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Bench-to-Bedside: A Translational Perspective on Murine Models of Sepsis

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Abstract

Background: Considerable research effort has focused on the development of novel therapies for the treatment of sepsis, yet after decades of clinical trials, few significant advances have been achieved. This limitation persists despite a wealth of data yielded by basic science that has expanded our knowledge of the biology of this disease exponentially.
Method: Review of the English-language literature.
Results: Translational researchers may address the resultant gap between the basic science laboratory and clinical research worlds. Herein, we review potential causes for the challenges of translating basic laboratory discovery into clinical benefit.
Conclusion: We propose conceptual platforms to further the development of translational sepsis research efforts.

Keywords: animal models; sepsis; translational science

Sepsis is common, debilitating, and deadly. Recent epidemiologic investigations estimate that annually more than 1.7 million people will experience sepsis in the United States, causing 270,000 deaths that represent 35% of inpatient hospital deaths [1]. Although a multitude of advances in modern medicine have improved nearly all of healthcare, the realm of sepsis has remained nearly immune to any improvement. This perspective is manifested by the fact that those dying of sepsis will receive the only therapeutics approved: Antibiotics, fluid resuscitation, source control when possible, and supportive care for failing organ systems.

Yet sepsis is no less than oncology, human immunodeficiency virus, and diabetes a focus of attention by both the basic and clinical sciences attempting to translate biological discovery into advances in healthcare. Research efforts have reached from focused and mechanistic investigation seeking to further the understanding of the disease to multi-center randomized clinical trials testing biologic targets and the agents used to modulate them. Unfortunately, none has proved of utility when subjected to clinical trial. In fact, more than 60 clinical trials have been conducted to test a variety of biologic therapies: Steroids, anti-cytokine antibodies, and coagulation network proteins [2]. Most of these studies were backed by the latest advances from the basic science laboratory but have failed to produce a novel therapy with confirmed benefit for septic patients.

Faced with an ever-present threat to their septic, critically ill patients, clinicians and researchers are still on the hunt for the next treatment breakthrough. However, the repeated failure to obtain benefit from treatments initially reported as effective in the laboratory has led investigators on both sides of the translational chasm to question current research foci and methodologies. One potential contributor to the failure of progress in therapeutic trials is the perceived disconnect between laboratory and clinical research.

Translational Research

Translational research embodies the overarching community of scientists who strive to connect the traditionally siloed realms of laboratory, patient-centered, and population-based research [3,4]. Its members, unique in the research they conduct, share the goal of forming novel connections between their traditional research disciplines so as to streamline the science that transforms basic discovery into medicine. The T1 research connects the laboratory with the patient, T2 research connects patient-centered research with population-based studies, and T3 research explores ways of applying

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population-based research to general practice, yielding knowledge about how interventions work in real-world settings [4]. ''Reverse translation,'' the moniker for the ''backwards'' flow of information from patient-centered research into the basic science laboratory, emphasizes that the ''bridge'' enabling the exchange of ideas is bidirectional [4,5]. Conceptually, the translational researcher aims to convert the discrete steps of scientific research into a more continuous curve or even a circle. The remainder of this article focuses primarily on the T1 translational research paradigm.

Laboratory versus Clinic: The Disconnect

Researchers with experience in clinical and epidemiologic research may harbor skepticism about laboratory research, especially given the recently publicized reports questioning the reproducibility of some findings [6,7]. Essential components of trial design (population, enrollment, exposure, intervention, endpoints) may not be clear in laboratory research manuscripts [8]. A lack of familiarity with the study endpoints and methods, combined with terminology that may be somewhat foreign, challenge interpretation, particularly as it relates to appreciating clinical relevance. However, it is crucial to realize that the hypotheses addressed shift as one progresses from cell and tissue to animal-based to human research. Basic science research at its core excels at exploring causality, whereas clinical trials focus on efficacy and effectiveness of certain treatments or strategies, often in the absence of the mechanistic biological underpinnings. There are limits to what can be studied and answered using basic laboratory techniques and a need by clinical scientists to appreciate them. In turn, laboratory scientists may be able to enhance the relevance of their investigations by open communication and collaboration with practicing clinicians so as to understand better what needs answering. So, although laboratory and clinical research pursue distinct but laudable immediate objectives, they share an overarching mission to broaden knowledge and improve the human condition; hence, efforts to narrow the void may facilitate a united effort to achieve this common goal. The remainder of this discourse focuses on moving basic investigation closer to the realm of clinical research.

