Trakia Journal of Sciences, No 1, pp 13-23, 2013

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Review

SEPSIS MODELS IN EXPERIMENTAL ANIMALS

D. Popov[1*], G. Pavlov[2]

[1]Department of Physiology, Medical Faculty, Medical University, Plovdiv, Bulgaria
[2]Department of Anesthesiology and Intensive Care, University Hospital "St. George" Plovdiv, Bulgaria

ABSTRACT

Sepsis develops as a common inflammatory response to various infections. It runs with the picture of a complex heterogeneous syndrome which often leads to the development of multiple organ dysfunctions and causes the death of millions of people worldwide. For various reasons, detailed research of sepsis in humans is difficult to conduct; therefore, a lot of efforts have been made to model it. PURPOSE: The aim of this paper is to present the most widely used experimental sepsis models developed with a view to explane the pathogenetic mechanisms of the disease, and it's clinical and paraclinical characteristics, as well as opportunities for its therapeutic management. METHOD: This review is based on a detailed survey of the available literature. RESULTS: The cited models differ by the type of laboratory animals, pathogens used, and the method of their introduction in the organism. Models can be defined as "non-surgical" (with parenteral introduction of endotoxins or pathogenic bacteria), and "surgical" – referring to preceding operative intervention which is aimed at inducing peritonitis such as ligation and subsequent puncture of the cecum, insertion of a stent in the wall of the ascending colon, implantation of bacterial cultures or of pathogens included in the composition of different carriers. Advantages and disadvantages of the reviewed models are considered, as well as the extent of resemblance to clinical sepsis in its various forms. CONCLUSION: The fact is that not one of the created models is capable of reproducing entirely the complex polymorphic and dynamic picture of sepsis. Nevertheless, anyone of them can provide reliable information on separate components of the septic process.

Key words: infection, inflammation, endotoxin, bacteremia, peritonitis, septic shock.

Sepsis is a complex and heterogeneous syndrome defined as a common inflammatory response to infection (1). It represents a very severe reaction of the immune system, activation of the pro-inflammatory cascades and the compensatory anti-inflammatory response (2). The resulting hemodynamic changes, microcirculatory disturbances and cellular disorders create a disparity between tissue perfusion and metabolic demands. The combination of these factors causes development of multiple organ dysfunction and death (3).

***Correspondence to**: *Dobrin Ivanov Popov, 15A, Vasil Aprilov Blvd., 4002 Plovdiv, Bulgaria*
tel.: +359 32 602 423, e-mail: dobrinpopov@gmail.com

Every year an estimated 18 million people worldwide develop sepsis with an approximate 30 % death rate, which makes it a serious healthcare and social problem (4). Research of sepsis in humans is difficult due to the complexity of pathologic processes, heterogeneity of the affected population, lack of firmly established diagnostic markers, and restrictions of methodological and ethical nature (5). Taking into account the above difficulties, sepsis models in animals have been created which are affordable and valuable research tools (6). They provide a unique opportunity to elucidate the mechanisms of the disease and outline the capacity and specific approaches of therapeutic intervention.

Several experimental models replicate to a various extent the clinical changes and alterations of laboratory parameters observed in sepsis. The created models differ mainly by the type of experimental animals, methods of infection, and the microorganisms used. In this review the most commonly used sepsis models will be presented. According to the method of infection they can be classified as non-surgical and surgical (7).

I. Non-Surgical Models

In these models, a systemic inflammatory response is caused by direct parenteral deposit of the agent which spreads rapidly in the body.

1. Models of endotoxin application. Endotoxin, a lipopolysaccharide (LPS), is a component of the outer membrane of Gram (-) bacteria. It is involved in the pathogenesis of sepsis and is widely used for research related to this pathology. LPS is a stable compound which can be stored in lyophilized form. It can be measured precisely and given to test animals as bolus or infusion (6). The compound is introduced intravenously, intraperitoneally, or intratracheally and thus, the simplest sepsis model are created. In critically ill patients the growing serum concentrations of the endotoxin refer to development of sepsis and correlate with the seriousness of the disease and lethality (6, 8).

