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Translational Sepsis Research: Spanning the Divide

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Our knowledge of the molecular mechanisms of sepsis has attained exponential growth. Yet, the pillars of its care remain antibiotics, fluid resuscitation, and physiologic support of failing organ systems (1, 2). The inability to bring biologic breakthroughs to the bedside is not for lack of effort. Over 60 clinical trials of novel therapies, each heavily supported by the momentum of biologic data suggesting clinical utility, have been conducted and have failed to identify benefit (2–5). This mass of “negative” clinical data abuts an equally towering mound of knowledge of sepsis biology, which collectively, have led investigators to ask, “what happened?” Here we present a synthetic review of some of the challenges in translating experimental animal models of sepsis to the bedside. We commence with the concept that the heterogeneity in the kinetics of the sepsis response serves as an important, often underappreciated but surmountable, source of translational impedance. Upon this groundwork, we discuss distinctions between animal experimentation and clinical trial design in the elements for hypothesis testing: cohort selection, power and sample size, randomization and blinding, and timing of intervention. From this concept, we develop a contextual framework for advancing the paradigm of animal-based investigations to facilitate science that transitions from molecule to medicine.

A SHARED COMMON GOAL

The path to translating biological discovery into clinical testing remains ill-defined and complex. Both scientific communities share an overarching mission to improve health, but their immediate objectives are distinct. Whereas the realm of basic science seeks to elucidate biological mechanisms as a step penultimate to understanding disease, clinical trials test the

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ramifications of an intervention on the course of human disease and answer the question of effectiveness. The clinical testing of anti-tumor necrosis factor-alpha (TNF- α) agents in the 1990s makes manifest the unforeseen consequences of these differences (6). The promising results of early mechanistic studies of anti-TNF α compounds fueled an eager jump to large-scale clinical trials to test this “magic bullet.” However, neither the biology nor the inflammatory profile of these early studies are representative of the complex systemic host response stemming from uncontrolled infection that characterizes human sepsis (7–11). Indeed, the clinical cohorts and trial platforms by which anti-TNF compounds were tested were distinct from those that had been utilized in the lab: human subjects, varying stages of sepsis, a spectrum of causal organisms, and a randomized design (3, 7, 12–20). When collectively viewed, the data make readily apparent that human studies were unlikely to represent the animal model upon which they were founded.

THE KINETICS OF SEPSIS

Animal studies are modeled in a manner that is inconsistent with the clinical presentation of septic patients and the design of clinical trials. Contemporary definitions of sepsis, and the biochemical and physiologic parameters from which they are derived, are perceived to represent a particular biological stage in the host response to pathogen. Yet the experimental design of animal investigation does not typically address the inherent variability and kinetics in the evolution of the host response to microbial invasion, and this provides an additional layer of consideration in modeling (3, 21, 22). Therapies are tested at arbitrary time points after the inciting insult rather than after ‘patient’ presentation and fulfillment of biochemical and physiologic inclusion and exclusion criteria. Although beyond the scope of this review, these aspects become more salient as animal models of sepsis become increasingly complex with the introduction of two-hit models to better understand the later phases of sepsis where host immunity may fail to effectively counter a second challenge, or the phase of “immunosuppression” in sepsis (23). Thus, platforms that refine the physiologic and biologic assessment of experimental animal subjects enable us to better define animal correlates of distinct stages of human sepsis. This would potentially afford the ability to identify and thereby test animal cohorts possessing a profile more representative of the human subjects enrolled in clinical research. This imperative possesses a clinical correlate, insofar that enhanced measures of endotyping human subjects may smooth the stepwise progression from basic to clinical investigation.

THE APPLICATION OF CONSORT TO ANIMAL MODELS

Animal models test mechanistic queries upon a reductionist experimental platform that ensures causality is accurately addressed. Assuring causality is paramount, and thus the model is predicated upon holding all other recognized events constant to eliminate the obfuscation that originates from heterogeneity. It is from this approach that biological mechanisms are characterized and integrated into contemporary biological paradigms of sepsis. However, once a potential therapeutic target has been identified, the actual testing of agents in pre-clinical animal models, an important translational step prior to clinical trial, should proceed differently.

Study Design

A fundamental assumption of hypothesis testing is chance, and chance is better assured with randomization and blinding. Although there are limitations to the randomized, controlled trial (RCT) and no study is flawless, a well-designed double-blind, placebo controlled RCT remains a gold standard of testing (24). If executed correctly, it eliminates bias and relegates the resultant data to one of two interpretations: 1) chance or 2) a rejection of the null hypothesis (25). Although there is growing recognition for enhancing rigor and reproducibility, many animal-based studies are not powered *a priori* (i.e., sample size estimates) for a primary endpoint, nor do they randomize animal subjects to an intervention or blind the investigators and those analyzing the resultant data (26).