Meeting in the Middle

Subjects

Efforts can and should be made to move animal studies closer to human trials, especially pre-clinical experimentation that is conducted prior to clinical testing. The ''subjects'' of animal studies merit discussion, as they often are a focus of criticism or projected blame when clinical trials fail to replicate the observations of the compelling basic discovery that supported them in the first place. Clearly, it is unfounded to utilize data derived from mechanistic laboratory science based on single molecules, cells, or tissue culture as the impetus for a clinical trial. However, on transitioning into preclinical animal-based models, the expectations are elevated, at least insofar as the path is perceived to lead to advances in the treatment of disease.

The rodent model is emblematic of basic laboratory investigation. The considerable genetic overlap with human beings renders mice and rats invaluable in conducting mammalian studies that the ethical or financial constraints of other models render unfeasible [9–12]. However, important biologic, genetic, and immunologic distinctions remain [2,13]. In the context of sepsis research, efforts are being made to increase the homology between murine and human models by generating ''humanized'' mice. Through the transplantation of human $CD34^+$ immune cells, scientists can create a murine model that better replicates the human being's adaptive and innate immune response to septic insult [14–16]. Similarly, sepsis afflicts the extremes of age particularly, yet nearly all experimentation, prior to recent efforts, has utilized relatively young (8–12-week-old) mice. The human correlate is a teenager. More recently, attention has been directed to characterizing the response of older mice to septic insult, and the results have been profound [17–19]. Clearly, this has been a source of translational impedance. Its remedy is simple, although the use of aged mice has not been adopted universally [18–21]. And lastly remains the question regarding if and when to progress into high-level vertebrate animals and what the preferred progression should entail [2,22]. Granted these animal subjects bring us closer to testing in humans, that alone does not guarantee translational success [2,23].

Models

Standardization of animal models will facilitate ease of interpretation by investigators and clinicians alike [24]. Model selection is crucial and may differ depending on the research intent and the hypothesis posed [25]. Cecal ligation and puncture (CLP) was developed nearly 40 years ago and remains a popular, indeed the standard, paradigm for studying intra-abdominal sepsis. However, it possesses significant sources of variability (amount of cecum ligated, number and gauge of puncture needles, antibiotic use and choice, fluid resuscitation protocol) [26–28]. The use of a single CLP model severity in mice of identical age and strain does not guarantee a homogeneous physiologic response in terms of the timing of onset and magnitude of systemic illness [29]. Even ''identical'' mice will manifest a temporally different unfolding of the physiologic changes consistent with a septic state after CLP [30]. Although it may not be possible to eliminate variability in the animal response to a septic insult, addressing this variability is key.

A platform of biotelemetry-enhanced CLP has been developed that enables monitoring animal physiology in real time. The method facilitates identification of distinct physiologic states, independent of the time after CLP at which they are attained, and randomization of mice to an interventional arm [25,29,30]. It possesses face validity as a model more representative of the physiology-based platform by which randomized clinical trials test agents. Recently, the authors of this paper presented data supporting construct validity that the model is highly sensitive to testing differences in therapeutic interventions; e.g., enabling seven domains by physical examination of the murine response to polymicrobial (CLP) sepsis: Appearance, level of consciousness, activity, response to stimuli, eyes, ventilation rate, and respiration quality of mice after CLP [31–33]. These are combined into a composite ordinate score that is highly predictive of shock, organ dysfunction, and death, although a notable limitation is

