Current Murine Models of Sepsis

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SEPSIS IS A COMMON CLINICAL ENTITY causing thousands of deaths and costing billions of healthcare dollars annually in the U.S. alone [1–4]. Further, sepsis and septic shock represent a heterogeneous spectrum of complex biology and pathophysiology [5–10]. The rise of early goal-directed therapy led to increased standardization of care for patients with severe sepsis or septic shock with corresponding improvements in mortality rates [11,12]. More recent trials, however, have since called into question the benefits of strict protocols for septic shock among those promptly recognized [13–15]. Novel therapies, outside of antibiotics, fluid resuscitation, and basic supportive care, have eluded clinicians and investigators for decades [16]. Yet, the search for new targets to enhance care for patients with sepsis continues. As it is not feasible to test each potential new therapy immediately in human patients, researchers have used animal models to provide platforms for testing, as they provide levels of sophistication far beyond that of cell and tissue culture.

Recently, murine models of sepsis have been a topic of considerable debate, particularly as they relate to human disease and guiding the development of novel biologic therapeutics. Detractors cite substantial differences between the murine and human response to sepsis at the genomic level [17,18]. In contrast, others argue that there are biologic similarities but also highlight the importance of model selection. For example, it is inappropriate to draw comparisons between the complex nature of the human condition and a homogeneous model of endotoxemia in a single genetic strain of mouse [7,19]. Models that result in universal and rapid death of experimental mice likely only compare with a minority of human sepsis patients, where death is of lower probability and likely to occur in the time frame of days rather than hours from disease onset [6,20]. In addition, pre-treatment experiments for sepsis translate poorly to human patients, who do not have the luxury of medical therapy *prior* to developing life-threatening infection [6]. This equipoise underscores the importance of model selection when conducting potentially translatable animal studies of sepsis.

Among animal models of sepsis, mouse models are often used because of the ease of experimentation, the availability of genetically engineered species, and the relative low cost. Herein, we summarize the currently available murine models of sepsis, with a focus on methodology, common variations,

issues, and limitations. The idea that a perfect murine model of sepsis exists is untenable, owing to the complex and heterogeneous nature of the condition, but a variety of good options for modeling exist depending on the focus of the research.

Lipopolysaccharide Injection: Models of Endotoxemia

Since the first description that injection of lipopolysaccharide replicates much of the physiology of severe sepsis, murine endotoxemia models have become a pillar in experimental studies of sepsis and have been utilized for nearly 100 y in an effort to re-capitulate human sepsis [21]. The model consists of injection of purified lipopolysaccharide (LPS), usually intra-peritoneally, into mice. Though simple to learn and expedient to perform, the model has inherent limitations. Lipopolysaccharide is a single component of the complex pathogen associated molecular patterns (PAMPs) released by gram negative organisms [20]. Furthermore, it neglects the host-pathogen interactions of gram positive organisms and polymicrobial sepsis. Bolus administration of LPS is essentially an intoxication model rather than a true septic state [22]. There is no microbial source for ongoing LPS or PAMP release. When compared with human sepsis, endotoxemia results in high plasma inflammatory cytokine concentrations, which peak earlier and at greater values and demonstrate faster resolution [6,23,24]. Lipopolysaccharide in large doses may resemble a small subset of human sepsis patients with fulminant conditions such as overwhelming meningococemia [16].

The murine response to LPS exhibits a dose-dependent spectrum; smaller doses produce a hyperdynamic state whereas large doses cause the hypotension and hypothermia characteristic of murine septic shock [6,8,20]. These doses are dissimilar to those producing similar effects in human beings, owing to a substantially greater LD50 for LPS in mice [16]. One potential explanation for differences between mouse and human responses to LPS may lie in varying expression of protective proteins such as hemopexin [16,20,23]. It is also critical to determine which LPS preparation is used, as purity varies between available products; products of lesser purity may contain other molecules such as Deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), which are known to modulate the host immune response.