Clinical trials are ‘powered’ to optimize the statistical ability to identify an *a priori* chosen effect size for the primary outcome of interest. Power is dependent upon 1) the magnitude of the between-group difference in means and 2) the standard error of the mean (SEM), which is a function of the sample size. Insufficient sampling or too small of an effect size enhance the risk of a type II error (failure to reject the null hypothesis when the alternate hypothesis is true; a false negative), and a potential therapeutic is never brought to clinical reality (27).

Cognizance of a type I (erroneously rejecting the null hypothesis when in fact it is true; a false positive) must also be exercised (27). The data yielded from basic science serve as the impetus for subsequent clinical testing. Potential sources of bias (e.g., not blinding) that foster a ‘positive’ study may erroneously support a false perception of success and the conduct of a clinical trial that consumes resources unnecessarily and exposes human subjects to risk. An example is randomizing an entire box of mice to an intervention but analyzing at the individual animal level; animals of the same cage are not independent and may possess characteristics rendering them unique relative to animals of other cages. The lack of independence artificially narrows the standard error and supports a type I error, whereas the latter may confound the results. Ideally, randomization of individual mice by sex, age, and in some cases, strain to an intervention should occur in addition to the blinding of the investigators performing the experimentation and data interpretation.

Subjects and the model itself

The vast majority of animal laboratories utilize rodent models (3). The genetic homology is substantial (approximately 80% of mouse genes possess an identifiable orthologue in humans), and when combined with the expansive techniques for genetic manipulation, renders the mouse a powerful tool for dissecting the biological mechanisms of mammalian life (28–30). Important distinctions still persist, which too have undermined translation of discovery to human disease (31, 32). For example, rodents are more robust and resilient to septic insult, and the mismatch between severity of the experimental model and that of the clinical disease may further distance the two.

Overcoming these limitations can be achieved, in part, by sequentially progressing into larger animal models. Determining ‘when’ and ‘how’ are difficult decisions that likely depend upon the question that is being addressed, the complexity of the disease studied and its biology (33). However, survival studies in large animals may pose ethical dilemmas, are

costly and resource-intensive, and yield small sample sizes, thereby rendering statistical inference challenging (3). Thus, prior to advancing to higher ordered species, rodent models are useful when they closely approximate the human disease for which therapy is being developed. Premature testing of anti-TNF α therapy in non-human primate models of endotoxemia may have yielded ‘promising’ results, whereas rodent models (i.e., CLP) more closely mirroring the sepsis of humans (i.e., intraabdominal catastrophe of perforated diverticulitis) may have highlighted the futility of such higher ordered experimentation, thereby sparing time, money, and importantly animals. This attention to model relevance includes an appreciation for the microbial epidemiology of a disease.

The majority of cohorts enrolled in clinical trials of sepsis are comprised of men and women, of older age, who possess a spectrum of comorbidities. Yet most basic laboratory investigation has used juvenile mice (8-12 weeks) that are healthy and typically of a single sex and strain. The process of aging is characterized by profound changes in immunity, and several studies highlight that aged mice exhibit vastly different biology than younger counterparts (34, 35). Indeed, most prior studies testing immunomodulatory agents used mice that did not approximate the age of the typical, adult patient populations presenting for sepsis care. The same biological heterogeneity has been observed when comparing different strains, gender and outbred mice (36, 37, 34, 38–41). Similarly, the presence or absence of comorbidities significantly influences the trajectory and outcome of sepsis and has been difficult to, and thus infrequently, replicated in murine models (42). However genetically modified mice that exhibit many of the comorbidities of our aging population are commercially available, and their increased use may foster successful translation.

It is beyond the scope of this discourse to discuss the innumerable permutations of animal models of sepsis (Table 1) or the obvious fact that not all sepsis is equal. Investigations aimed at dissecting biological mechanisms typically adopt a reductionist approach: the use of microbial components (e.g., lipopolysaccharide) and genetic manipulation of cognate receptors (e.g., toll-like receptor TLR-4) to understand the biological ramifications on host inflammation and survival. Yet, as was observed with anti-TNF compounds and most recently TLR4 antagonists, these studies, though biologically insightful, may yield disappointment when subsequent clinical trials fail to determine efficacy (6, 43). The model of ‘sepsis’ itself also influences the degree to which the results predict future success of human testing. For example, certain models such as CLP approximate human disease better than endotoxemia (44–46). The breadth of variability (i.e., puncture size and number, length of ligation) facilitates standardizing the model to a particular severity (22, 46–49). Contemporary advocates of re-exploration for removal of the necrotic cecal segment and source control note the closer approximation to clinical care; however, this additional step has not been broadly practiced (45, 46, 50–55). Similarly, the colon ascendens stent peritonitis model attempts to recreate the conditions of a free perforated viscus with diffuse peritonitis, and proponents argue to its greater clinical relevance to actual disease (56, 57). The same can be said for the other predominant forms of sepsis for which clinicians provide care: urinary tract infections and pneumonia. Logically the more closely the animal model resembles the disease situation, the more likely relevant the data will be that is generated from them.