the bias introduced by the need for animal handling. A final method by which to stratify the septic response has been achieved with rapid immunoassay of inflammatory cytokines, which, although also prognostic, is limited to a single cross-sectional time point of assessment [34–36]. Clearly, the heterogeneity of the murine response to sepsis is recognized, as is the need for methods to characterize this variability better (i.e., inclusion and exclusion criteria) to enable a more targeted and clinically relevant model platform to test therapeutic discoveries. However, consensus regarding the optimal stratification methods remains to be achieved. Recently, the ''Minimum Quality Threshold in Pre-clinical Sepsis Studies'' (MQTiPSS) project has been developed in the wake of the publication of the Sepsis-3 guidelines. It, too, calls for a more standardized approach to animal sepsis modeling, including stratification of animals according to the degree of physiologic derangement after septic insult [22].

Buy-in for animal modeling research may be improved by clearly stating the clinical disease entity the investigators are attempting to recreate. The correlation between mouse models of pneumonia or urosepsis and human disease are readily apparent. However, a clinician may less readily appreciate the clinical correlate of a CLP model, which has been likened to perforated diverticulitis or appendicitis with an intra-abdominal abscess. In contrast, the colon ascendens stent peritonitis model more closely approximates free perforation of a hollow viscus with diffuse peritonitis and produces a biologic and physiologic response distinct from that generated by CLP [37,38]. Making pre-clinical experimentation terminology relatable to the uninitiated reader enhances the interpretability of the science.

Not all sepsis is the same, and the heterogeneity in etiology should be reflected in pre-clinical therapeutic testing strategies. A good example is sepsis at the mucosal interface of the lung versus the peritoneal cavity, where macrophages and the local environment are very different [39]. Simply to find positive results in a single model and apply it broadly to sepsis of all etiologies may be erroneous. The number of different models and the optimal progression through those models could be a further focus of standardization in pre-clinical sepsis modeling [22]. In addition, the decision to enroll an animal in a pre-clinical therapeutic ''trial'' ideally could be based on similar physiologic entry criteria between models, which may manifest at various time points depending on the model selected. Theoretically, this would help avoid testing treatments on mice that have not yet mounted a systemic response to an infectious insult and similarly may help avoid testing therapies on mice that are moribund and not expected to survive regardless of therapy reducing type I and type II errors. Further, use of objective enrollment criteria such as physiologic changes more closely mirrors the enrollment of patients in human clinical trials and enhances the translational relevance of experimental results.

Study design

Regardless of the hypothesis being tested in the laboratory, practices that are standard in clinical trials such as randomization, blinding, and unbiased study design should be implemented by any laboratory. Although the laboratory focus may be on discovery and innovation, these practices can eliminate bias and render results reproducible without compromising creativity. Integrity in the randomization and blinding process helps ensure the fidelity of experimental results. However, as mentioned above, the enrollment criteria for animal trials also should be considered. Sample size and power estimations based on the expected magnitude of difference between experimental groups are key elements in planning clinical trials but unfortunately all too often either are not performed or at least are not reported in many pre-clinical studies.

Reporting

The SPIRIT and CONSORT statements have been put forth to improve reporting standards in human clinical trials. Similarly, the ARRIVE guidelines offer a framework to standardize the reporting of animal-based research, although adoption has not been as universal as initially hoped [40–43]. Checklist-based approaches such as these may elevate reporting standards by offering a minimal set of necessary information that should be included in manuscripts, facilitating interpretation by readers of all backgrounds. Ultimately, it will be up to scientists and journals to decide to what degree all published standards and guidelines are utilized, and this will be the primary determinant of their longitudinal impact.

Training the Next Generation

A final consideration in bridging the translational gap lies in the training of researchers who possess the necessary skills to speak the languages of both the basic and clinical research domains. In the past, this role frequently was performed by physician-scientists, although a trend toward increasing the number of specialized Ph.D. researchers and decreasing physician involvement in research has broadened the gap between the laboratory and the clinical environment [44,45]. The National Institutes of Health have responded by the creation of the National Center for Advancing Translational Sciences in 2012. As a result, more than 50 program hubs have been established to offer formal training in clinical and translational sciences [46]. This training will need to be customized to the specific needs of each investigator, taking into account previous experience and training [4]. Mentorship from a complementary multidisciplinary team will play a pivotal role [4]. Establishing a training pipeline to produce dually trained translational scientists will help abrogate the translational gap.

