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BASIC RESEARCH IN SHOCK AND SEPSIS

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INTRODUCTION

Animal-based research aims to elucidate complex physiological phenomena, understand pathophysiology of various diseases, and test therapeutic interventions and drugs for their potential progression to clinical trials. Increasingly, findings from animal studies translate into therapeutic benefits both in the human and veterinary medicine (1). Animal models of acute diseases have accompanied historical and modern medical research since its inception (2). Most recently, animal models were on the forefront of combating coronavirus disease 2019 (COVID-19) pandemic (3). Similar to other areas of medicine, animal models have gained appreciation for their role in research on sepsis, shock, and trauma, and in vivo findings triggered many important advances in those fields (4,5). However, it should be readily acknowledged that animal-based research in not free from errors, nonoptimal investigative approaches and lack of standards in some areas. Such shortcomings lead to erroneous findings, which may subsequently translate into false conclusions misguiding further animal-to-human decision making (e.g., go/no-go decisions for launching phase 1 clinical trials). In addition, suboptimal and poor-quality animal research fuels criticism from the lay public. The purpose of this communication is to provide an update on the chosen aspects of preclinical sepsis and shock research, to delineate its key shortcomings and suggest realistic improvements.

How can minimal quality in sepsis models be achieved?

A crisis in data reproducibility has been described by both pharmaceutic companies and national funding agencies. Many groundbreaking studies across various disciplines cannot be reproduced. This failure to reproduce may be due to lack of repeatability, that is, failure to provide sufficient experimental detail so that the results cannot be reproduced even in the same laboratory. Another reproducibility failure is failure of replication where the results cannot be replicated in another, independent laboratory. These problems were highlighted by several reports from pharmaceutical companies. Before taking a drug to market, companies will usually attempt to reproduce the findings from the published literature. Prinz et al. (6) queried his colleagues about their experience replicating pivotal studies from the literature. There were attempts to replicate 67 different experiments; only 25% of these studies could be reproduced. Begley (7) also queried his colleagues at Amgen about their ability to reproduce landmark studies from the literature, and the results were even more alarming: only 11% of the studies could be replicated in the laboratories.

Part of the problem relates to lack of uniform standards. An analysis of published papers showed a lack of standards in the published literature where the results from 271 studies were examined (8). Although it was noted that 95% of publications stated the purpose of the study and 74% reported the sex of the animals, serious deficiencies were noted. Among the issues were that 68% of the studies were the result of a single experiment (although it should be noted that several animals were included in the single experiment). This indicated that there was not an attempt to reproduce the study in the original laboratory. Of even greater concern, more than 85% of the studies did not report that the experiments were randomized and/or blinded, which are critical elements to ensure reproducibility and reduce bias. Failure to randomize or blind studies has been demonstrated to induce bias in the experimental results. A study using an experimental model of autoimmune encephalitis showed that a lack of randomization resulted in reporting greater improvement with an intervention (9). Lack of blinding also resulted in reporting greater improvement. Design flaws in preclinical studies can lead to drugs progressing to clinical trials that then fail. For example, studies that did not randomize or blind showed that a treatment for stroke showed a reduction in infarct volume (10), but studies that did randomize and blind did not report positive results. Subsequent clinical trials failed to show efficacy. The following experimental design features have been suggested to improve scientific rigor and reproducibility: (1) Randomize experimental subjects, (2) blind observers to which animals were treated, (3) select a model that most closely replicates the human condition, and (4) report complete results, instead of selected data.

The need to standardize a shock model

It is stated that imperfections in preclinical studies lead to poor translation of good experimental results. This also applies to sepsis, where a variety of animal models was developed in the last decades to study various aspects of a highly complex human syndrome. A solution to this problem is to set a series of well-formulated standardized requirements to increase the similarity to the clinical setting. Therefore, the currently accepted technical recommendations include the need for an adequate monitoring to quantify the severity of the condition (11). The size of a model typically defines

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possibilities on how to detect organ function changes during the (clinically relevant) time course of the disease. Therefore, it is not surprising that the experimental results of relatively well-investigated mouse models are still difficult to adapt to other species, including humans.