Standardizing animal models may also facilitate ease of data interpretation and afford opportunities to apply Bayesian and meta-analyses (58). To that end, some investigators have called for the development of standard murine sepsis models. One such effort is the “Minimum Quality Threshold in Pre-clinical Sepsis Studies” (MQTiPSS), which aims to produce a standard murine model of intraabdominal sepsis (59). Adding guidance specifying when and how to progress to higher vertebrate models would generate an instructional manual of step-wise translational experimentation performed with the intent of optimizing the ‘success’ of subsequent clinical trials.

Enrollment and the testing of interventions

Enrollment for clinical trials is guided by physiologic and biochemical inclusion and exclusion criteria that presumably reflect a particular biological stage in the host response to uncontrolled infection (3). For example, inclusion criteria may include all subjects 18 years or older with definitive microbiological or clinical evidence of acute infection, hypothermia or hyperthermia, tachycardia, tachypnea, and hypotension or other evidence of end-organ dysfunction. Exclusion criteria may include immunocompromised state and anticipated survival < 48 hours (60). Rarely are such parameters applied to murine studies, and their absence may serve as one barrier to successful translation.

Animal models administer and test interventions at *fixed* time intervals after the inciting insult (e.g., cecal puncture). This approach may not account for the inherent variability in magnitude and temporality of the animal’s response to sepsis. At any specific time, only a fraction of the animals may be at a particular biological stage (61). In this 21-gauge, double puncture model of CLP (Figure 1), testing at a 12-hour timepoint would include n=6 mice with no physiologic evidence (i.e., hypothermia, bradycardia) of sepsis. Even at 24 hours after CLP, these 6 mice exhibit little physiologic evidence of sepsis and may not have met the inclusion criteria of a clinical trial (60). Consequently animals are being ‘enrolled’ and tested at very different points in the biological response to sepsis (Figure 1), and salient to human trials that often use physiologic triggers to administer new treatments, murine models incorporating solely fixed schedules may show less clinical relevance (3, 21, 52).

Recent efforts are refining the platform of animal experimentation, the assessment of the kinetics of the septic animal, and enabling the identification of phenotypes that represent biological stages in the evolution of the host response to pathogen. The murine sepsis score (MSS), a scale comprised of seven behavioral and physical characteristics was developed to enable the prediction of shock and death, a better comparison of disease and treatment outcomes in animal models of sepsis, and provide a murine correlate of the sophisticated scoring systems applied in human illness: APACHEII and SOFA (Table 2) (62–64). Using a dose-response cecal slurry model, the developers reported moderately strong discriminatory power for survival, organ injury and cytokine concentrations and excellent inter-rater reliability. A similar score has been developed for *Klebsiella pneumoniae* pneumonia: the mouse clinical assessment for sepsis (M-CASS) (63). A notable limitation is the use of a single strain (C57BL/6J) of mice that were young (8-12 weeks). The MSS also utilized a single sex (male) (63). Resolution remains dependent on the frequency of mouse scoring, which can introduce bias from handling (65). Nevertheless, the MSS and M-CASS are

perceived to advance the *status quo*, facilitating the separation of mice that are significantly ill from those that are not.

Implantable wireless biotelemetry has enabled the development of a physiology-oriented murine model (20, 66–76). Coupled with CLP, biotelemetry can help quantify physiologic differences in the murine response to sepsis that are indicative of significant biological differences. The model more precisely identifies a similar point along the spectrum of the host response to sepsis for each animal, independent of lapsed time after the insult. In doing so, it yields a more homogeneous cohort of testable mice at a similar physiologic state and accurately excludes mice that do not meet physiologic inclusion criteria. A follow-up study validated the platform in female and male, young (12 week) and aged (40–50 week), and different strains (C57BL/6J, BALB/C) of mice. Furthermore, the model possesses face validity in enabling real-time physiologic assessment in more complex (e.g., 2-hit models) models and studying later phases of sepsis where it may be even more important to assess individual animal responses. The authors quantified the reduced animal usage and potential ethical benefits in addressing the 3 R's (Replacement, Reduction, and Refinement) first proposed in the *Principles of Humane Experimental Technique* (77–81). A notable limitation is cost, yet the investigators provide a cost analysis of incorporating the technology into laboratory practice.