Since compared to humans, laboratory animals show relatively lower sensitivity to LPS, a higher dose is needed so that an inflammatory response can be elicited. In studies which compare the effects of LPS, the dose causing a similar cytokine response is 250 times higher in mice than in humans (9).

Alternatively, various animal species have different sensitivity to endotoxin. Rabbits, sheep and primates show high sensitivity while rodents, dogs and cats are relatively resistant (6, 10). In them, through preliminary sensitization with killed microorganisms or D-galactosamine, the dose of LPS causing inflammatory response is reduced (11).

Application of LPS causes systemic inflammation which simulates many of the initial clinical signs of sepsis, including increase of proinflammatory cytokines, such as TNF-α, IL-1, IL-6 and IL-8; however this reaction is temporary (12-14). Production of cytokines reaches peak values 1.5 - 4.5 hours after application, and, thereafter, decreases as opposed to sepsis in humans (15). An exception is meningococcal sepsis, in which cytokine levels are comparable to those in LPS models (16).

In sepsis models with endotoxin various animals are used, and doses and duration of application vary substantially. Introduction of high LPS doses in rats – 40 mg/kg (17), and rabbits – 5 mg/kg (18), causes a hypodynamic circulatory state with reduced cardiac output, rapidly developing collapse and subsequent death (6, 17, 18). With lower doses of LPS in rats (0.1 mg/kg) (19), and rabbits (1-3 μg/kg) (20), a hyperdynamic reaction occurs with early increase of cardiac output. Application of high-dosed LPS in big mammals such as sheep – 4 μg/kg (21), dogs – 2 mg/kg (22), and primates – 10 mg/kg (23), causes hypodynamic circulation with a sharp drop of arterial pressure, cardiac output, hepatic perfusion, tachycardia, increase of total vascular resistance and plasma lactate levels. A collapse develops rapidly and lethal outcome occurs. Low doses, in sheep 0.5-0.75 μg/kg (21, 24) cause a two-stage response: initial decrease of cardiac output, severe pulmonary hypertension and hypoxemia, and a few hours later are observed an increase of cardiac output, mild pulmonary hypertension, and increased permeability of the pulmonary capillaries (25). In primates, when a low dose (2 mg/kg) is used, sepsis develops characterized by early cardiovascular and metabolic disorders, coagulopathy and progressive multiple organ dysfunction (26).

Fish and Spitzer (27) studied the effect of chronic application of LPS in rats by introducing it continuously with the help of an osmotic pump whereby animals developed hyperdynamic sepsis accompanied by anorexia, leucocytosis and lactacidemia. Investigating the myocardial function of a working heart preparation isolated from rats treated with endotoxin, they found myocardial dysfunction characterized by a decrease of the peak systolic pressure, cardiac output and increased oxygen consumption per unit of myocardial work (28).

The endotoxin models are popular because they are convenient and reproducible. LPS can be

measured precisely and its application is readily standardized during experiments (6). When using animals, the applied dose and duration of effect vary significantly, thence the induced changes also vary. Application of high toxin doses causes uncontrolled aggression in the animals (12, 29), while with medium and low doses they recover very quickly, for no longer than 48h. Continuous or multiple introductions of LPS causes tolerance to endotoxin (6, 27, 30).

In experimentally induced LPS sepsis it is not possible to reproduce exactly the characteristic features of clinical sepsis. For example, cardiovascular changes described in animals do not correspond to those observed in humans. Moreover, the cytokine responses in animals appear sooner and are stronger, but shorter (14, 31). On the whole, clinical course and development of the disease in rodent LPS models are more dynamic by far than those observed in humans (14, 31).

Currently, there is a unanimous opinion that application of LPS can be used to study the pathophysiological processes in endotoxemia and as model of endotoxic shock, but not of sepsis (32).

