The disconnect between animal models of sepsis and human sepsis

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Abstract: Frequently used experimental models of sepsis include cecal ligation and puncture, ascending colon stent peritonitis, and the i.p. or i.v. injection of bacteria or bacterial products (such as LPS). Many of these models mimic the pathophysiology of human sepsis. However, identification of mediators in animals, the blockade of which has been protective, has not translated into clinical efficacy in septic humans. We describe the shortcomings of the animal models and reasons why effective therapy for human sepsis cannot be derived readily from promising findings in animal sepsis. *J. Leukoc. Biol.* 81: 137–143; 2007.

Key Words: *cecal ligation and puncture · lipopolysaccharide · rodents*

INTRODUCTION

Sepsis with its complications is still a major challenge in contemporary medicine. Approximately 700,000 patients are affected annually at huge costs for the healthcare system. Depending on the standards of medical care, the worldwide mortality rates in septic humans range from 30% to 70% (with an aggregate mortality rate of ~50%) [1, 2]. Despite more than 20 years of extensive research and development of numerous therapeutic approaches used in clinical settings, the incidence of sepsis and the number of sepsis-related deaths are rising [2]. For a long a time, it was believed that sepsis was caused by an overwhelming immune response of the patient to invading microorganisms, but in only <50% of the patients showing symptoms of sepsis, bacteremia can be detected by standard culture methods [3]. This was taken into account when the term, systemic inflammatory response syndrome (SIRS), was established in 1992 (see below). Thus, SIRS describes a hyperinflammatory state of the immune/inflammatory systems represented by elevated levels of proinflammatory mediators with development of multi-organ dysfunction syndrome and multi-organ failure (MOF) [4]. A tight regulation of the immune/inflammatory system is crucial for maintaining the balance between protective and tissue-damaging responses. SIRS and sepsis are characterized by a loss of control over inflammatory responses, which can be provoked by a variety of causative insults.

Several different animal models of sepsis have been developed, in most of which a local intra-abdominal infection is generated to initiate systemic inflammation. Common experimental models used in sepsis research currently pursue two different strategies: a septic focus originating from injection or release of feces into the peritoneal cavity or injection of bacteria or microbial components (e.g., LPS) into the abdominal cavity or bloodstream. These approaches attempt to mimic pathophysiological changes typically seen in septic patients, ranging from bacteremia to SIRS to septic shock to multi-organ dysfunction and subsequently, death. Numerous therapeutic attempts have targeted proinflammatory mediators and have had promising effects when used in animal models of sepsis, but virtually all have failed to demonstrate clinical efficacy in human clinical trials [5, 6]. Although typical symptoms such as hyperthermia (progressing to hypothermia), tachycardia, and tachypnea can be observed in septic animals, other parameters such as levels of pro- and anti-inflammatory cytokines differ between animals and humans with sepsis, providing a possible explanation as to why human clinical trials based on effective treatment strategies in animals have failed. As a result, the initial euphoria and optimism to find a potent therapeutic strategy for septic patients were dampened, and pharmaceutical companies often consider sepsis research to be a "graveyard" rather than a promising investment with payoff.

In this review, the more frequently used models of experimental sepsis are discussed with their advantages and disadvantages and limitations. We try to bridge the gap between animal and human sepsis to address the question as to why insights from animal studies cannot be transferred categorically from the laboratory to the clinical setting.

HUMAN SEPSIS

SIRS is defined by the combination of typical clinical symptoms in the presence (sepsis) or absence (SIRS) of microbial (bacterial, fungal, or parasitic) infection: hypo/hyperthermia (<36°C or >38°C), tachycardia (>90 beats per minute), tachypnea (>20 breaths per minute or P_aCO_2<32 mmHg), and leukocytosis/leukopenia {white blood cell count >12,000/mm^3 or <4000/mm^3 or presence of >10% immature (band) forms; **Table 1**; ref. [4]}. The most frequent sources of infection are lung, abdomen, and urinary tract, but in only 40–60% of

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patients with sepsis does bacteremia occur. Originally, the leading cause of septic shock was considered to be an infection with gram-negative bacteria, but gram-positive infections in sepsis are currently as frequent or more so than gram-negative infections, followed by fungi and parasites as causative agents of sepsis [7]. It should be noted that the frequency of fungal sepsis has been on the rise [8].