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Models of endotoxemia are of scientific utility in interrogating specific biological mechanisms and pathways, such as the immune response to prototypical stimuli of specific toll-like receptor (TLR) pathways, such as LPS and TLR4. However, when compared with more complex and arguably more physiologically relevant models such as the cecal ligation and puncture (CLP) model, LPS injection can be titrated to achieve similar mortality rates and hematologic alterations. However, at the molecular level, important differences remain. Inflammatory cytokines such as Interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), keratinocyte chemoattractant (KC), and macrophage inflammatory protein-2 (MIP-2) all show different temporal profiles, with cytokines peaking earlier, at greater concentrations and for shorter durations with endotoxemia [22,25]. Perhaps these differences were highlighted by clinical trials of TNF- α inhibitors, which initially showed impressive protection and thus great therapeutic promise when tested in murine LPS models. Unfortunately, later human clinical trials failed to show any mortality rate benefit, which could have been predicted from murine CLP models that similarly demonstrated reduced survival [22,24,26]. These contrasting outcomes emphasize the importance of testing in multiple, different animal models prior to advancing therapeutic agents into large-scale clinical trials.

Bacterial Injection Models

Bacterial injection, either intra-peritoneally or intravenously, has been performed to mimic sepsis in mice. A variety of bacterial species have been employed. *Escherichia coli* is commonly used to replicate gram negative sepsis, whereas *Staphylococcus* and *Pseudomonas* represent other common, gram positive human pathogens. Large inocula are needed because of the inability of most strains to replicate in vivo [6]. A large dose of bacteria is also necessary in the mouse in order to overcome host defenses, which efficiently clear low doses of bacteria [21,23]. Observed cytokine responses are more similar to LPS injection models [6]. The magnitude of the host response observed in these models is dependent on the route of bacteria administration, with the intra-peritoneal route showing attenuated inflammatory responses as compared with the intra-venous route [23]. The decision to use intra-venous versus intra-peritoneal injection routes typically is balanced against the relative increase in difficulty of tail vein injections in mice when compared with the quick and relatively facile intra-peritoneal injection. Other investigators have inserted vascular catheters and infused bacteria over a period of several hours, in an effort to avoid some of the "bolus effect" observed with faster inoculations and to better replicate sepsis [20].

The model is highly dependent on fluid resuscitation and antibiotic administration, both of which are necessary if mice are expected to survive for a duration enabling the measurement of other important endpoints, such as organ injury [20]. Even with antibiotics, mortality rates are high if fluid administration is omitted, a likely consequence of the large inflammatory response to a bolus dose of bacterial pathogen leading to hemodynamic collapse [20]. Critics of the bacterial injection model note that like endotoxemia, it does not mimic the human septic condition; there is usually a steady stream of bacteria present in human sepsis as opposed to the bolus

usually observed in these mouse models [20]. Though conditions related to most human sepsis patients are poorly recreated by these models, they continue to have some utility in mechanistic studies, such as interrogating a specific toll-like receptor pathway with its cognate microorganism (e.g., *E. coli* and TLR4) [8].

Bacteria and Fibrin Clot Implantation

An added level of sophistication to the bacterial injection model came with the inclusion of standardized amounts of bacteria into fibrin clots, which are subsequently implanted into the peritoneal cavity [27,28]. The fibrin clot serves as a reservoir for bacterial seeding of the bloodstream and elsewhere in the body, similar to what is observed in human abdominal sepsis [8]. Thus, the inclusion of fibrin clots allows one to modulate the rate of bacteria clearance and titrate mortality rates based upon the bacterial load delivered [21,27]. Early investigations observed that as opposed to the near 100% early mortality rate observed with bacterial injection, inclusion of fibrin clot resulted in mice that remained sick for up to several days before dying [27]. This model is highly reproducible as bacterial quantities can be controlled, and source control procedures can be employed during a second laparotomy for fibrin clot removal [20,27]. This model has been used to show the importance of early antibiotic administration in sepsis, where delays after the onset of septic shock in experimental animals were associated with substantially greater mortality rates [29].

Cecal Slurry Injection

The cecal slurry model of sepsis is based upon intra-peritoneal injection of cecal contents from a donor rodent that have been standardized in quantity (i.e., fecal mass) and suspended in fluid [30]. Proponents of this technique claim that it can be more consistent than CLP because of the ability to standardize the bacterial inoculum, which is not possible in CLP [31]. However, although mortality rates from cecal slurry can be titrated to mimic that of a particular CLP model, the kinetics of mortality between these two models still vary [32] (Table 1). In addition, the models have been determined to produce distinct genomic and cytokine response patterns after injury and should not be considered to be directly interchangeable [32]. One obvious difference in technique between the two models is that mice subjected to cecal slurry lack the surgical tissue trauma and ischemic tissue generated by standard CLP methods.