2. Models with intravascular administration of pathogens. Sepsis models exist in which many characteristic features of clinical sepsis are simulated by intravascular introduction of live microorganisms. Various types of cultured bacteria, most frequently *E. coli*, are used. In the various models, dose, frequency and duration of effect, and last but not least, the resuscitation applied, vary significantly. These factors have their impact on the course of disease and the outcome thereof (6, 14, 31). For example, in rats a dose of 4-5•10^8 *E. coli* causes a transitory hyperdynamic state with increase of the cardiac index, decrease of the total peripheral vascular resistance and arterial blood pressure. Treatment with the substantially higher dose of 12-15•10^8 *E. coli* causes a two-stage response. A hyperdynamic circulatory reaction is initially observed with an increase of the cardiac index, decrease of the total peripheral vascular resistance and arterial blood pressure. Subsequently, a hypodynamic circulatory state with a reduced cardiac index and increased total peripheral vascular resistance is registered (33).
In experiments with big animals high doses of microorganisms are always used (32). In the case of baboons the intravenous administration of *E. coli* in doses of 4-10•10^{10} CFU/kg causes excessively great production of cytokines, severe changes in the coagulation and fibrinolytic systems, cardiovascular collapse and death (34-36).

The interdependence between the bacterial species used and the changes which occur in the experimental animals was demonstrated by Dehring *et al.* using pigs. They compared the effects of equal doses Gram (+) and Gram (-) bacteria. The continuous intravenous infusion of *S. aureus* caused minimal hemodynamic and pulmonary changes, while *E. coli* or *P. aeruginosa* brought about hemodynamic shock and acute respiratory insufficiency (37).

A successful model reproducing the picture of severe clinical sepsis, as observed with meningococcemia, has been created in dogs by intravenous administration of a lethal dose (1.2•10^{10} CFU/kg) of *E. coli*. Early severe cardiovascular disorders are observed accompanied by hypotension, very small cardiac output, splanchnic hypoperfusion, and strongly expressed metabolic changes (32). Cardiovascular collapse quickly occurs followed by death. In this model local changes prevail over general ones as a result of severe microcirculatory disorders. Such disparity between general and local changes is demonstrated both in experimental and in clinical investigations (38, 39).

Shaw and Wolfe (40) describe a dog model of sepsis in which, the development of cardiovascular collapse had been prevented by prompt fluid resuscitation. Twice, every other hour, lethal doses of *E. coli*, 10^{10}, and 5•10^9 respectively, were introduced intra-arterially, whereupon the animals were resuscitated with 1000 ml of Ringer-lactate solution. A short hyperdynamic state less than 24 hours was observed and complications related to anorexia and dehydration, also common for other models of peritonitis and abscess, were avoided. The model is predictable and reproducible. It

replicates hormonal, hemodynamic and metabolic states, similar to clinical sepsis.

Breuille *et al.* (41) describe a highly reproducible sepsis model with intravenous injection of bacteria in which were manifested continuous metabolic disorders typical of sepsis. A single dose of *E. coli* ($6.8 \bullet 10^8$) caused symptoms of a serious disease with rapid transitory hypothermia which was observed in several rat models of sepsis (27, 42). The animals were lethargic, anorexic, with strong piloerection, chromodacryorrhea and diarrhea. Three days after inoculation their clinical condition began to improve, in that chromodacryorrhea and diarrhea stopped, and motor activity increased. This model reproduces the hypermetabolic stage of sepsis marked, in this case, by increased values of plasma glucose, insulin and lactate which are also found in humans but are difficult to reproduce in small animals (29, 30, 42). In the first two days acute loss of body mass was observed in the animals. It lasted between 6 and 8 days whereupon growth recovery began. Six days after the infection the muscle protein mass of the infected animals was 60 % of that of the control group. Researchers report correlation between the concentration of TNF-α measured an hour and a half after the infection and the change of body mass observed nine days later. The model described reproduces continuous metabolic disorders characteristic of sepsis which makes it appropriate for the study of nutritive intervention, particularly in terms of the effect of specific nutrition support.

1.

Some authors question the relevance of the models with intravascular inoculation of live bacteria to clinical sepsis because patients rarely have massive bacteraemia and present more often with a septic focus from which bacteria are released intermittently (6, 31). In general, a single application of a high dose of bacteria causes effects close to those observed after the intravenous injection of a high dose of LPS. The clinical course runs quickly – a hypodynamic circulatory state and violent growth of serum cytokine levels are observed, and in the absence of adequate resuscitation, the outcome is early death. In this case, an endotoxicosis model is achieved rather than a real model of infection, which is due to the quick lysis of bacteria (14, 31).

