Published in final edited form as:

Crit Care Med. 2013 January ; 41(1): 356–357. doi:10.1097/CCM.0b013e318270e3ee.

Cecal-Ligation Model of Sepsis in Mice: New Insights

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A large number of small and large animal models of sepsis and acute lung injury (ALI) have been used by investigators for the past four decades. The animal models have been designed to simulate common clinical disorders associated with clinical lung injury, including pneumonia, sepsis (pulmonary or non-pulmonary source), aspiration syndromes (gastric acid, fresh and salt water), and ischemia-reperfusion lung injury (1). A recent workshop provided consensus guidelines for animal models of ALI (2). Nevertheless, most animal models have several limitations because they cannot recapitulate the complex nature of sepsis and ALI in a critically ill patient (3). While mouse models offer some advantages over other animal models because specific mechanisms can be tested with genetic manipulations, it is difficult to measure respiratory and hemodynamic variables on a continuous basis during the course of the experiments and many mouse studies have been limited to shorter term experiments. In contrast, Patel et al (4) recently reported an excellent acid-induced lung injury model in mice studied for 10 days that reflected both the evolution and resolution of ALI, with measurements of extravascular lung water, alveolar fluid clearance, arterial blood gases, biochemical markers of lung injury and lung histology.

In this issue of *the Journal*, Iskander and colleagues developed a novel approach to study sepsis and ALI in mice using a plasma biomarker to predict mortality in order to stratify mice according to their likelihood of survival (5). This approach presents a new way of designing pre-clinical studies in animal models. Traditionally, murine studies of sepsis and ALI are limited by an inability to induce severe injury while trying to allow for survival of the mice. Make the injury too severe and the mice will die, but if not severe enough then the mice will not develop sepsis and/or ALI. In this study, plasma IL-6 levels were measured early in the course of cecal-ligation-induced peritonitis and sepsis in order to assign the mice to one of two groups: those with high levels of plasma IL-6 and a subsequent 100% mortality rate by 5 days and those with low levels of plasma IL-6 and a predicted survival of 100% by 5 days. The mice were followed closely for 48 hours and several parameters of lung injury and inflammation were measured. By stratifying the mice according to the likelihood of survival, it was possible to make comparisons between mice that lived compared to those that died. Using this design, the investigators were able to draw meaningful conclusions about the development of lung injury and its association with

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The authors have not disclosed any potential conflicts of interest

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mortality in this septic model, information that would not have been possible using a traditional design.

Using this new approach the authors established that development of lung injury does not contribute to mortality in cecal-ligation induced peritoneal sepsis. By standard indices, there was little evidence of ALI in these mice with peritoneal sepsis. Are these conclusions applicable to human sepsis and ALI or is this simply a result of their approach to studying sepsis? The answer is probably both. First, some clinical studies reported that ALI does not confer an increased risk of death in patients with severe sepsis. One study of 4,530 critically ill patients in a surgical ICU reported that ARDS was not an independent predictor of mortality in septic patients but multi-organ failure was associated with increased mortality (6). Another study that examined the changing patterns of ARDS over the course of 16 years showed that in recent years (2006-2009) a majority of patients die from multi-organ dysfunction syndrome (70%) and a small fraction die primarily from refractory hypoxemia (7%) (7). The reasons for this are not entirely clear. Perhaps in the era of mechanical ventilation it has been possible to support septic patients with respiratory failure and ALI allowing them to survive long enough to develop additional organ failures, leading to death. In addition, there are comorbidities, genetic and environmental factors and process of care variables that contribute to the development and outcomes from ALI (8-10).

Alternatively, it may be that ALI does contribute directly to mortality from sepsis but the current experimental study did not detect an association. In this study mice were sacrificed at 24 and 48 hours although the majority of mice did not die until day 3. It is possible that mice develop ALI between days 2-3 which could have been missed in this analysis. The time interval for development of ALI in patients is variable. Shari *et al* found that of patients who ultimately develop ALI in the hospital, one-third have ALI on admission while the remaining two-thirds develop ALI approximately 30 hours after hospital admission (11). Considering that patients are typically ill for some time before they seek medical care, it is likely that in a majority of patients the onset of illness precedes the development of ALI by several days. So conceivably, the development of ALI was missed in the current study. However, it is more likely that ALI did not develop because peritoneal sepsis was associated with systemic hypotension and pulmonary vascular filling pressures were probably low, as reported in one mouse model of severe pneumonia (12). In the setting of clinical sepsis, most patients receive substantial intravascular volume resuscitation that probably results in more accumulation of pulmonary edema in the setting of an increase in lung vascular permeability (13, 14). Most mouse models of sepsis do not include volume resuscitation.

As was the case in this study, it has been difficult to pinpoint the cause of death in mouse models of sepsis. The likely explanation is shock and hypotension complicated by hypothermia and acidosis, but the challenge in measuring these variables continuously in mouse models has made it difficult to draw firm conclusions. As recently discussed (3), mouse models of sepsis do not recapitulate what can be measured and monitored in patients with clinical sepsis.

In summary, the study by Iskander *et al* demonstrates that the standard cecal-ligation model of peritoneal sepsis is not a good model of acute lung injury, although their experiments generated a novel design that incorporated an early measurement of a plasma biomarker (IL-6) to risk stratify septic mice for mortality. Given the severe limitations of current animal models for sepsis, new approaches are needed. Iskander and colleagues should be complimented for developing a new model system that may be a small step in making mouse models more clinically relevant to human sepsis.

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