Reporting in Animal Trials

Efforts to standardize protocol development and the reporting of results of human trials have been promulgated by the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) and CONSORT (Consolidated Standards of Reporting Trials) statements (82, 83). Historically the platform of animal-based investigation has lacked this principled approach (26). The recently developed ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines offer a similar framework for standardizing the conduct and quality reporting of animal studies (79). Nearly every point on the ARRIVE checklist has a recognizable analogue in the CONSORT checklist (Table 3). While adopted by many journals, utilization of the ARRIVE guidelines still have room for improvement (84). Perhaps a greater adoption of ARRIVE by basic scientists will facilitate disseminating fundamental quality metrics of animal research. The corollary is equally valid, however; attention by basic scientists to the CONSORT statements of clinical trials may facilitate reverse translation into basic investigation.

Adding biology to human clinical trials—The exchange of data and ideas is bidirectional, and the concept of “reverse translation” emphasizes that we can bring clinical observations back into the laboratory for further study (85, 86). The initial trials of gefitinib targeting the epidermal growth factor receptor (EGFR) revealed only modest benefit in the treatment of lung cancer (87, 88). Posthoc analyses, however noted that gefitinib conferred a survival benefit for particular subgroups (89). Laboratory investigators, convinced that these unique sub-cohorts experiencing benefit shared a common biological feature, performed mutational testing on tumor specimens from enrolled subjects. They determined that patients with particular somatic mutations in EGFR obtained benefit from the drug (89). Had this reverse translation not occurred, the overall conclusion for the utility of the drug in question

would have been dramatically different, and many patients may have lost the potential benefit afforded by gefitinib (90).

Despite inclusion and exclusion criteria, the populations of clinical trials still possess heterogeneity at the biological level. The resultant variability, many argue, is another principle factor underlying the high prevalence of negative trials of sepsis. Recent trials underscore the potentially profound ramifications when the biological mechanisms characterized through basic investigation and that guided drug development are incorporated. The Monoclonal Anti-TNF: A Randomized Controlled Sepsis (MONARCS) study continued the testing of anti-TNF compounds, however, stratified subjects by base-line serum IL-6 concentrations (60). The design strategy stemmed, in part, from basic science demonstrating that IL-6, which is released in response to TNF α and IL-1, is consistently detected in the blood of patients with sepsis, and elevated levels are associated with adverse outcome. Thus, an intermediary hypothesis is that elevated IL-6 concentrations may be useful in identifying those patients most likely to benefit from anti-TNF α therapy. This perspective is supported by animal-based investigation validating that early quantification of IL-6 concentration stratifies low- and high-risk outcomes, reduces heterogeneity and refines selection for therapy (91). In the overall population (n=2634) there was no significant difference in 28-day mortality for placebo and afelimomab groups (35.9% and 32.3%). However, in the cohort with elevated IL-6, the adjusted 28-day mortality was reduced with anti-TNF α therapy: adjusted absolute reduction in mortality 5.8% (p=0.041) (60). More recent meta-analyses now support anti-TNF compounds (92). Thus, circumstances in which clinical trials are ‘brought closer’ to the murine models upon which they are based may help illuminate the path between the basic and clinical sciences.

CONCLUSION

The translational divide between the basic laboratory and clinical research realms persists yet cannot be ignored if we are to capitalize upon the wealth of biologic discovery and our ever-growing knowledge to develop novel sepsis therapeutics that beneficially alter human disease. The scientific community must continue discourse and action that standardize methodologies, develop more clinically analogous and relevant laboratory research platforms, enhance the reporting and dissemination of research findings, and incorporate biologic mechanisms into the design of clinical trials. It is the bridging of these two impressive worlds that is needed.

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Figure 1. Physiology early after CLP: heart rate and core temperature

C57BL/6 mice underwent laparotomy, CLP, and implantation of a DSI HD-X11 telemetry device and were then monitored for 24 hours or until death. A, Heart rate. B, Core temperature. Each line represents a single mouse (n=17). Vertical red lines represent time points 6 and 12 hours after CLP.