Sepsis models which use live bacteria have certain advantages. This method allows for the controlled inoculation of one type or a combination of various microorganisms resulting in an easily reproducible infection. The bacterial strain and applied dose can be standardized. Inoculation of cultured bacteria in predetermined doses can cause easily reproducible clinical manifestations. Moreover, use of different doses allows for the regulation of the severity of the disease, together with the study of various sepsis manifestations. This model enables researchers to cause specific types of sepsis, i.e. with Gram (+), or with Gram (-) microorganisms. Models with intravascular inoculation of live pathogens are easy to run. They do not require surgical intervention, thus avoiding inconveniences related to such a procedure, and the side effects which it inherently brings about.

Both models with endotoxin and with bacterial infection can be modified so that they reproduce to a great extent the clinical picture of disease.

II. Surgical Models

In the reviewed sepsis models surgical procedures for causing peritonitis are used. Some of the models require implantation of contaminated materials while in others the intestinal wall is disturbed in order to cause growth of mixed bacterial flora in the peritoneal cavity.

1. Models using cecal ligation and puncture. The most widely used sepsis model is achieved by cecal ligation and puncture (CLP) which is recognized as one of the models with the greatest compatibility in clinical terms (14, 43-45). Although it produces a complex series of pathologic manifestations, the CLP procedure is relatively simple and always guarantees results.

"Early" sepsis models in dogs and pigs have been described as been induced by ligation of the cecum but without puncturing it (46, 47). In rodents, however, ligation without puncture causes an intra-abdominal abscess without subsequent general symptoms (12). Therefore, in rats a model with a cecal puncture was suggested and later adapted to mice (48). Surgical procedure is as follows: the cecum is

exteriorized through a small abdominal cut, a ligature is placed distal to the ileocecal valve, and a puncture is made with a needle. If the intestine is punctured without ligation the tiny perforations may close and no peritonitis occurs. In an experimental environment, a simulated manipulation is carried out with the animals of the control group where the cecum is pooled out and later returned to the abdominal cavity without ligation and puncture.

Cecal puncture causes polymicrobial perotinitis with subsequent translocation of bacteria to the blood. Inflammation develops which leads to immune, hemodynamic and biochemical changes, septic shock, multiple organ failure, and death (12, 44). In rodents, the CLP model reproduces many of the clinical manifestations of sepsis. Several hours after CLP the animals show clear symptoms of the disease, such as tachypnea, tachycardia, hypotension, hypothermia, piloerection, lethargy, hunched posture, diarrhea, anorexia, and behavioral changes. Symptoms of the early, hyperdynamic stage of sepsis develop such as increased organ perfusion, hyperglycemia, and hyperinsulinemia, followed by the late, hypodynamic stage – reduced organ perfusion, hypoglycemia and lactacidemia (49, 50).

The immune response to the CLP-induced sepsis is similar to that in clinical sepsis. The cytokine profiles and their dynamics during the two immunologically different stages, the pro-inflammatory and compensatory anti-inflammatory phase in animals, correspond to those reported in humans (15, 51, 52). The levels of IL-6 correlate exactly with survivability after CLP, a phenomenon also observed in humans. In acute models severe sepsis leads to a moribund state typical for the first three days after CLP, whereas in chronic ones the picture is not so serious and the animals recover after several days (49, 53).

The extent of inflammatory response and seriousness of symptoms are closely interrelated with four essential factors i.e. length of the cecal ligation, number of perforations, their diameter, and the postoperative therapy. The manifestations of sepsis may be affected by a change in the technical procedure employed. The relationship between the length of the cecal ligation, expression of pro-inflammatory cytokines, and lethality among animals has been proved (54). In certain experimental protocols the necrotic cecum is cut out, and if this is done at the onset of sepsis, lethality drops (48). Changing the size of the needle used and the number of punctures can also alter the course of disease. Tests with mice indicate that in case of two punctures with 18 G needle lethality is 100%. If a 21 G needle is used lethality falls to 50%, and with 25 G – to 5%. The quantity of pro-inflammatory cytokines in the peritoneum and plasma also depends on the size of needle (49).