Conclusion

We need to acknowledge the inherent limitations of each type of sepsis research, but this does not require us to be complacent in accepting the present disconnect between the laboratory and the clinic. Researchers must work to bring the pre-clinical research models and methodology closer to the reality of the clinical world. In turn, the clinical trial world needs to expand efforts to understand the biological reasons for differences in observed effectiveness, bringing the clinical research world closer to the laboratory. Training researchers with specific roles in translational science is key. These translational interpreters will act as ambassadors to unite laboratory and clinical research in the quest to improve patient outcomes after sepsis.

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References

1. Rhee C, Dantes R, Epstein L, et al. Incidence and trends of sepsis in us hospitals using clinical vs claims data, 2009–2014. JAMA 2017;318:1241–1249.
2. Fink MP. Animal models of sepsis. Virulence 2014;5:143–153.
3. Woolf SH. The meaning of translational research and why it matters. JAMA 2008;299:211–213.
4. Rubio DM, Schoenbaum EE, Lee LS, et al. Defining translational research: Implications for training. Acad Med 2010;85:470–475.
5. Efron PA, Mohr AM, Moore FA, Moldawer LL. The future of murine sepsis and trauma research models. J Leukoc Biol 2015;98:945–952.
6. Begley CG, Ioannidis JPA. Reproducibility in science: Improving the standard for basic and preclinical research. Circ Res 2015;116:116–126.
7. Yaffe MB. Reproducibility in science. Sci Signal 2015;8:eg5.
8. Han S, Olonisakin TF, Pribis JP, et al. A checklist is associated with increased quality of reporting preclinical biomedical research: A systematic review. PLoS ONE 2017;12:e0183591.
9. Osuchowski MF, Remick DG, Lederer JA, et al. Abandon the mouse research ship? Not just yet! Shock 2014;41:463–475.
10. Takao K, Miyakawa T. Genomic responses in mouse models greatly mimic human inflammatory diseases. Proc Natl Acad Sci USA 2015;112:1167–1172.
11. Mouse Genome Sequencing Consortium, Waterston RH, Lindblad-Toh K, Birney E, et al. Initial sequencing and comparative analysis of the mouse genome. Nature 2002;420:520–562.
12. Rhesus Macaque Genome Sequencing and Analysis Consortium, Gibbs RA, Rogers J, Katze MG, et al. Evolutionary and biomedical insights from the Rhesus macaque genome. Science 2007;316:222–234.
13. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci USA 2013;110:3507–3512.
14. Melkus MW, Estes JD, Padgett-Thomas A, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med 2006;12:1316–1322.
15. Unsinger J, McDonough JS, Shultz LD, et al. Sepsis-induced human lymphocyte apoptosis and cytokine production in ''humanized'' mice. J Leukoc Biol 2009;86:219–227.
16. Laudanski K, Lapko N, Zawadka M, et al. The clinical and immunological performance of 28 days survival model of cecal ligation and puncture in humanized mice. PLoS ONE 2017;12:e0180377.
17. Howell GM, Gomez H, Collage RD, et al. Augmenting autophagy to treat acute kidney injury during endotoxemia in mice. PLoS ONE 2013;8:e69520.
18. Saito H, Sherwood ER, Varma TK, Evers BM. Effects of aging on mortality, hypothermia, and cytokine induction in mice with endotoxemia or sepsis. Mech Ageing Dev 2003;124:1047–1058.
19. Turnbull IR, Wlzorek JJ, Osborne D, et al. Effects of age on mortality and antibiotic efficacy in cecal ligation and puncture. Shock 2003;19:310–313.
20. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303–1310.
21. Hyde SR, Stith RD, McCallum RE. Mortality and bacteriology of sepsis following cecal ligation and puncture in aged mice. Infect Immun 1990;58:619–624.
