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Sepsis

Polymicrobial sepsis models: CLP versus CASP

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Sepsis is a disease syndrome covering many different aspects of the host immune system. Our understanding of sepsis is still incomplete. Several animal models of sepsis have been developed and much of our current knowledge on the molecular basis of the disease has originated from these models. Two of the most reliable and clinically relevant rodent models to mimic human

(CASP).

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Introduction

Treatment of sepsis is still a major issue for our health care system and morbidity and mortality among patients are very high. Treatment options of septic patients are scarce and have not changed dramatically over the past three decades. These strategies rely predominately on the right choice as well as the timely application of broad-spectrum antibiotics, hemodynamic resuscitation and support of organ function if necessary [1]. However, to date many bacterial strains resistant to certain antibiotics emerge as imminent problems in our health care system. Therefore, there is a dire need for new therapeutics eliminating the primary 'threat' as well as modulating the host immune response. Unfortunately immune suppressive treatment of septic patients with 'biologicals' targeting proinflammatory molecules such as TNFα as well as procoagulant molecules such as tissue factor proved to be ineffective and failed with only a few exceptions. The development of these 'biologicals' based on initial studies mostly in the 1990s using different animal models of sepsis. Unfortunately although most of those agents showed promising results in animal studies either the choice of the model or species differences precluded successful trials in

Sepsis

Sepsis is clearly defined by a systemic host response to an invading pathogen. The systemic inflammatory response syndrome (SIRS) may also be induced by other noninfectious insults such as burns and trauma. Sepsis is further characterized by clinical signs of hyperthermia or hypothermia, tachycardia, tachypnea, leukocytosis or leukocytopenia [2]. Concomitantly the compensatory anti-inflammatory response syndrome (CARS) is initiated to limit the inflammatory response and protect the host from excessive inflammatory tissue damage. Sepsis is a multifaceted disease syndrome with a multitude of factors and parameters involved and therefore the disease is still barely understood. Homeostasis of proinflammatory as well as counter-acting anti-inflammatory mechanisms is mandatory to prevent the dysfunction of host organs such as lung, liver or kidney. Moreover it is important to categorize patients to develop personalized treatment strategies. In some immune-suppressed patients the initial infection cannot be contained, spreads and causes sepsis. In another group of patients the host inflammatory response is exacerbated, continues and becomes dysregulated leading to SIRS, sepsis and subsequently septic shock. This course of the disease results in

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30–50% lethality at least for septic patients in North America [1]. The initial triggering cause of the disease may be multifaceted. The majority of the septic complications are due to various bacterial infections. However, alternative but less frequent causes for sepsis are viruses, fungi and other parasites. The pathogens may enter the host via different body surfaces due to dysfunctional barrier protection. Among those the lungs as well as the intestines are commonly found entry routes. Influx of bacteria into the peritoneal cavity after leakage of the intestines due to disease, trauma or surgery leads to peritonitis, which may result in sepsis [3]. Therefore different animal models such as 'cecal ligation and puncture (CLP)' and 'colon ascendens stent peritonitis (CASP)' mimicking this type of disease progression have been developed during the past 30 years.

Cecal ligation and puncture

The CLP murine model of peritoneal, polymicrobial sepsis is the so-called 'gold standard model'. It has been widely used over the past 30 years to study the pathobiology of sepsis [4,5]. CLP was developed among others by the group of Chaudry in the 1970s [5]. In PubMed more than 1600 hits for 'cecal ligation puncture AND sepsis' are found. CLP has several advantages to offer. It requires a simple experimental procedure, which is represented by polymicrobial infection of the peritoneum with a localized infectious focus. Furthermore, bacteria as well as pathogen associated molecular patterns (PAMPs) are released into the host periphery. Taken together this will eventually lead to septicaemia at later stages of the disease. In recent publications the model has been described together with an educative video [6,7].

In short the procedure to induce CLP in mice is characterized by midline laparatomy, the cecum is prepared and ligated directly located below the ileocecal valve (Fig. 1). This will lead to necrosis of the cecum, which is a chronic source of inflammatory stimuli in the further progression of the disease [8]. Cecal ligation is followed by the puncture of the cecum by a defined needle size.