Unlike CLP, this model can be performed in neonatal mice, a cohort corresponding to human neonates, which are substantially affected by sepsis [31,33,34]. Cecal ligation and puncture is frequently not possible in neonatal mouse models of sepsis because of small organ size and incomplete intestinal development [33]. Thus, cecal slurry certainly fills an important gap when it comes to modeling sepsis in neonates. The technique continues to be refined. Previously, it was necessary to extract cecal contents from a donor and prepare a standardized slurry in a solution of 5% dextrose in water with each experiment performed, but newer techniques have emerged to store slurry preparations in glycerol, which can be stored frozen for at least 6 mo with minimal losses in pathogen viability [31].

TABLE 1. MORTALITY MODELS OF CECAL SLURRY, CECAL LIGATION AND PUNCTURE, AND COLON ASCENDENS STENT PERITONITIS STUDIES

<i>Cecal slurry</i>			
Group	Cecal Slurry Parameters	6–8 Day Mortality	Antibiotic Used
Gentile et al. 2014	1.1 mg cecal contents per gram of body weight	40–50%	no
Starr et al. 2014	40 mg cecal contents per mouse	60%	no
Starr et al. 2014	30 mg cecal contents per mouse	0%	no
Starr et al. 2014	20 mg cecal contents per mouse	0%	no
<i>CLP model</i>			
Group	CLP Parameters	6 to 8 Day Mortality	Antibiotic Used
Ebong et al. 1999	Distal 1/3 ligated, 25-gauge double puncture	0%	yes
Newcomb et al. 1998	1 cm ligation, 25-gauge double puncture	55%	no
Newcomb et al. 1998	1 cm ligation, 25-gauge double puncture	28–56%	yes
Turnbull et al. 2004	Entire cecum ligated, 23-gauge double puncture	~50%	no
Wynn et al. 2007	1 cm ligation, 22-gauge double puncture	70%	no
Turnbull et al. 2004	Entire cecum ligated, 21-gauge double puncture	~75%	no
Vyas et al. 2005	Entire cecum ligated, 21-gauge double puncture	64–75%	yes
Lewis et al. 2016	1 cm ligation, 21-gauge double puncture	83%	no
Lee et al. 2007	1 cm ligation, 20-gauge double puncture	100%	no
Hermann et al. 2013	Distal 20% ligated, 20-gauge quadruple puncture	83%	no
Turnbull et al. 2004	Entire cecum ligated, 19-gauge double puncture	~80%	no
Maier et al. 2004	1.5 cm ligation, 18-gauge single puncture	60%	no
Maier et al. 2004	1.5 cm ligation, 18-gauge double puncture	65%	no
Vianna et al. 2004	Entire cecum ligated, 18-gauge single puncture	70%	no
Vianna et al. 2004	Entire cecum ligated, 18-gauge single puncture	0–10%	yes
Vianna et al. 2004	Entire cecum ligated, 18-gauge triple puncture	100%	no
Vianna et al. 2004	Entire cecum ligated, 18-gauge triple puncture	60–70%	yes
Xiao et al. 2006	Unspecific ligation length, 18-gauge double puncture	25%	yes
<i>CASP model</i>			
Group	CASP Parameters	6 to 8 Day Mortality	Antibiotic Used
Zantl et al. 1998	22-gauge stent	38%	no
Zantl et al. 1998	18-gauge stent	64%	no
Maier et al. 2004	18-gauge stent	50%	no
Maier et al. 2004	16-gauge stent	70%	no
Zantl et al. 1998	14-gauge stent	100%	no
Maier et al. 2004	14-gauge stent	100%	no

Selected studies listed utilized juvenile/young adult (6–12 wks old) mice. Studies were selected from references in this manuscript, which provided data on mortality outcomes in a 6–8 day time period.

Cecal Ligation and Puncture

Widely used, the CLP model of sepsis involves performance of a laparotomy under general anesthesia followed by ligation of a portion of the cecum in conjunction with creation of one or more cecal colotomies via needle puncture [35,36]. This model results in three insults to the host: 1) Surgical trauma to the tissues, 2) ischemic tissue from the ligated cecum, and 3) polymicrobial sepsis from fecal spillage after needle puncture(s) [37]. The infectious insult resulting from performance of the CLP procedure can be modulated based upon the length of cecum ligated and the size and number of punctures performed (Tables 1 and 2). However, this also serves as a drawback, as the variety of insults detracts from standardization of the procedure between laboratories [38,39]. Some labs ligate the entire cecum, whereas others ligate a specific length during each procedure, and still others yet ligate a percentage of the total length of the cecum. Similarly, needle sizes used for puncture run the gamut of available sizes, most commonly in the 18- to 25-gauge range.