Postoperative therapy is particularly important for the course and outcome of sepsis. Adequate antibiotic treatment and fluid resuscitation reduce lethality in the CLP-induced model (55, 56). Antibiotics may reduce dissemination of bacteria, thus preventing the development of septic shock and restricting the multiple organ damage (57). Postoperative application of fluids is recommended due to the severe hypotension in animals and the risk for shock (58). Alternatively, it is necessary to induce a hyperdynamic circulatory state characteristic of the early stage of sepsis (12, 50). Taking into consideration the above mentioned factors allows for a flexible approach in the implementation of the CLP model and the establishment of variations in terms of seriousness and course of this heterogeneous disease process.

The main advantage of the CLP model is the easy run: a simple surgical procedure is used, culturing of bacteria, their quantitative determination, and inoculum preparation are not needed. Moreover, with this sepsis model the peritoneum is contaminated with mixed flora in the presence of devitalized tissue, and demonstrates apparent similarity to the clinical situation in perforated appendicitis or diverticulitis (59). A serious asset of the model created by CLP is that it resembles to a great extent the development of clinical sepsis characterized by an early hyperdynamic state followed by a pronounced hypodynamic one and hypermetabolism.

The CLP model also has certain disadvantages in that, it is difficult to control the amount of bowel

leakage and thus the septic manifestations may vary in quite a wide range. Furthermore, the intestinal flora of different animal species is not uniform. As a result of the CLP procedure significant amount of tissue necrotizes and an abscess is formed. The effects of the devitalized tissue add to the pathological changes caused by the infection. Thus, CLP establishes an abscess model and affects the course of sepsis in a direction different from that of most cases of clinical sepsis (14, 45).

In the CLP model, lethality of animals is high (12, 29), and in modifications with longer survivability the severity of the clinical course varies substantially. Moreover, the model requires surgical intervention which in itself causes a catabolic state, and differentiation of sepsis from surgical intervention is not easy. It creates difficulties for the standardization of this model for metabolic research (41). The postoperative therapeutic approach is not unified. The support treatment with fluids and antibiotics varies substantially in the different laboratories, and is almost always insufficient because a single dose fluid and/or antibiotic is usually applied (60). This, in its turn, becomes a precondition for significant variations in the clinical course of sepsis.

The CLP model is not standardized. Therefore, comparisons between studies should be made very carefully, taking into account variations in surgical procedure and postoperative care. Nonetheless, CLP induced sepsis is the most widely-used model of that heterogeneous syndrome.

2. Models with insertion of stent in the wall of the ascending colon. One of the problems which may occur with CLP induced sepsis is the formation of uncontained pus collection, thus obtaining a model of intra-abdominal abscess rather than a septic one. To solve this problem, a model has been created which induces peritonitis after inserting a stent in the ascending colon (Colon ascendens stent peritonitis – CASP).

CASP is a relatively new model of polymicrobial sepsis. It was first described in mice, and later adapted to rats. In both animal species the procedure is as follows: the ascending colon is laid open by median laparatomy and a stent is inserted through the wall at some distance aboral to the ileocecal valve. The stent is stitched to the intestine. It provides continuous leakage of intestinal contents into the peritoneal cavity causing polymicrobial sepsis and peritonitis (61, 62). After the CASP procedure bacteraemia occurs as the number of colony forming units grows exponentially between the third and twelfth hour, thereafter forming a plateau. Dissemination of bacteria in the internal organs is observed several hours later (61). LPS is present in the plasma as early as the second hour and grows in proportion to the increase of the quantity of bacteria. Direct comparison between the CLP and CASP models indicates stronger cytokine expression and higher bacteraemia in the CASP model, which is expected because of the severe diffuse peritonitis it induces. The inflammatory and anti-inflammatory syndromes develop rapidly and simultaneously in the CASP model (61, 63). The inflammatory response grows constantly, which is established by the increase of serum cytokines from 6 to 18 hours after the CASP procedure (63). In about 12 hours the animals demonstrate signs of severe disease (62). Death occurs in one to two days as a result of multiple organ failure including lungs, liver and kidneys (61-64). Both in the CPL model and in CASP variations in surgical procedure have an impact on the course of disease. The sepsis severity and lethality are dependent on the stent diameter (61-63). Control over the source of infection and continuity of fecal leakage may be carried out by random removal of the stent. In mice, removal of the stent up to the ninth hour after implantation reduces lethality (61).