At particular risk to develop SIRS or sepsis are elderly and immunocompromised patients. The average age of patients with sepsis or septic shock ranges from 60 to 65 years [2]. As would be expected, the risk of mortality rises with increasing age [1, 9]. In addition, a variety of underlying diseases as well as presence of organ dysfunction and the individual's genetic predisposition may increase the risk of SIRS and sepsis [10].

The initial insult or infection can lead to an overwhelming reaction of the innate immune system with activation of proinflammatory cascades (e.g., the complement system) and appearance of various mediators (TNF-α, IL-1, IL-6, C5a, and many more), resulting in SIRS and progressive MOF [11, 12]. At some point in sepsis, anti-inflammatory factors [such as IL-4, IL-10, IL-1 receptor antagonist (IL-1RA), and others] are released, perhaps representing a compensatory, anti-inflammatory response, although this is debatable. Too much proinflammatory mediator release may trigger an uncontrolled, inflammatory response, resulting in consumptive depletion of the clotting system, and excessive release of anti-inflammatory mediators may contribute to immunosuppression or anergy, which occurs in humans with sepsis. In this regard, there may be iatrogenic reasons for compromised immune system function in septic patients. Inotropic agents, morphine, and allogenic blood transfusions are known to induce immunosuppression [13–16]. The coincidence of the imbalance of the immune and inflammatory responses during SIRS and sepsis is still poorly understood and requires further intensive, basic research.

COMMON EXPERIMENTAL MODELS OF SEPSIS

LPS model

The systemic administration of endotoxin as a model of sepsis underlies the notion that it is the host response that causes the clinical features of sepsis and not the intact pathogen per se. Endotoxin is part of the outer membrane of the cell envelope of gram-negative bacteria. The term endotoxin is often used exchangeably with LPS, as LPS represents the main biologically active component of endotoxin. The i.v. infusion of LPS as well as its instillation into the abdominal cavity causes sepsis-like symptoms, accompanied by similarities to pathophysiological responses in patients with sepsis, such as hematological alterations [17]. Furthermore, LPS infusion induces an increase of proinflammatory cytokines in serum [18, 19], another parallel to septic patients, whose elevated cytokine levels correlate with severity of the disease [20]. TNF-α and IL-1 have emerged to play a crucial role in the development of in vivo responses to LPS and have become targets of therapeutic interventions in septic humans [21]. The injection of recombinant TNF-α or IL-1 can induce many of the features caused by LPS and by sepsis itself [22, 23]. Based on promising results of TNF-α blockade in LPS animal studies, several clinical trials were performed using TNF-α antibodies or soluble receptors (TNF-SR p55, p75). In trials using the SR p55, there was no improvement of survival [24]. A study with p75 even showed a dose-dependent increase of mortality in septic patients [24]. Overall, these trials failed to improve survival, although a trial meta-analysis of a cohort of the more seriously ill septic humans suggested a beneficial effect of TNF-α blockade [25]. The need to identify a subset of septic humans who would benefit from TNF-α blockade substantially limits this type of intervention in septic humans. Similarly, the blockade of IL-1 by the so-called IL-1RA did not significantly reduce sepsis-related mortality in large clinical trials [26]. Only patients with the highest risk of death seemed to benefit from use of IL-1RA [27]. Finally, it turns out that the LPS experimental model and sepsis in humans differ in several key points, especially in the profile of cytokine release. Cytokine levels (TNF-α, IL-6, CXC chemokines) peaked much later and occurred at much lower levels in human patients with sepsis as well as in the model of cecal ligation and puncture (CLP; described in detail below) when compared with effects of LPS infusion [12, 17]. Such findings suggested that the LPS model may not accurately reflect sepsis in the CLP model or in septic humans. Another confounding factor related to the linkage of TNF-α with sepsis is the finding that in CLP mice, the interception of TNF-α with antibody actually made survival worse [28–30]. The hypothesis