22. Osuchowski MF, Thiemermann C, Remick DG. Sepsis-3 on the block: What does it mean for preclinical sepsis modeling? Shock 2017;47:658–660.
23. Nemzek JA, Hugunin KMS, Opp MR. Modeling sepsis in the laboratory: Merging sound science with animal well-being. Comp Med 2008;58:120–128.
24. Dyson A, Singer M. Animal models of sepsis: Why does preclinical efficacy fail to translate to the clinical setting? Crit Care Med 2009;37(1 Suppl):S30–S37.
25. Lewis AJ, Seymour CW, Rosengart MR. Current murine models of sepsis. Surg Infect 2016;17:385–393.
26. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock: A review of laboratory models and a proposal. J Surg Res 1980;29:189–201.
27. Baker CC, Chaudry IH, Gaines HO, et al. Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. Surgery 1983;94:331–335.
28. Hubbard WJ, Choudhry M, Schwacha MG, et al. Cecal ligation and puncture. Shock 2005;24:52–57.
29. Lewis AJ, Yuan D, Zhang X, et al Use of biotelemetry to define physiology-based deterioration thresholds in a murine cecal ligation and puncture model of sepsis. Crit Care Med 2016;44:e420–e431.
30. Lewis A, Zuckerbraun B, Griepentrog J, et al. Reducing animal use with a biotelemetry-enhanced murine model of sepsis. Sci Rep 2017;7:6622.
31. Shrum B, Anantha RV, Xu SX, et al. A robust scoring system to evaluate sepsis severity in an animal model. BMC Res Notes 2014;7:233.
32. Huet O, Ramsey D, Miljavec S, et al. Ensuring animal welfare while meeting scientific aims using a murine pneumonia model of septic shock. Shock 2013;39:488–494.
33. Nemzek JA, Xiao H-Y, Minard AE, et al. Humane endpoints in shock research. Shock 2004;21:17–25.
34. Remick DG, Bolgos GR, Siddiqui J, et al. Six at six: Interleukin-6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. Shock 2002;17:463–467.
35. Osuchowski MF, Welch K, Siddiqui J, Remick DG. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. J Immunol 2006;177:1967–1974.
36. Osuchowski MF, Connett J, Welch K, et al. Stratification is the key: Inflammatory biomarkers accurately direct immunomodulatory therapy in experimental sepsis. Crit Care Med 2009;37:1567–1573.
37. Zantl N, Uebe A, Neumann B, et al. Essential role of gamma interferon in survival of colon ascendens stent peritonitis, a novel murine model of abdominal sepsis. Infect Immun 1998;66:2300–2309.
38. Maier S, Traeger T, Entleutner M, et al. Cecal ligation and puncture versus colon ascendens stent peritonitis: Two distinct animal models for polymicrobial sepsis. Shock 2004;21:505–511.

39. Gordon S, Plüddemann A, Martinez Estrada F. Macrophage heterogeneity in tissues: Phenotypic diversity and functions. Immunol Rev 2014;262:36–55.
40. Chan A-W, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: Defining standard protocol items for clinical trials. Ann Intern Med 2013;158:200–207.
41. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. BMJ 2010;340 (4 Suppl):c332.
42. Kilkenny C, Browne WJ, Cuthill IC, et al. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biol 2010;8:e1000412.
43. Baker D, Lidster K, Sottomayor A, Amor S. Two years later: Journals are not yet enforcing the ARRIVE guidelines on reporting standards for pre-clinical animal studies. PLoS Biol 2014;12:e1001756.
44. Butler D. Translational research: Crossing the valley of death. Nature 2008;453:840–842.
45. Keswani SG, Moles CM, Morowitz M, et al. The future of basic science in academic surgery: Identifying barriers to success for surgeon-scientists. Ann Surg 2017;265:1053–1059.
46. National Institutes of Health. NCATS Scholar and Research Programs. (Cited Dec 13, 2017). *Available at*: https://ncats.nih.gov/ctsa/training/programs

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