CASP is a relatively new and uncommon model of polymicrobial diffuse septic peritonitis. It reproduces important signs of acute clinical peritonitis, and in particular the profile of certain cytokines and LPS levels (61). In addition, the CASP procedure does not disturb the cecal blood flow, with subsequent necrosis and formation of abscess which occur in the CLP model (62).

The main disadvantage of CASP as compared to CLP is the more complex surgical procedure. Awkward insertion of the stent leads to blockage and abscess formation (14). In this model the acute hyperinflammatory reaction is closer to the strong immune response to endotoxemia while the inflammatory reactions in the CLP model are

similar to the ones observed in clinical sepsis (65). No appropriately defined protocol is available for the run of the CASP model which makes it hard to reproduce (65). The hemodynamic response in CASP induced sepsis is still not properly described (14). Given the fact that cardiovascular parameters are critical for the validity of a certain model, in CASP there is a need for further studies in order to make it more widely accepted in sepsis research.

3. Models with implantation of pathogens. These models also aim at causing sepsis by provoking peritonitis. Initially, tests to provoke peritonitis in experimental animals by implanting their own fecal matter in the abdominal cavity were often unsuccessful. The reason is the tolerance they demonstrate towards their own intestinal flora (66). To solve this problem different approaches were applied such as mixed fecal material from different animals, human feces, barium sulfate, biliary salts, and adding autologous hemoglobin to the fecal matter (59). It was found that when treating rats with a mixture of fecal matter from different animals polymicrobial peritonitis occurred which caused hyperdynamic sepsis with high lethality (29).

A variant of this model is the inducement of peritonitis by applying fecal pellets. It resembles to a great extent the sepsis induced by intraperitoneal introduction of feces (67). Instillment of feces without concomitant therapy leads to early death or fast recovery of animals (44). In many aspects the model with fecal pellets resembles CLP. In both cases the quantity of released microorganisms varies, which affects the severity of the course and reproducibility of the model. Therefore, models with implantation of pure bacterial cultures in the peritoneal cavity have been created. This allows for strict control both of the type of the microorganisms used and their combination, and of the dose applied. In some cases monocultures are used (68, 69), and in others – mixed bacterial cultures which mimic more precisely the gastrointestinal flora (70). Bacteria may be introduced in the peritoneal cavity by infusion, or included in the composition of different carriers.

To create conditions closer to fecal contamination, Nakatani *et al*. used autoclaved fecal pellets containing *E. coli* and *B. fragilis* (42). They describe synergy between the two bacterial types and development of a localized intra-peritoneal abscess. A variant of this model is the application of sterile feces of rats contaminated with *E. coli* and wrapped in gelatin capsules (71).

Intra-peritoneal infusion of bacteria in rats induces many of the clinical and histological changes characteristic of sepsis in humans (72, 73), although the course of the disease is very severe with high lethality. Therefore, a technique is being developed whereby bacteria are introduced in the peritoneum included in a fibrin clot. Ahrenholz and Simmons (73) compare lethality in rats injected intraperitoneally with a suspension of *E. coli* in physiological solution, and those implanted by a clot containing the same number of bacteria. In the first case lethality within 24 hours is 100%, and in the second it reaches 90% as late as the tenth day. Fibrin impedes the release of bacteria from the clot and a chronic intra-peritoneal abscess develops as septic focus (6). Based on this method a reproducible model has been created described both in small and in big mammals. In general, it replicates the features of clinical sepsis: larvated beginning, hyperdynamic circulatory state, reversible left ventricular dilatation, intensity of cytokine response, and substantial, but delayed lethality (68, 69, 74).