TABLE 1. Human Sepsis Criteria

SIRS:	
hyperthermia *or* hypothermia	core body temperature $< 38°C$ *or* $<36°C$
tachycardia	>90 beats/min
tachypnea	>20 breaths/min *or* $P_aCO_2 < 32$ mmHg *or* respiratory support
leukocytosis *or* leukopenia	>12,000 cells/mm^3 *or* <4000 cells/mm^3
sepsis:	
SIRS + infection	
severe sepsis:	
sepsis + organ failure (lactacidosis, kidney failure, ARDS,[a] liver failure, thrombocytopenia)	
septic shock:	
severe sepsis + hypotension	

[a] ARDS, Acute respiratory distress syndrome.

TABLE 2. Comparison of LPS Infusion and CLP Model in Rodents

LPS infusion	Cecal ligation and puncture
clinical symptoms (rapid hypothermia) develop within a few hours	clinical symptoms develop within 12–24 h
leukopenia followed by leukocytosis	leukocytosis or leukopenia
early, high transient levels of proinflammatory cytokines (IL-1β, TNF-α, IL-6)	late, moderately high levels of proinflammatory cytokines (IL-1β, TNF-α, IL-6, C5a)
protective effects of anti-TNF-α	anti-LPS or anti-TNF-α without protection
	serum LPS levels very low to nondetectable

that circulating endotoxin (LPS) causes shock in sepsis is contestable [31], as when endotoxin is detected in the blood of patients with sepsis, the levels are low [32]. It is not surprising that blocking of endotoxin (with antibody) in septic humans as well as other therapeutic interventions failed to show beneficial effects [33]. Endotoxemia has been used in baboons [34] and in human volunteers [19, 35] to define the temporal patterns and diversity of mediators appearing in the blood compartment. Infusion of live, gram-negative bacteria (*Escherichia coli*) has been used in nonhuman primates [34]. In these models, blockade of TNF-α has generally been protective, but the real question here is whether such findings can be applied to septic humans. As a result of these many observations, the LPS model may not represent a valid model to simulate sepsis, although it can be used to determine the pathophysiology of endotoxemia (**Table 2**).

Fecal pellet model and bacterial inoculum model

The model of fecal pellet peritonitis mimics sepsis induced by i.p. administration of feces [36]. A pellet most commonly within a fibrin clot is used [37, 38]. Without additives, feces instillation leads to rapid death or recovery of the animals [39]. In many respects, the fecal pellet model, with the mixed aerobic and anaerobic flora, resembles the CLP and the colon ascendens stent peritonitis (CASP) models (below). The fecal pellet model has been widely replaced by other models, such as the bacterial inoculum model.

The bacterial inoculum model features a known number of *E. coli* CFU, which are infused i.p. [40]. Usually, "adjuvants" may be added [40], but the procedure can also be performed without such additives [41, 42]. Injection of pure cultures of bacteria without a carrier is considered to represent another model of endotoxin shock rather than of peritonitis [43]. Numerous variants of i.p. bacterial delivery exist, such as using different species of bacteria (e.g., *Bacteroides fragilis*) or a combination (*E. coli* plus *B. fragilis*) [44]. The mortality rate depends on the species of animals used and the number of bacteria administered, so that this model becomes more controllable and reproducible than the fecal pellet deposition. The fecal pellet and bacterial inoculum models mimic abscess formation following peritonitis rather than abdominal sepsis, especially when *B. fragilis* is involved [45]. This could be the reason why treatment with antibiotics and fluid resuscitation reduces the mortality rate to 20%; in this model, the abscess is often walled off [46].

