

For CLP surgery, mice are anesthetized. The abdominal cavity is opened via a midline laparotomy incision in an aseptic fashion and the cecum is exposed. The cecum is ligated distal to the ileocecal valve, taking care to maintain bowel continuity. Further, the ligated cecum is punctured (Fig. 1). Next, sufficient pressure is applied to the cecum to extrude fecal material from puncture site.

The abdomen is closed, and mice are resuscitated with sodium chloride 0.9%. Until mice wake up from anesthesia they are kept warm on a heater mat. Animals receive buprenorphine after surgery and every 8 h. Monitoring of animals at defined time points.

### Cecal slurry (CS) model

Laparotomy of donor mouse and harvesting of cecal slurry. Preparation of stool suspension by adding sodium chloride 0.9% and straining (Fig. 3). Intraperitoneal (i.p.) injection of stool suspension in recipient mouse for dose-dependent severity of sepsis (Fig. 4). Animals receive buprenorphine after surgery and every 8 h. Monitoring of animals at defined time points.

### Selected publications

- von Knethen A, Schäfer A, Kuchler L, Knape T, Christen U, Hintermann E, et al. Tolerizing CTL by Sustained Hepatic PD-L1 Expression Provides a New Therapy Approach in Mouse Sepsis. *Theranostics* 2019; 9:2003-2016.
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- Netzer C, Knape T, Kuchler L, Weigert A, Zacharowski K, Pfeilschifter W, et

al. Apoptotic Diminution of Immature Single and Double Positive Thymocyte Subpopulations Contributes to Thymus Involution During Murine Polymicrobial Sepsis. *Shock* 2017;48(2):215-226.

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- Schmidt MV, Paulus P, Kuhn AM, Weigert A, Morbitzer V, Zacharowski K, et al. Peroxisome proliferator-activated receptor  $\gamma$ -induced T cell apoptosis reduces survival during polymicrobial sepsis. *Am J Respir Crit Care Med* 2011;184(1):64-74.

3 *Experimental setup of the CS model. Harvesting of cecal slurry from a donor mouse (A) and preparation of stool suspension by adding sodium chloride 0.9% (B) followed by an i.p. injection of stool suspension in recipient mouse (C) for dose-dependent severity of sepsis (D) © Goethe University Frankfurt / Katharina Schwarzkopf, Urs Christen.*

4 *Survival of mice after stool injection © Goethe University Frankfurt / Katharina Schwarzkopf, Urs Christen.*