To improve one aspect of such a between-species adaptation, our laboratory has developed a protocol with a standardized fecal inoculum in rats and pigs. The ultimate aim of the protocol is to increase the level of clinical relevance of single-hit sepsis models. We used a rat sickness score (RSS) system, similar to that used in mice (12) to assess the severity of symptoms and to determine the criteria for termination. The unrestrained animals receive adequate analgesia and fluid therapy after intraperitoneal peritonitis induction, and the general anesthesia is maintained during the last 2 hours of the experiment. Next, a Rat Organ Failure Assessment (ROFA) score is calculated by determining (1) respiratory, (2) cardiovascular, (3) renal, (4) liver, and (5) metabolic (plasma lactate) parameters through invasive monitoring (13,14). In the 24-hour pig sepsis model, a pig-specific sequential organ failure assessment score is used, which is based on respiratory, cardiovascular, renal, hepatic, and coagulation (platelet count) parameters; the invasive monitoring commences during the last 8 hours of the study (i.e., after an intraperitoneal fecal inoculation). In both rat and pig models, changes in sublingual and small intestinal microcirculation are recorded. In the liver, kidney and brain mitochondrial function, inflammatory cytokine level, and leukocyte activation are measured.

Most importantly, our studies demonstrate a critical importance of the microbial composition of the initiating fecal insult on the outcome. In septic pigs, the pig-specific sequential organ failure assessment scores demonstrated strong correlation with Colony Forming Units in the hemocultures. However, rats injected intraperitoneally with a quantitative standardized fecal inoculum have different degrees of RSS and impact on ROFA scores contingent on whether the bacterial loads are of monomicrobial or polymicrobial dominance (15). As sepsis progressed, the bacterial colony-forming units (quantitative parameters) and diversity of the strains (qualitative factors) were progressively reduced in the ascites, whereas the RSS and ROFA values of the surviving animals are steadily improving for 12 to 72 hours from the sepsis onset. Based on these findings, we suggest that a numerical scoring of severity facilitates standardization and that an additional knowledge of the bacterial composition of the inoculum (via microbiological testing) will further improve translational research success.

How to implement clinically extrapolated endpoints in animal research?

Understanding the pathogenesis of critical care diseases is an important first step in improving outcomes in patients. The contribution of animal research to medical progress including critical care illnesses is undeniable. In the recent years, however, there has been substantial criticism, both from professional and lay organizations, concerning preclinical disease modeling of all fields pointing out poor reproducibility of findings and meager bench-to-bedside translation (16). Such a criticism challenges the role and utility of experimental animals in research, which should be addressed and remedied (11,17). Recapitulation of clinical disease phenotypes and current care standards in the animal models is the central point in a successful translation of pre-clinical findings to the clinic (18,19). The recapitulation process in animal-based research is complex, as it must simultaneously transpire on several levels and in a synchronized fashion. There are two general levels, which facilitate an appropriate implementation of clinical endpoints into animal modeling: (1) the design of the disease model and (2) execution of the study using a given model. Quality of both elements is pivotal in achieving the desired translatability. The difficulty of reproducing a given critical disease model depends on our poor versus thorough understanding of pathophysiology of a given condition.

Whereas various models of trauma (e.g., bone fractures, traumatic brain injury) are relatively easy to recapitulate in animal models, infectious diseases (e.g., sepsis, COVID-19) are much more challenging because of their complicated pathophysiology. For example, early (and improper) sepsis models based on bolus injections of lipopolysaccharide (LPS) led to many false conclusions regarding its pathophysiology (e.g., dominant hyperinflammation) and provoked futile treatment trials (anticytokine drugs) (20,21). The key dangers in the design of such disease models include a reliance on a single, seemingly proper setup and employment of a single species. Such an approach constitutes a high risk for idiosyncratic and artificial data generation with little translational value. At least two improvements should be considered. First, the tested models should include a relatively wide repertoire of triggers leading to a desired acute illness (e.g., in/direct induction of acute lung injury via intratracheal LPS, acid aspiration, intravenous bacteria) (22). Second, the illness should be tested in more than a single species (typically the mouse) to increase probability of detecting a broader range of responses (e.g., in experimental drug testing). The difficulties related to the study execution center on several modules; omission/inclusion of which may be decisive regarding the final value of an animal experiment. These components include exemplary design elements such as presence/absence of (1) comorbidities critical in a given disease (e.g., obesity or type 2 diabetes and COVID-19), (2) commonly used medications (e.g., antimicrobials in sepsis), (3) standard care practices (e.g., mechanical ventilation in acute lung injury), and (4) sex and age-based comparisons. In addition, all the above must be supported by best practices in avoiding experimental bias including blinding, randomization, allocation concealment, and proper power analyses (23) — the lack of these culminates in generation of useless findings and distortion of the scientific advance. Importantly, to strive for the best quality in animal modeling, these studies should submit to the concept of the so-called reverse translation; both on the level of disease phenotypes and pathophysiology (e.g., similar dynamics/direction of studied responses and markers) as well as study design practices commonly used in the clinical research (e.g., stratification of patients, theranostics). Only maximally fine-tuned animal models will continue to serve as a useful preclinical tool for comprehending pathophysiology and testing of novel therapeutic concepts against various critical care diseases.