CASP model

A CASP model has been used to induce sepsis in mice. The idea behind this model is that intestinal leakage (e.g., after major abdominal surgery) leads to bacterial invasion of the peritoneal cavity followed by organ failure, septic shock, and death. A stent is inserted into the ascending colon, generating a septic focus [47]. With a maximum mortality of 100% within the first 48 h, the use of stents of different sizes can modify mortality rates. The CASP model has been shown to result in organ dysfunction similar to that seen in septic patients. Acute lung injury, renal failure, and bone marrow cell dysfunction have been reported following CASP [48–50]. Proinflammatory mediators such as IL-1 and IFN-γ are thought to play an important role in this model of sepsis as well [47, 51]. Survival in the CASP model seems to be TNF-α-independent as opposed to the CLP model (see below) [47]. Although pathophysiological changes after CASP appear to be similar to those in human sepsis, the nature of the model limits its use. Stenting the colon ascendens creates a constant leakage of bowel content, mimicking the situation of an insufficient anastomosis following bowel surgery, which is often followed by sepsis.

CLP model

Similar to CASP, an abdominal septic focus leads to a polymicrobial infection of the peritoneum in the model of CLP, eventually resulting in bacteremia, SIRS, sepsis, septic shock, and usually death. This model features ligation immediately below the ileocecal valve followed by through-and-through needle puncture of the cecum. Subsequently, animals develop typical symptoms of sepsis or septic shock and a high mortality rate [28, 49]. Since the first description of the CLP procedure, it has been used extensively as a model for experimental sepsis [52]. Besides analysis of survival, the complex pattern of cytokine expression has been investigated extensively. The main proinflammatory cytokines, IL-6 and TNF-α, have been shown to increase following CLP. Further, it has been reported that high levels of IL-6 strongly correlate with survival after CLP, a phenomenon that also occurs in human sepsis [53–55]. We have investigated the role of the anaphylatoxin C5a and its receptor C5aR during sepsis. Using the CLP model, we have found that blocking C5a or C5aR by administration of antibody significantly improves survival and prevents the development of organ dysfunction [56, 57]. Similarly, blockade of IL-6 results in increased survival of septic mice following CLP [58]. In contrast, if TNF-α were inhibited by administration of TNF-α antibodies or using p55 (TNF-αR I)-deficient mice, the mortality increased [57–60]. As described above, levels of TNF-α peak earlier and are much higher in the LPS model than in the CLP model.

Despite its clinical relevance and widespread use in sepsis research, a concern of the CLP model is consistency. The outcome after CLP is strongly associated with several factors

during the procedure. The length of the cecum ligated is a major determinant of mortality [59]. Moreover, not only does the distance of cecum ligated influence survival, serum levels of proinflammatory cytokines such as IL-6 and TNF-α are increased markedly with increasing length of cecum ligated [59]. Other factors affecting mortality following CLP are size of needle used for the puncture, the number of punctures, fluid resuscitation, and antibiotic treatment. As a consequence, it is of great importance that the procedure of CLP is performed with high consistency and reproducibility to evaluate findings obtained from septic animals. In summary, the model of CLP has become the most widely used model for experimental sepsis and is currently considered the "gold standard" in sepsis research, with the caveat that mixed aerobic and anaerobic gram-negative bacteria predominate (Table 2).

Nonhuman, primate models of sepsis

Sepsis models involving the i.v. infusion of live *E. coli* have been used in baboons. Endpoints have included survival curves, biochemical evidence of disseminated intravascular coagulation (DIC), and appearance in serum/plasma of proinflammatory cytokines (IL-6, IL-8, TNF-α). Treatment with anti-TNF-α or with antithrombin III markedly improved survival, reduced evidence of DIC, and reduced blood levels of proinflammatory cytokines [60–62]. Treatment of *E. coli*-infused monkeys with anti-C5a also improved survival and biochemical parameters of sepsis [63, 64]. Infusion of LPS into baboons or humans results in appearance of proinflammatory mediators in blood and evidence of DIC [65, 66]. The infusion of live *E. coli* has been described as a type of "acute intoxication" in which similar blood levels of *E. coli* are not seen in septic humans [67]. The levels of TNF-α occurring after infusion of LPS or live *E. coli* into animals (including mice) are high and sustained, in contrast to the transient levels of TNF-α in septic humans [68]. All of these observations raise the question as to whether the nonhuman, primate models of sepsis are surrogates for human sepsis. Because of ethical reasons as well as the lack of access to intensive care facilities, models of bacterial peritonitis have not generally been used in nonhuman, primates, creating a gap between rodent models of bacterial peritonitis and septic humans.