Proponents of CLP cite its theoretical and biological relevance to human sepsis: A polymicrobial insult, the hyper- then hypodynamic hemodynamic transition, the generation of detectable bacteremia, and the elevation in damage-associated molecular patterns such as high-mobility group protein 1 (HMGB1) [6,16,39–41]. Cecal ligation and puncture results

TABLE 2. ADJUSTING SEVERITY IN THE CECAL LIGATION AND PUNCTURE MODEL

	Puncture quantity	Puncture diameter	Cecal ligation length
Least severe	1*	25 gauge*	0.5 cm*
	2*	22 gauge	1.0 cm*
	3	21 gauge*	1.5 cm
Most severe	4+	18 gauge*	Entire cecum*

*Commonly used parameters. Mortality and shock kinetics vary between reports for any given combination.

in concomitant activation of pro-inflammatory and anti-inflammatory networks in the host [37]. Similar to the bacteria-impregnated fibrin clot model, there is a continuous reservoir for bacterial release, which is more clinically relevant than simpler bolus-based sepsis models [6,35]. In addition to dissemination of bacteria, the ligated and ischemic cecal tissue also contributes to the observed immune dysfunction [42]. Critics of the CLP model point out that frequently no source control procedure is employed in this model, making it essentially a model of tissue injury and incompletely treated peritonitis [6,8,21]. However, a number of groups have successfully incorporated a second procedure to resect and debride the de-vitalized cecum, in an effort to render the model more clinically relevant to human abdominal sepsis [39,43,44]. Cecal resection has been specifically studied and shown to improve mortality rates in the CLP model [43].

The physiologic response to CLP is dependent on fluid resuscitation; in its absence, mice will fail to enter a hyperdynamic circulatory state, and mortality rates will be increased [20,35,39,45]. It is well documented that commonly used strains of mice (C57BL/6, Balb/c, CD-1) experience hypothermia after undergoing CLP alone [26,41,46]. The inclusion of routine antibiotic administration and the specific antibiotic employed can substantially alter the model. Antibiotics influence the survival of mice after CLP, with greater relative risk reduction as the magnitude of the septic insult increases [47]. One study found improved survival after usage of imipenem as compared with a triple antibiotic regimen of gentamicin, clindamycin, and ciprofloxacin [48]. The same study also showed that the triple antibiotic regimen exacerbated the pre-existing hypothermia after the dose was received in a manner independent of LPS or inflammatory cytokine concentrations [48]. An alternate study showed consistent improvements in survival after antibiotic administration in the CLP model but did not note any differences between a regimen of imipenem versus a ciprofloxacin-clindamycin combination [49]. As has been suggested in other studies, the timing of antibiotic administration relates directly to overall survival. When antibiotics were given at 12 h after CLP, mice with IL-6 concentrations greater than 14,000 pg/mL had 0% survival, whereas delivery of antibiotics at 6 h to mice with similar IL-6 concentrations increased overall survival to 25% [50]. Consistency in the decision to include or omit antibiotic administration in the model and also the timing of antibiotic delivery is essential to maintenance of comparability between experiments.

Choice of anesthetic, though initially given little attention in discussions of murine models of sepsis, matters considerably. The original group describing CLP used isoflurane anesthesia to minimize the post-operative recovery time and minimize the cardiovascular effects that can be observed with other anesthetic agents (e.g., ketamine and xylazine combinations or pentobarbital) [39,41]. However, isoflurane may not be without side effects. Some groups have noted enhanced survival in CLP sepsis as well as protection from renal and hepatic injury when volatile anesthetics such as isoflurane or sevoflurane are used [51,52]. The exact mechanisms remain to be determined; however, studies have noted decreases in serum markers of inflammation with volatile anesthetics. Ketamine, another popular agent for animal studies, has also been reported to decrease nuclear factor- κ B (NF- κ B) activity and the pro-inflammatory cytokine production in rat CLP sepsis [53]. Although the ideal anesthetic

agent has not been definitively determined for use in the CLP model, it is worth considering that any anesthetic agent likely confers effects on the model and represents a source of inter-laboratory and inter-experiment variability.