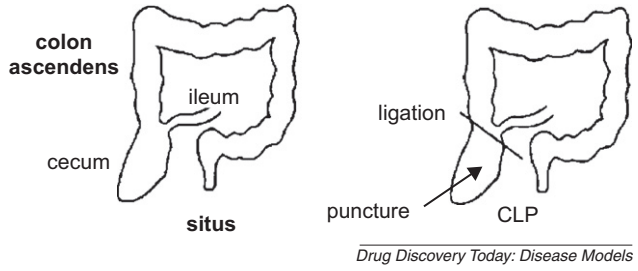

Figure 1. Schematic representation of cecal ligation and puncture model. CLP is induced by isolation, ligation and puncture of the cecum right below ileocecal valve. The location of the ligation and the type of puncture determine the severity of the disease model. Sham operation is performed by isolation of the cecum without ligation and puncture.
Modified from Ref. [23].

The severity of peritonitis can be increased by a thorough through and through puncture. Moreover, the cecum may be punctured more than once. Wound patency is warranted in order that feces can leak into the peritoneal cavity. In theory needle size as well as the number of punctures define the severity of the disease [5]. However, there is conflicting data in the literature on that matter claiming that needle size as well numbers of punctures do not alter the severity of the model [9]. At later stages of CLP the disease is irreversible. This is shown by the late excision of the ligated cecum, which did not prevent mortality of mice in the model [10,11]. Interestingly, necrotic tissue of the ligated cecum does not interfere with innate immune functions of the animal. A recent study could show that the fecal content, in a TLR4 independent manner, but not the necrotic tissue causes impaired bacterial clearance upon secondary infection with *Pseudomonas* [12].

CLP shows only transient bacterial dissemination in the peritoneal cavity and in particular other organs [13,14]. Therefore, septicemia is not as pronounced as in the CASP model but may be detected at later stages of the disease [9,15].

Several important inflammatory molecules have been shown to be indispensable for CLP peritonitis. For instance, TNFα plays a pivotal role in the development of this disease model [13,14]. Downstream of PAMP recognition the release of cytokines is required to mount the host defence against the CLP-induced bacterial infection. However, the important cytokine IL6 shows conflicting results in this respect. On one hand IL6 is required for survival in experimental sepsis models using *Listeria* and *Klebsiella* [16,17]. By contrast IL6 is an essential molecule in the pathobiology of tissue or organ damage in the CLP model. Blockade of IL6 using antibodies increased the survival of septic mice. In addition reduction of complement activation as well as neutrophil depletion leading to reduced IL6 levels also protected the animals in the CLP model [18,19]. Another example for an essential innate immune cascade which is involved in host defence that has been analyzed using CLP is the complement system. The complement system is an important antibacterial as well as proinflammatory protease cascade. Blockade of the complement factor C5a or the C5a receptor leads to organ protection and increased survival in the CLP model [20–22]. Taken together it can be summarized that the CLP has been extensively used to study molecules that have been implicated in the host immune reactions to bacterial infection.

More recently a second murine peritoneal sepsis model has been developed, namely the CASP. Although it has been developed to circumvent some of the flaws of the CLP model such as reproducibility, it turned out to be entirely different in terms of pathogenesis and disease progression.

Colon ascendens stent peritonitis

The CASP model of peritoneal, polymicrobial sepsis is not as commonly used as CLP. Only in recent years this model began to replace or rather complement the 'gold standard model' CLP. In PubMed less than 100 articles containing (colon ascendens stent AND sepsis) can be found. This clearly indicates that the scientific community still heavily relies on CLP to model peritoneal sepsis. The CASP model was first introduced as an alternative sepsis model in 1997/98 by Zantl *et al.* [23]. Recently the model has been thoroughly described including an interesting as well as educative video [24].

In short the procedure to induce CASP is characterized by the fixed insertion of a stent (catheter) into the colon ascendens by laparotomy (Fig. 2). Fecal content is milked from the cecum into the stent and finally may leak from the stent into the peritoneal cavity, which leads to polymicrobial peritonitis and subsequently sepsis [24]. The severity of the disease is defined by the diameter of the stent ranging from 14 gauge (100% lethality) to 20 gauge (50 or less % lethality).

As mentioned above all of the mice with an implanted 14G catheter succumbed to the disease and died within the first 48 hours postsurgery [9]. Interestingly the model system can be modified by removing the stent after a certain period of time to mimic surgical intervention in patients with the aim to eliminate the source of infection. However, it has been shown that a particular time interval postsurgery the removal does not improve survival (approx. nine hours). This has been shown for the 14G CASP model [23]. In effect this is comparable to the late excision of the ligated and necrotic cecum, which eventually did not rescue CLP mice from death.