DISCUSSION

In spite of substantial research in experimental sepsis models, few if any of these promising findings have been shown to be therapeutically efficacious in septic patients based on clinical trials [69]. Only three therapeutic strategies that improve patient outcomes in sepsis are currently being used on intensive care units. These include administration of low-dose glucocorticoids, intensive insulin therapy, and infusion of activated protein C (APC) [70–72]. However, APC represents one of the first approved drug (with restrictions) for the treatment of sepsis which may affect the inflammatory response. APC specifically targets the clotting pathway and may affect parameters of the inflammatory response. Patients with sepsis only benefit from an early intervention, even if the therapy consists solely of supportive, nonspecific treatment [73]. Therefore, the question remains as to whether the animal models currently used to study sepsis differ too much from septic humans or if researchers and physicians get lost in translation when attempting to adapt their findings into daily practice.

The Shock Society and the International Sepsis Forum dedicated a symposium about that issue held on Oak Island, Nova Scotia, in 2005. There is an abundance of preclinical models of shock and sepsis, but we have yet to determine if and how promising findings obtained from these models can be translated to the setting of human sepsis. Obviously, there are clear differences between laboratory animals and patients. Mice and rats are housed in specific pathogen-free areas, may often be inbred strains, have the same age and weight, and most importantly, do not have comorbidities (such as diabetes, hypertension, and pre-existing immunosuppression among others) seen in septic humans. In light of the fact that most humans with sepsis are >50 years old, and as most mice used in sepsis are <3 months old (with an average lifespan of 24 months), it is possible that there is a "disconnect" between the study of mice and humans with sepsis. Furthermore, the experimental models have a precisely known time period. In contrast, we encounter patients of different ethnicities, ages, and weight, and most of the time, we are uncertain when the symptoms first emerged. In addition, there are differences between rodents and humans on the molecular level. For instance, human C-reactive protein (CRP), an acute-phase protein and popular marker for inflammation, is known to be an activator of the complement system in humans, but in contrast, rat CRP does not influence the complement system [74, 75]. The fact that there are differences between TLRs in mice and in humans could also affect interpretation of sepsis studies in the two species [76]. Therefore, the simple transfer of knowledge from animals to humans is highly illusive. To investigate underlying pathomechanisms that will allow us to develop effective therapies for sepsis patients, we must aim at models of sepsis that resemble human sepsis as closely as possible.

Almost all of the sepsis models currently in use involve the primary administration of bacteria, bacterial cell wall components, or other organisms that subsequently trigger the immune system. However, there are many other initial events such as burns, major trauma, or surgical procedures that can lead to a systemic inflammatory response. Therefore, findings obtained from the CLP model, for example, can be applied to patients with abdominal perforations as the initial event leading to peritonitis and sepsis. However, other patients may have experienced different insults, which may eventually result in sepsis as well, but other pathways may have been activated, perhaps explaining why so many clinical trials have failed.

Many immunomodulating strategies have been used on the basis of experimental data from animal studies. However, the conditions seen in experimental animal models of sepsis do not necessarily reflect the pathophysiological situation of patients with severe sepsis or septic shock. As outlined above, sepsis in animals and in humans depends on many variables and often differs in its severity, which in turn may be closely linked to beneficial effects of immunomodulating strategies. As already mentioned, except for APC, none of the anti-inflammatory strategies, which showed promising effects in experimental