Critics of the CLP model note a high degree of variability when protocols from different laboratories are compared [31]. Sources of variability include but are not limited to the length of cecum ligated, the size and number of punctures created, the amount of stool extruded from the cecotomy, the anesthetic choice, whether antibiotics are included and if so which drug and regimen, the timing and volume of fluid resuscitation after CLP, the individual surgical skill of each operator in regards to length of operation and quantity of tissue trauma, and the dietary status of the mouse (i.e., NPO vs. fed, and choice of feed) [39]. Though relevant to nearly all models, the severity and duration of organ injury induced, such as acute kidney injury (AKI), varies between young and aged mice [54–56]. Acute kidney injury increases as time passes after CLP and mice approach death [55]. In fact, many biological processes are altered during the process of aging and may alter clinically relevant endpoints. Decreased autophagy observed in aged mice may contribute to greater levels of AKI after CLP, and use of agents in an effort to induce greater levels of autophagy improved renal function in aged experimental mice [54]. Others have also noted that younger mice do not have histologic changes consistent with kidney injury after CLP when compared with older mice, but this does not necessarily indicate that some degree of renal dysfunction is not present [56,57]. There is some disagreement in the literature on whether or not lung injury occurs as a result of CLP sepsis; those groups claiming that it does typically employ a highly lethal model [58–60]. It is important to note that when comparing these three cited studies on the particular issue of lung injury, one group used outbred mice, whereas the other two used inbred C57BL/6 mice; therefore some differences may be related to the choice of mouse strain utilized.

Colon Ascendens Stent Peritonitis

Colon ascendens stent peritonitis (CASP) is a relatively newer method for producing abdominal sepsis in rodents that more closely mimics free intestinal perforation as opposed to the abdominal abscess condition created by CLP [61]. In this model, a mouse undergoes general anesthesia and midline laparotomy, followed by insertion of a plastic stent (created from an intra-venous catheter) into the ascending colon of the mouse, 1 cm distal to the ileocecal junction. The plastic stent is secured to the ascending colon with two sutures, and stool is then capable of continuous flow from the colon into the abdominal cavity. Generalized peritonitis is induced by a constant efflux of feces from the colon, bacteremia, and seeding of distant organs [61,62]. This model also appears to reproduce the organ dysfunction observed in human sepsis, as changes in lung, kidney, and bone marrow function have been reported [24,63–65]. The magnitude of the septic insult can be titrated by altering the size of the stent catheter [61,62] (Table 1). Though initially described in mice, it has since been performed in rats as well [66].

Colon ascendens stent peritonitis produces much of the same pathophysiology as the CLP model; hypothermia occurs and is predictive of animal mortality [26,46,67,68]. Like the CLP models that include a second operation for cecal

debridement, source control can also be employed with CASP, as the stent can be removed and the colotomy sutured closed [61]. However, important differences also have been noted between the CLP and CASP models. In contrast to CLP, survival in CASP sepsis is independent of TNF- α , as determined by CASP experimentation in TNF- α receptor deficient knockout mice [61]. The exact mechanism to account for this difference is as yet unclear. One interesting study compared CASP and CLP models and confirmed that CLP mice had more localized and contained inflammation in the abdominal cavity as compared with the generalized peritonitis observed in CASP mice [62]. Perhaps this difference in the infectious insult may account for varying roles of TNF- α . The study noted that CASP resulted in a more pronounced and steadily increasing amount of systemic bacterial dissemination that yielded greater inflammatory cytokine concentrations at all time points measured [62]. This is especially notable as a relatively severe, 18-gauge double puncture, 1.5 cm ligation length CLP model was chosen for comparison. All CASP catheter sizes were more severe in magnitude of septic insult when compared with this severe CLP sepsis model. The study confirmed that stent size affected overall mortality rates, but did not change the average time to death in mice with sepsis. The addition of CASP into the overall arsenal of sepsis models adds a reproducible mimic of free intestinal perforation producing high levels of inflammation in the host.

