P401

Development of a Cecal Ligation and Puncture Induced Acute Lung Injury and Acute Respiratory Distress Syndrome Mouse Model

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Abstract

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), occur when an event (such as pneumonia, sepsis, inhalation injuries, or transfusion reactions) triggers a chain reaction of inflammation in the lungs. This cascade involves increased permeability, inflammation, and surfactant dysfunction.

Commonly used ALI/ARDS mouse models include sterile injury (lipopolysaccharide) models and infectious (Streptococcus pneumoniae) models. The lipopolysaccharide model offers control and standardization but lacks the complex immune response and organ dysfunction seen in ALI/ARDS. On the other hand, the Streptococcus pneumoniae model, although more clinically relevant, exhibits greater variability due to differences in bacterial virulence and mouse susceptibility, and logistical challenges around using live cultured bacteria.

The cecal ligation and puncture (CLP) model is the gold standard rodent sepsis model due to its clinical relevance. It involves ligating and puncturing the cecum, leading to a polymicrobial infection in the abdomen that generates a strong immune response and multiple organ dysfunction, including inflammatory lung injury. Although it is relatively simple to perform, CLP can be challenging to reproduce and varies in severity.

We established a mild-severity CLP model to induce ALI/ARDS. The lung perfusion procedure was optimized to minimize Evans blue dye contamination during the harvest of lung samples. Python imputation and feature engineering techniques were utilized to establish equivalent timepoints and identify key characteristics within the datasets.

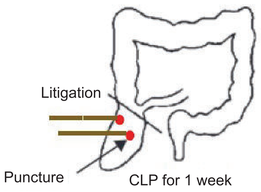
We closely monitored several datasets to determine the humane end point through the study. This included tracking body temperature and activity using a RFID microchip system (UID® Identification Solutions), as well as assessing Murine sepsis score (MSS). The survival rate of the mice subjected to our CLP model was approximately 75%, and the mice experienced decreased body weight in the days following CLP.

End point samples were harvested on day 7 post-CLP. Significant changes in biomarkers were observed in the mice subjected to our CLP model. These changes included enhanced expression of NLRP3, caspase-11, caspase-1, and gasdermin D (GSDMD), elevated levels of plasma interleukins 1β (IL-1β) and 18 (IL-18), increased pulmonary edema, lung permeability, and neutrophil infiltration (assessed by lung myeloperoxidase [MPO] activity and expression), as well as reduced expression of vascular endothelin (VE)-cadherin (correlated with increased degradation of VE-cadherin).

Materials & methods

Mild severity CLP to induce ALI/ARDS

- All animal study protocols were reviewed and approved by Merck's Institutional Animal Care and Use Committee (IACUC)
- Approximately 2- to 3-month-old male C57BL/6NJ mice housed in static caging under a 12 h/12 h light/dark cycle with food and water available ad libitum. No fast before surgery
- Perform a 1-cm midline laparotomy and expose the cecum
- The cecum is tightly ligated with a silk suture at about 1 cm from the distal end of the cecum and is perforated twice with a 23 gauge needle
- The cecum may be gently squeezed to extrude a small amount of feces from both the perforation sites

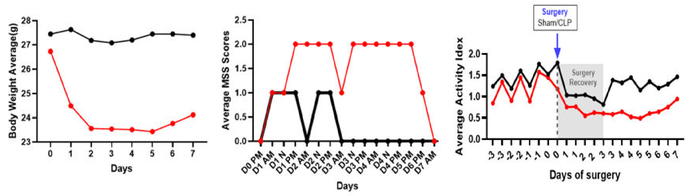


- The cecum is returned to the peritoneal cavity and the peritoneum and skin are closed by layers
- At study end points, lung was perfused by pumping PBS into the inferior vena cava and exiting from the abdominal aorta

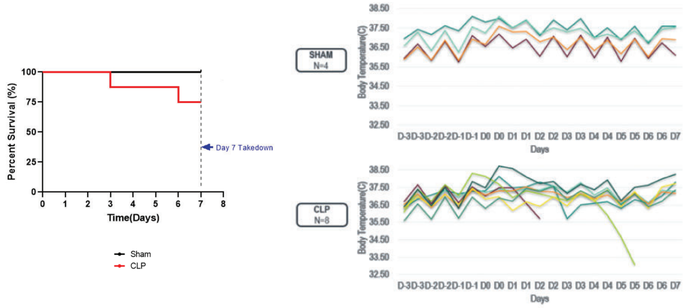
Results

Figure 1. Datasets to determine the humane endpoints

a. Average body weight, Murine sepsis score, and activity change



b. One week survival rate and body temperature change



c. Python imputation and feature engineering techniques

Change point detection (CPD) algorithms are used to detect abrupt shifts in timeseries trends. The results shown here are from an offline model, meaning they do not use live streaming data and instead require the full timeseries for statistical analysis. The plots show continuous temperature and activity level measurements collected from RFID chips (left y-axis), compared with observational MSSs (right y-axis). The change in background colors indicates a change point as identified by the model. During the timeframe of 7 days, we see evidence within the CLP group of sepsis based on higher MSS overall. The CPD algorithms need further fine-tuning to capture these changes, as more evidence of time lags is seen. Future work will include exploring online models so changes can be detected real time.