This model requires significant surgical intervention when implanting the fibrin clot in the peritoneal cavity in order to achieve a deeply located infection focus. Being easy to reproduce, the model provides an opportunity for the study of the dose dependent intensity of the inflammatory response and its dynamics by modifying the size of inoculum (70).

CONCLUSION

Sepsis is amongst the most difficult models of clinical conditions since the animal models have to reproduce the very complex picture of sepsis in humans. Ideally, the models should reproduce the course and seriousness of disease with manifestations of the typical hemodynamic, immunologic and metabolic stages, as well as show the pathohistological changes in key organs. It is apparent that not a single model can meet all these requirements as it does not

reproduce all aspects of sepsis. Anyone of them, however, may provide reliable information on separate components of the very complex septic process.

Although animal models are often subject to criticism in terms of correlation of experimental data to clinical sepsis, they will undoubtedly continue to play an important role in the systematic study of sepsis. A detailed review of the advantages and drawbacks of each model will help the researcher make a choice consistent with the particular objective of the investigation, and, consequently, increase the use of models in scientific research.

REFERENCES

1. Abraham, E., Matthay, M.A., Dinarello, C.A., Vincent, J.L., Cohen, J., Opal, S.M., Glauser, M., Parsons, P., Fisher, C.J. Jr. and Repine, J.E., Consensus conference definitions for sepsis, septic shock, acute lung injury, and acute respiratory distress syndrome: time for a reevaluation. *Crit Care Med*, 28:232-235, 2000.
2. Van der Poll, T., Immunotherapy of sepsis. *Lancet Infect Dis*,1:165-74, 2001.
3. Giantomasso, D.D., May, C.N. and Bellomo, R., Vital blood flow during hyperdynamic sepsis. *Chest*, 124:1053-9, 2003.
4. Slade, E., Tamber, P.S. and Vincent, J.L. The Surviving Sepsis Campaign: raising awareness to reduce mortality. *Crit Care*, 7(1):1-2, 2003.
5. Cohen, J., Guyatt, G., Bernard, G.R., Calandra, T., Cook, D., Elbourne, D., Marshall, J., Nunn, A. and Opal, S., New strategies for clinical trials in patients with sepsis and septic shock. *Crit Care Med*, 29:880-886, 2001
6. Fink, M. P. and Heard, S. O., Laboratory models of sepsis and septic shock. *J Surg Res***,** 49:186-196, 1990.
7. Nemzek, J.A., Hugunin, K.M. and Opp, M.R., Modeling sepsis in the laboratory: Merging sound science with animal well-being. *Comp Med*, 58(2):120-8, 2008.
8. Hurley, J.C., Guidet, B., Offenstadt, G. and Maury, E., Endotoxemia and mortality prediction in ICU and other settings: underlying risk and co-detection of gram negative bacteremia are confounders. *Crit Care*, 7;16(4):R148, 2012.
9. Copeland, S., Warren, H.S., Lowry, S.F., Calvano, S.E. and Remick, D., Acute inflammatory response to endotoxin in mice and humans. *Clin Diagn Lab Immunol*, 12:60-67, 2005.
10. Michie, H.R., The value of animal models in the development of new drugs for the treatment of sepsis syndrome. *J Antimicrob Chemother*, 41:47-9, 1998.
11. Galanos, C. and Freudenberg, M.A., Mechanisms of endotoxin shock and endotoxin hypersensitivity. *Immunobiology*, 187: 346-356, 1993.
12. Wichterman, K.A., Baue, A.E. and Chaudry, I.H., Sepsis and septic shock--a review of laboratory models and a proposal. *J Surg Res*, 29:189-201, 1980.
13. Remick, D.G. and Ward, P.A., Evaluation of endotoxin models for the study of sepsis. *Shock*, 24(Suppl 1):7-11, 2005.
14. Buras, J.A., Holzmann, B. and Sitkovsky, M., Animal models of sepsis: setting the stage. *Nat Rev Drug Discov*, 4:854-865, 2005.
15. Remick, D.G., Newcomb, D.E., Bolgos, G.L. and Call, D.R., Comparison of the mortality and inflammatory response of two models of sepsis: lipopolysaccharide vs. cecal ligation and puncture. *Shock*, 13:110-116, 2000.
16. Brandtzaeg, P., Kierulf, P., Gaustad, P., Dobloug, J., Mollnes,T.E., and Sirnes, K., Systemic meningococcal disease: a model infection to study acute endotoxinemia in man. *Prog Clin Biol Res*, 272: 263-71, 1988.
17. Brackett, D.J., Schaefer, C.F., Tompkins, P., Fagraeus, L., Peters, L.J. and Wilson, M.F., Evaluation of cardiac output, total peripheral resistance, and plasma concentration of vasopressin in the conscious unrestrained rat during endotoxemia. *Circ Shock*, 17:273-84, 1985.
18. Wyler, F., Neutze, J.M. and Rudolph, A.M., Effects of endotoxin on distribution of cardiac output in unanesthetized rabbits. *Am J Physiol*, 219:246-51, 1970.
19. Reidy, J., Wright, I., Boom, S.J., Barclay, R., Di Padova, F. and Ramsay, G., Prevention of cardiovascular effects of endotoxaemia by monoclonal antibodies specific for core endotoxin. *Br J Surg* 79(10):1087-90, 1992.
20. Fink, M.P., Morrissey, P.E., Stein, K.L., Clement, R.E., Fiallo, V. and Gardiner,