Extra-Abdominal Models of Sepsis: Pneumonia and Urosepsis

When testing potential therapeutics, it is important to consider models other than abdominal sepsis. Sepsis because of pneumonia is the most common source in human beings, and pneumonia can be induced in mice by intra-nasal or intra-tracheal delivery of bacteria or via inhalation of aerosolized mist [1,8,69,70]. Similar to peritoneal bacterial inoculum, the clinical course of these experimental mice is dependent on the bacterial strain selected and dose of inoculum [8]. Not surprisingly, antibiotics also influence survival in these models [71]. An interesting “two hit” model of sepsis is also possible in these pneumonia models. An insult such as abdominal sepsis (possibly followed by source control) or sterile traumatic insult can be delivered, followed by later administering bacteria to the respiratory system to mimic the development of hospital-acquired pneumonia in human patients after trauma or abdominal sepsis [8,72,73]. Urinary tract sources of sepsis can also be created in mice via injection of bacteria into the bladder to produce ascending urinary tract infection [74–78]. This model has been in published use for several decades to study urinary tract infection.

Limitations of Translating Mouse to Human: Efforts to Improve the Murine Model

There are many limitations that must be recognized when utilizing murine models in the study of sepsis. As mentioned earlier, considerable sources of model variation include age, gender, strain of mice used, time of day of experimentation, anesthetic choices and timing of delivery, narcotic or other analgesic choices, antibiotic choices, and the volume and timing of fluid resuscitation [8,10,20,39]. There are also no universally agreed upon magnitudes for septic insult. Using

CLP as an example, length of cecal ligation and the range of needle puncture gauges and quantities vary substantially between studies and the outcomes of interest: Systemic inflammation versus organ injury versus mortality rate.

The age of mice used in experimentation merits further discussion. Despite sepsis occurring mostly at the extremes of age in the human population (i.e., neonates and the elderly), the majority of animal experimentation is performed on young adult mice [1,18,23,24,37]. The typical 8–12-wks old mouse approximates an adolescent human being, which has the least risk of sepsis and sepsis-related morbidity across the spectrum of human ages. Aged human beings, and mice to some extent, have had previous illnesses, trauma, and have co-morbid conditions, which have developed over their lifetime; this type of exposure and stress is poorly replicated in young adult animals [6,8,24]. In mice, CLP mortality rate increases with advancing age [79,80]. Aged mice do not always compare well with younger cohorts in the same species: Experiments with a cecal slurry model showed substantially different mortality rates, immune cell activation, and bacterial clearance between neonatal mice, young adult mice, and aged mice [34]. Comparisons in the LPS injection and CLP models between young adult mice (4 mo old) and aged mice (24 mo old) showed substantially greater inflammatory response and mortality rates in the aged group [81]. In addition to greater mortality rates, antibiotics also appear to have decreased efficacy in older mice [82]. Thus, it may be inappropriate to extrapolate findings in young mice to the human sepsis population, unless one accounts for these differences.

The housing of experimental animals is most commonly carried out in specific pathogen-free environments, which obviously does not correlate with the microbe- and pathogen-rich environment of the “real world” [24]. Most experiments utilize mice of a single gender, even though it is known that males and females of a species will have varying responses to septic insult and trauma [6,83–85]. As we briefly highlighted in the earlier discussion of lung injury in CLP models, there are varying responses to sepsis between strains of mice, both inbred and outbred populations [6,58–60]. There is some question of equivalency in time passage between mice and human beings: Do mice experience the passage of a given time interval in the same relative manner as do human beings [18]?

Another issue is the standard use of fixed time intervals after septic insult to administer or test new therapies. Essentially all published studies to date have used a fixed amount of time after septic insult (2 h after CLP, for example) to administer antibiotics or other therapeutic measures [30,38,39,43,44,47,50,53,54,56,59,60,80,82,86–88]. This is problematic as mice, like human beings, have neither the same magnitude of response nor same timing of immune response to septic insult, even if the magnitude of the insult is kept at a constant level [24,46,88]. This is perhaps one possible explanation for the failure of many past successful laboratory experiments to translate into improvements in treatment for human patients [16]. It historically has been suggested that the inter-animal differences in mouse sepsis studies can be overcome by simply increasing the sample size [20]. However, human sepsis trials use clinically apparent physiologic triggers for enrollment criteria rather than arbitrarily defined set time intervals. It stands to reason that for animal models of sepsis to attain a greater level of translatable relevance, they should also incorporate a similar physiology-based timing of testing treatments [16,20,46].