Disseminated intravascular coagulation and thrombosis: What does the animal setting suggest for the human host?

An inflammatory host response is invariably accompanied by activation of the coagulation system. Disseminated intravascular

coagulation (DIC) is defined as systemic intravascular activation of coagulation leading to an excess generation of thrombin and the deposition of fibrin, with formation of microthrombi in organs with ensuing organ failure and formation of macrothrombi obstructing vessels. During the coagulation process, consumption of coagulation factors and aggregation of platelets occur, leading to a prolonged prothrombin time and low platelet counts. A combination of these laboratory-based findings is used to define DIC. However, the question is whether this classification actually reflects a single specific condition. Disseminated intravascular coagulation never occurs by itself as a specific illness and is always secondary to an underlying disorder. Very different conditions can elicit DIC, including sepsis, cancer, obstetric problems, and trauma, but with very different prevalence (24,25). In sepsis, 50% of cases have DIC, whereas DIC complicates only 7% in a cohort of cancer patients. Whereas the clinical definition based on derangement of general laboratory tests would classify all those conditions as DIC, a more in-depth analysis shows that different causes of DIC result in different underlying coagulopathies. In sepsis, lysis is inhibited, whereas, in trauma, lysis can be inhibited but also enhanced (24,26–28). Also, risks of thromboembolic events differ between different causes. Thereby, the clinically used DIC score is actually a container term, encompassing distinct entities. This is where animal models can help us gain a more precise insight regarding characterization of the different DIC forms that may exist. Most DIC models to date have used injection of procoagulants or LPS to generate a DIC-like syndrome (29). These models may serve to study the pathophysiology of coagulation derangements in DIC, as these inducers elicit the coagulation cascade. However, it is questionable whether treatments for DIC should be tested in those models. Given that different DIC forms may exist, it would most probably be an improvement if the insult eliciting DIC was modeled, for example, using bacteria to generate sepsis or inflicting polytrauma. In clinically relevant sepsis models, a recent insight into the DIC pathogenesis is the important role of factors of the contact activation system. Blocking of factor XII dramatically reduced DIC, organ failure, and mortality (30). Another important recent insight is the tight interaction between hyperinflammation-induced shedding of von Willebrand factor (vWF) from the endothelium and a relative decrease of vWF cleaving enzyme ADAMTS13, resulting in a imbalance of the ratio vWF/ADAMTS13 (31). Experiments such as these unravel the complex pathophysiology of DIC and have the potential to generate novel treatment targets for DIC, moving from anticoagulant interventions to inhibiting of hyperinflammatory host response systems.

CONCLUSIONS

It is evident that we have been steadily progressing toward a new era of clinical development, in which only quality and standardization-based preclinical modeling of acute conditions will have an important translational impact. To effectively support a beneficial animal-human-animal investigative interaction, the preclinical findings must derive from appropriately designed, reproducible, and clinically relevant animal models of sepsis, trauma, and shock. To facilitate these, both basic and clinical scientists should jointly work on standardization of the preclinical fields of intensive care research by (1) defining specific guidelines for given animal models and (2) ensuring practical operationalization and compliance of the developed guidelines.

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