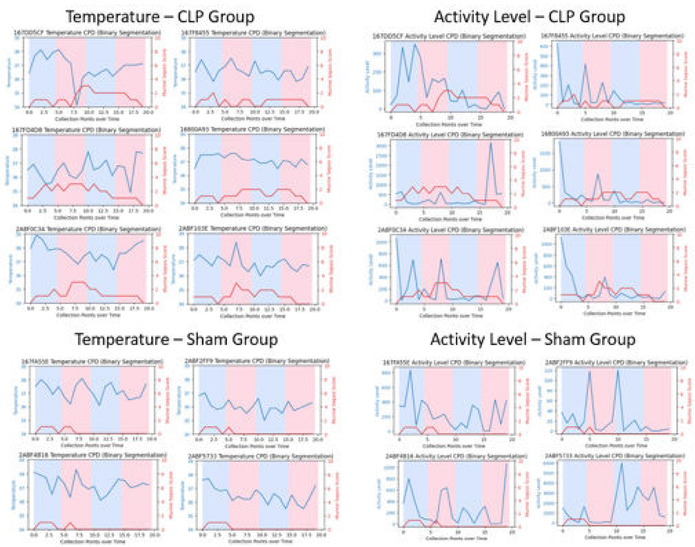
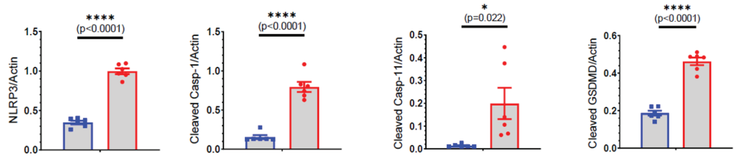
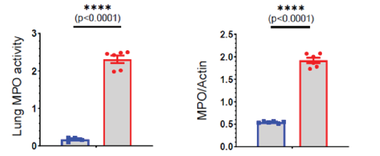


Figure 2. Endpoint-biomarker changes on day 7 post CLP

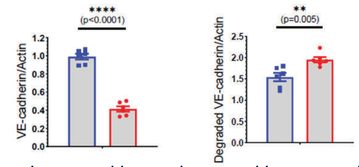
a. Enhanced expression of NLRP3, cleavage of caspase-1 and -11, and GSDMD in lung lysates



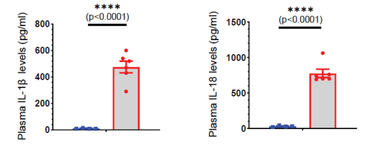
b. Increased neutrophil infiltration (assessed by lung MPO activity and expression)



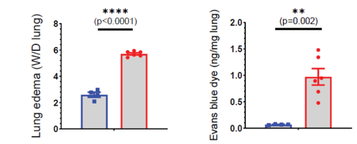
c. Reduced expression of VE-cadherin correlated with increased degradation of VE-cadherin in lung

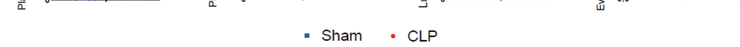


d. Elevated levels of plasma IL-1β and IL-18



e. Increased lung edema and lung vascular permeability





Conclusions

- The datasets presented in this study allowed us to identify the key mediators involved in the NLRP3 inflammation pathway and the cascade of events leading to ALI/ARDS
- 1-week CLP activated the canonical NLRP3/GSDMD pyroptotic signaling pathway, as indicated by the enhanced expression of NLRP3, caspase-11, caspase-1, and GSDMD
- 1-week CLP resulted in typical characteristics of ALI/ARDS, including the release of IL-1β and IL-18, increased neutrophil infiltration and degradation of VE-cadherin
- NLRP3, along with caspase-11, caspase-1, GSDMD, IL-1β, IL-18, VE-cadherin, and neutrophil infiltration, play a significant role in the pathophysiology of ALI/ARDS by promoting inflammation, increasing lung vascular permeability, and causing lung edema
- These findings indicate the successful establishment of a CLP-induced ALI/ARDS model, which provides a valuable foundation for future research in this area

References

1. Matthay MA, et al. *Nat Rev Dis Primers.* 2019;5(1):18.
2. Chimenti L, et al. *Intensive Care Med Exp.* 2020;8(suppl 1):62.
3. Xiong S, et al. *J Clin Invest.* 2020;130(7):3684-3698.
4. Komarova YA, et al. *Circ Res.* 2017;120(1):179-206.
5. Lampugnani MG, et al. *Cold Spring Harb Perspect Biol.* 2018;10(10):a029322.

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