TABLE 3. COMMON SEPSIS BEHAVIORAL SCALE PARAMETERS

Response to stimulation
Appearance of fur (piloerection, soiling, etc.)
Presence or absence of activity (foraging, eating, etc.) and assessment of ataxia
Posture
Evaluation of respiratory rate and associated noise/gasping
Appearance of eyes (open, closed, secretions)
Quality of bowel movements
Weight loss, level of food/water intake
Self-mutilating behavior
Bleeding from an orifice

Summary of parameters taken from Huet et al. (2013), Shrum et al. (2014), and Nemzek et al. (2004)

Multiple behavioral scales have been developed in an effort to overcome the variability in mouse response to sepsis by classifying the onset and severity of each animal's immune response [89–91] (Table 3). Unfortunately, behavioral/observational scales can introduce some bias because of handling or stimulation of animals, and grading the scale components is inherently subjective. Although these scales have been validated to determine which mice are in a terminal state of illness, they lack the sensitivity to determine the exact point at which an animal begins to show a physiologic response to septic insult and when experimental treatments should be tested.

Cytokine quantification has also been a subject of much focus in stratifying experimental animal responses. Perhaps best known is the IL-6 criteria, which has since been expanded to include predictive mortality cutoff concentrations for other cytokines as well [87,88,92]. These strategies have also been developed by other labs, using different cytokine thresholds and alternative strains of mice [47]. Yet, others have utilized peritoneal washings from cecal debridement procedures to stratify animals and attempt to predict mortality outcomes [44]. The conserved element between all of the aforementioned stratification studies has been the measurement of a cytokine of interest at a fixed point after septic insult, with animals then being classified into groups expected to live or die based upon this cytokine quantification. Like the behavioral scales, these cytokine measurements at fixed time points lack the susceptibility for a precise point of onset of physiologic changes in a particular host, but they are reliable in predicting eventual death in a large percentage of a cohort of mice. Though they succeed in stratifying the magnitude of responses from the experimental mice, they lack a clinical correlate in the human patient realm, where initial decisions to treat are largely based upon clinical physiologic measurements.

Recently, it is been proposed to use physiologic criteria as determinants for animal sepsis development [20]. The availability of implantable wireless biotelemetry devices has enabled the characterization of the murine physiologic response to sepsis in real time, and our lab has recently published validated criteria for the determination of physiologic deterioration thresholds after CLP [46]. We believe that the established physiologic criteria may serve as an analogue of the human “9-1-1 call,” where patients experience physiologic changes substantial enough to prompt them to seek medical treatment. In this way, treatment timing can be customized to each individual animal at the time they begin

to show signs of sepsis, analogous to the decision to initiate treatment in human clinical trials and patient care. The use of telemetry data to characterize each individual animal's response to CLP eliminates the added stress of animal manipulation, restraint, and blood sampling that is required in the behavioral and cytokine stratification methods previously described. Additionally, animals are continuously monitored so treatment can be initiated at the precise moment the deterioration criteria are met, avoiding the wait time assay results that are intrinsic to cytokine measurement methods. The main drawback to the physiologic criteria method for animal studies at the present time is the relatively high cost for devices and laboratory equipment needed for animal monitoring.

Summary

Scientific investigators cannot reach consensus in methods and results between sepsis experiments in mice, and it seems unrealistic to expect great leaps in translational human trials based upon these animal data. This is not to say that animal studies are not worth undertaking but rather serve as a call to standardize model protocols in the literature so that results can be more readily compared between different laboratories. In addition, a systematic multiple-model approach in testing potential therapies should also be developed and standardized. Testing can begin in fast, easy-to-use models and proceed through greater levels of complexity as the research progresses [6,10,21]. One only needs to appreciate the failure of anti-TNF antibody trials to see the logic behind this suggestion [26]. Despite the increased time and monetary cost of more complex animal models, they are still generally easier to perform than full-scale human clinical trials, even when multiple animal models are employed.

In addition to improvements in standardization and incremental increases in complexity, animal models should strive to replicate the conditions of human trials. For example, testing of novel therapeutic agents in sepsis should also include antibiotics and adequate fluid resuscitation in the model, as they represent the standards of patient care [6,21]. It is difficult to assess the value of new treatment agents if they are not tested in a context relevant to this standard. Others advocate for the use of “humanized” mice that have been transplanted with human cells lines to better replicate the human response to disease [16]. Finally, the stratification of experimental animal responses to septic insult ensures that we are not testing therapies in mice that have gone past the point of salvage or in animals that have not developed a clinically substantial response to the infection [21,46,87]. As more sophisticated methods are constantly being developed, the search will continue for the ideal pre-clinical therapeutic testing platform.

Author Disclosure Statement

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