In contrast to CLP, CASP does not seem to strictly rely on TNFα secretion [23]. The CASP model strongly depends on the activation of the innate immune system via TLRs, in particular TLR2 and TLR4. In addition the activation of the TLR adaptor molecule MyD88 is important for the development of sepsis induced by the CASP model [25]. In a more recent study administration of a single bolus of an inhibitory anti-TLR4/MD2 antibody could prevent CASP induced death [26]. Among the inflammatory cytokines and bactericidal molecules implicated in sepsis IL12 and inducible nitrogen monooxide synthase (iNOS) have been studied using the CASP model. Gene deficiency for IL12 as well as iNOS rendered mice more susceptible to CASP peritonitis underscoring the importance of these molecules in the antibacterial immune defence [27]. Downstream of bacterial recognition and the initial inflammatory response by immune competent cells, activation of the complement system is also important in CASP. Administration of exogenous complement factor C3 protected mice from lethality (or symptoms) of CASP induced sepsis [28]. The coagulation cascade and associated anticoagulation molecules have been shown to be important in inflammatory as well as infectious diseases. One of those molecules, the activated protein C (aPC), which not only terminates the coagulation cascade but also has interesting cytoprotective features, is already in clinical use for the treatment of septic patients. Recently it could be shown that

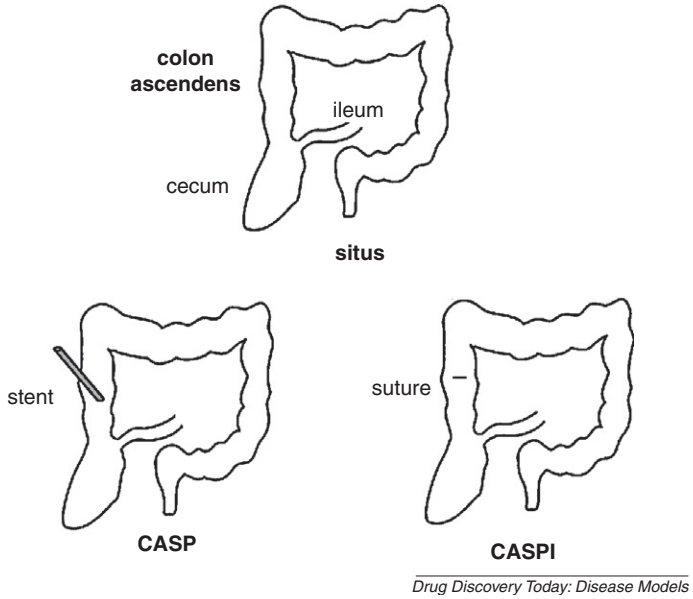

Fig. 2. Schematic representation of the colon ascendens stent peritonitis model. CASP is induced by the fixed insertion of a stent into the ascending colon right next to the cecum. Sham operation is performed by fixing the catheter to the intact intestinal wall. CASPI is defined by the removal of the stent after CASP induction (less than nine hours).
Modified from Ref. [23].

administration of an aPC variant with only minor anticoagulatory but normal cytoprotective and anti-inflammatory properties could significantly prolong the survival of CASP mice [29]. This finding might be useful for septic patients, because common side effects of treatment with recombinant aPC are serious bleeding complications. Taken together this clearly shows that CASP is an important model to mimic human sepsis. However, the scientific community is still not convinced in the regular use of it.

Model comparison

Possible flaws of CLP and CASP sepsis models

One of the most important issues applying the CLP or the CASP model is the high variability among the animals included in a particular study. Experimental variability may obscure a hidden phenotype. However, published data indicate that the CASP model is more reliable than CLP in this respect [9]. High *n*-numbers are needed for both CLP and CASP to achieve certainty whether the observed effect is based on a real phenomenon. First and foremost the animals selected for a particular experiment must be housed under the very same conditions for an extended period of time (at least one week). Littermate controlled experiments are preferred. In addition, reasons for inter-animal differences may be the experimental handling of mice and surgical expertise of the experimenter. Another possibility might be simple anatomical differences among animals. For instance the cecum even of inbred littermate mice may be different in size or fill level. However, the size of the ligated cecum determines the degree of severity of sepsis. This is very important to consider for the CLP model. The fecal content, the condition as well as the actual amount of stool present in the cecum can vary quite a bit. The same is true for the colon to a certain extent for the CASP model. Similarly differences, in the fecal material that leaks out either the puncture (CLP) or the stent (CASP) might account for additional inter-animal variability.