Table 1

Experimental Murine Sepsis Models and their Clinical Analogues

Model	Technique	Clinical Analogue
<i>Lipopolysaccharide injection</i>	Injection of LPS into the peritoneal cavity or bloodstream	None, although it may resemble fulminant meningococcemia in some cases
<i>Bacterial injection</i>	Injection of bacteria in the peritoneal cavity or bloodstream	None. Lacks the constant source of bacteria characteristic of human sepsis and instead administers a single bolus
<i>Cecal slurry</i>	Injection of a standard amount of donor mouse cecal contents into the peritoneal cavity	Perforated viscus, though it lacks a continuous source of contamination
<i>Cecal ligation and puncture</i>	Ligation of a specified portion of the cecum then cecal puncture and extrusion of contents	Perforated diverticulitis or appendicitis with abscess formation
<i>Colon ascendens stent peritonitis</i>	Insertion of a stent into the ascending colon with continuous flow of stool into the abdomen	Freely perforated viscus
<i>Pneumonia</i>	Intratracheal injection or inhalation of a bacterial inoculum	Pneumonia
<i>Urosepsis</i>	Intravesical injection of a bacteria inoculum	Urosepsis from ascending urinary tract infection

Table 2

The Murine Sepsis Score (MSS) to assess animal response to abdominal sepsis models

Variable	Score and Description
<i>Appearance</i>	0 – Coat is smooth 1 – Patches of hair piloerected 2 – Majority of back is piloerected 3 – Piloerection may or may not be present, mouse appears “puffy” 4 – Piloerection may or may not be present, mouse appears emaciated
<i>Level of consciousness</i>	0 – Mouse is active 1 – Mouse is active but avoids standing upright 2 – Mouse activity is noticeably slowed. The mouse is still ambulant 3 – Activity is impaired. Mouse only moves when provoked, movements have a tremor 4 – Activity severely impaired. Mouse remains stationary when provoked, with possible tremor.
<i>Activity</i>	0 – Normal amount of activity. Mouse is any of: eating, drinking, climbing, running, fighting 1 – Slightly suppressed activity. Mouse is moving around bottom of cage 2 – Suppressed activity. Mouse is stationary with occasional investigative movements 3 – No activity. Mouse is stationary 4 – No activity. Mouse experiencing tremors, particularly in the hind legs
<i>Response to stimulus</i>	0 – Mouse responds immediately to auditory stimulus or touch 1 – Slow or no response to auditory stimulus; strong response to touch (moves to escape) 2 – No response to auditory stimulus; moderate response to touch (moves a few steps) 3 – No response to auditory stimulus; mild response to touch (no locomotion). 4 – No response to auditory stimulus. Little or no response to touch. Cannot right itself if pushed over
<i>Eyes</i>	0 – Open 1 – Eyes not fully open, possibly with secretions 2 – Eyes at least half closed, possibly with secretions 3 – Eyes half closed or more, possibly with secretions 4 – Eyes closed or milky
<i>Respiration rate</i>	0 – Normal, rapid mouse respiration 1 – Slight decreased respiration (rate not quantifiable by eye) 2 – Moderately reduced respiration (rate at the upper range of quantifying by eye) 3 – Severely reduced respiration (rate easily countable by eye, 0.5 s between breaths) 4 – Extremely reduced respiration (>1 s between breaths)
<i>Respiration quality</i>	0 – Normal 1 – Brief periods of labored breathing 2 – Labored, no gasping 3 – Labored with intermittent gasps 4 – Gasping

* From: Shrum B, Anantha R V, Xu SX, et al.: A robust scoring system to evaluate sepsis severity in an animal model. BMC Res Notes 2014; 7:233.

Table 3

Comparison of the CONSORT and ARRIVE guidelines

CONSORT	ARRIVE
<i>Title and abstract</i>	<i>Title and abstract</i>
<i>Introduction</i>	<i>Introduction</i>
Background and objectives	Background and objectives
<i>Methods</i>	<i>Methods</i>
Trial design	Study design
Participants	Experimental animals
Interventions	Experimental procedures
Outcomes	
Sample size	Sample size
Randomization sequence generation	
Allocation concealment	Allocation of animals to experimental groups
Randomization implementation	
Blinding	
Statistical methods	Statistical methods
	Housing and husbandry
	Ethical statement
<i>Results</i>	<i>Results</i>
Participant flow diagram	
Recruitment	
Baseline data	Baseline data
Numbers analyzed	Numbers analyzed
Outcomes and estimation	Outcomes and estimation
Ancillary analyses	
Harms	Adverse events
<i>Discussion</i>	<i>Discussion</i>
Limitations	Interpretation and scientific implications (including limitations)
Generalizability Interpretation	Generalizability/translation
<i>Other Information</i>	
Trial registration	
Location of full protocol description	
Funding source(s)	Funding source(s)