sepsis, has improved the outcome in studies of patients with sepsis [6]. This may be partly a result of the inhomogeneity of patient populations in most of the sepsis clinical trials. Here, inclusion and exclusion criteria seem to be crucial. Although the above-mentioned definitions for SIRS and sepsis are criticized for being too sensitive and not adequately specific, they are still commonly used as the standard criteria for the enrollment of patients into clinical trials [77]. This may explain why some clinical trials involving small numbers of patients showed significant results, but such beneficial effects failed to materialize in controlled, randomized Phase III trials, as the benefit of anti-inflammatory therapies in septic animals as well as in humans with sepsis is tightly linked with the severity of disease [78]. In other words, the most desperately ill patients had the greatest benefit. Therefore and as a result of the fact that an early intervention may be beneficial [73], the establishment of a reliable system for diagnosing and staging sepsis is required, just as it is represented by the tumor node metastasis (TNM) system for classification of malignant tumor diseases. An attempt in this direction was undertaken 2001 with the creation of the PIRO system as a tool for clinicians as well as for researchers to diagnose sepsis [10]. Besides the initial injurious or infective insult and the presence of organ dysfunction, the patient's predisposition and current status of the immune response are taken into account. In the future, a system for diagnosing and prognosing sepsis should be endorsed by biomarkers such as mediator profiles or receptor status [79]. Based on a "molecular fingerprint" providing information about the individual situation and stage of disease, patient-targeted, therapeutic strategies could then be pursued.

With regard to the human sepsis syndrome, sepsis models such as CLP closely resemble the course of sepsis observed in patients, characterized by an early hyperdynamic, hypermetabolic state, followed by a pronounced hypodynamic, hypometabolic state. However, we need to realize that there is no single, ideal model of sepsis. The sepsis syndrome is a complex and a therapeutically challenging disorder involving several organ systems. It is in the nature of basic research that usually, single pathways or mechanisms are being investigated in a rather isolated manner in experimental models, whereas a septic patient presents as a complex and interconnected system that is out of balance. Although one of the attributes of using mice for basic research lies within the availability of gene knockouts, one has to be aware that results obtained from animals cannot suffice to draw conclusions on an individual patient. Nevertheless, animal models represent an important and indispensable tool to derive a better understanding of the underlying molecular and genetic mechanisms of SIRS and sepsis. Thereby, the limitations of the particular animal model used to investigate sepsis must be kept in mind.

WHERE DO WE GO FROM HERE?

How to bridge and extrapolate data from animal sepsis to the setting of human sepsis is a daunting challenge. As indicated above, the interventions that have been protective in septic animals have not shown clinical efficacy in humans. This does not mean that animal models of sepsis are irrelevant or that they provide irrelevant information for application to humans. It is likely that animal models will be useful, provided we understand their limitations. First, we need to determine what the most relevant animal models of sepsis are. As suggested above, the most relevant model is probably not endotoxemia or massive infusion of live organisms. CLP may be one reasonable surrogate of human sepsis, but it may not yield information relevant to the increasing incidence of sepsis caused by gram-positive bacteria and fungi. Accordingly, it would seem that several models of sepsis featuring live organisms should be used. Furthermore, there is nothing wrong with the use of products from live organisms, such as staphylococcal toxins, provided limitations in the interpretation of such data are acknowledged. Second, we need to consider if the use of nonhuman primates will provide data that are different from information generated from septic rodents. The major difficulty here is the expense of such studies, the substantial animal welfare concerns, and the need to have intensive care-type facilities if nonhuman primates are to be used. It would certainly be important to know if the use of the larger animals resulted in divergent data about the pathophysiology of sepsis and the mediators involved as compared with findings in rodents. Third, the age of the animals may lead to different data, as described above. Should animal sepsis studies be redesigned to reflect more accurately the corresponding age of septic humans where advanced age is clearly known to be a risk factor? Mediator profiles in aging mice might be quite different from those in young mice and might therefore require a reassessment of the most important mediators. Fourth, should animals undergoing sepsis studies be treated in manners similar to septic humans? In addition to fluid resuscitation and broad-spectrum antibiotic treatment, should they receive agents such as morphine, inotropic drugs, or allogenic blood transfusions, all of which can lead to immunosuppression as well as other outcomes?

Clearly, there are no definitive answers, but these observations underscore the exciting challenges that lie ahead if we are able to make new progress into more effective, therapeutic interventions in human sepsis.

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