CLP versus CASP

Both models do have several advantages as well as disadvantages (Table 1).

As pointed out CLP and CASP are diverse models covering different aspects of sepsis. The response of the host to the CLP model is the attempt to fend off the infection by walling off the source of infection. This abscess formation has often been observed in CLP [5,9]. By contrast the CASP model is clearly a model for polymicrobial peritonitis with bacteremia detectable as early as 12 h postsurgery. Systemic and circulating levels of cytokines and chemokine such as TNF, IL-1, IL-12, IL18, IFNγ, KC/GRO-α and MCP-1 are observed within a short period of time (three hours) after stent implantation. But not only proinflammatory cytokines indicative of SIRS are present early on. Also anti-inflammatory IL10, which is a sign of CARS is expressed after three hours post-CASP induction [15,30].

These differences between the two models have been shown for several various gene-deficient mouse strains that have been subjected either to CLP or CASP. These published data show contrary results for different important molecules involved in the murine innate inflammatory response to infection. TNFα deficient mice are protected in CLP, whereas TNFRp55/TNFR1 deficient show no difference as compared to wildtype mice in the CASP model [14,23]. Although several reviews point that out, one has to be careful with that assumption, because only one TNFR is eliminated. Apparently there is a distinct phenotype of TNFR1 (mediates TNFα cytotoxic effects) and TNFR2 (protective) deficient mice, at least when subjected to CLP [31]. The opposite has been found for IFNγ and IFNγ receptor deficient mice in the CLP and CASP model [23,32]. Similarly deficiency of the important innate immune cytokine IL12p40 results in higher susceptibility to the CASP model [27]. By contrast no significant effects have been found when using the CLP model [32]. In this respect one has to mention distinct results that

Table 1. Characteristics of animal models of peritonial sepsis.

	Cecal ligation and puncture (CLP)	Colon ascendens stent peritonitis (CASP)
Pros	- Easy procedure - Less time consuming - Analysis of inflammatory cell recruitment - Partly reflects human disease	- Analysis of bacteremia and sepsis development - Analysis of SIRS - Partly reflects human disease - Similar disease staging as compared to humans
Cons	- Invariability - High *n*-numbers required - Restriction to the peritoneum and invariable bacterial dissemination - Necrosis of the cecum	- Invariability (less than CLP) - High *n*-numbers required - Procedure is more demanding in terms of experimental skills - Time consuming
Best use of model	Study of peritoneal abscess formation accompanied by local peritoneal infection, inflammation and infiltration	Study enterobacterial peritoneal infection accompanied by bacteremia, SIRS and sepsis progression

accumulate in the literature when using the very same model. A more recent paper using IL12p40 deficient mice in sublethal CLP provide evidence that these mice are more susceptible to CLP-induced sepsis [33].

Comparison of some of the key players in the innate immune defence revealed that there are specific differences in the outcome which may be attributed to the model of choice. However, still some of the discrepancies found in the literature are not due to the CLP or the CASP model. The precise reproducible experimental setup is of utmost importance to achieve comparable data sets in different laboratories.

Conclusion

Animal models of acute inflammation and infectious diseases, especially in rodents, have long been used to study and understand the pathophysiology of human sepsis. Furthermore a great number of therapeutics has been evaluated in studies using animal models of sepsis. To date only a limited number of those studies have proven to be translated successfully from the murine model to the treatment of human patients in the clinic. In many of those disappointing failures the wrong choice of the model precluded successful trials. In the meantime models such as CLP and CASP have been used extensively. These models are better characterized by now and quite a bit of knowledge on the parameters involved have been accumulated over the past ten years of research. They offer the opportunity to re-evaluate some of the treatment strategies (most of those failed) that have been developed some years ago.

However, it is clear that sepsis is a disease covering a multitude of immunologic, inflammatory as well as coagulatory aspects (and many others not even mentioned in this review). It shows a multifactorial disease pattern, which implies multifactorial therapy. To study these combinations of possible treatment options *in vivo*, CLP as well as CASP offer advantages to analyze different aspects of sepsis.

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