W.M., Systemic and regional hemodynamic effects of cyclo-oxygenase and thromboxane synthetase inhibition in normal and endotoxemic rabbits. *Circulatory Shock*, 26:41-57, 1988.
21. Esbenshade, A. M., Newman, J.H., Lams, P. M., Jolles, H. and Brigham, K.L., Respiratory failure after endotoxin infusion in sheep: lung mechanics and lung fluid balance. *J Appl Physiol Respir Environ & Physiol*, 53:967-976, 1982.
22. Spapen, H., Zhang, H., Wisse, E., Baekeland, M., Seynaeve, C., Eddouks, M. and Vincent, J.L., The 21-Aminosteroid U74389G enhances hepatic blood flow and preserves sinusoidal endothelial cell function and structure in endotoxin-shocked dogs. *J Surg Res*, 86:183-91, 1999.
23. Schuler, J.J., Erve, P.R. and Schumer, W., Glucocorticoid effect on hepatic carbohydrate metabolism in the endotoxin-shocked monkey. *Ann Surg*, 183(4):345-354, 1976.
24. Talke, P., Dunn, A., Lawlis, L., Sziebert, L., White, A., Herndon, D., Flynn, J.T. and Traber, D., A model of ovine endotoxemia is characterized by an increased cardiac output. *Circ Shock*, 17:103-8, 1985.
25. Sugi, K., Newald, J., Traber, L.D., Maguire, J.P., Herndon, D.N., Schlag, G. and Traber, D.L., Cardiac dysfunction after acute endotoxin administration in conscious sheep. *Am J Physiol*, 260:H1474-H1481, 1991.
26. Lindsey, D.C., Emerson, T.R. Jr., Thompson, T.E., John, A.E., Duerr, M.L., Valdez, C.M., Kuo, H.S., Bouffard, R.B., Irwin, R.G., Canivel D et al. Characterization of an endotoxemic baboon model of metabolic and organ dysfunction. *Circ Shock*, 34:298-310, 1991.
27. Fish, R.E. and Spitzer, J.A., Continuous infusion of endotoxin from an osmotic pump in the conscious, unrestrained rat: a unique model of chronic endotoxemia. *Circ Shock*, 12:135-149, 1985.
28. Fish, R.E., Burns, A.H., Lang, C.H. and Spitzer, J.A., Myocardial dysfunction in a nonlethal, nonshock model of chronic endotoxemia. *Circ Shock*, 16(3):241-252, 1985.
29. Lang, C.H., Bagby, G.J., Bornside, G.H., Vial, L.J. and Spitzer, J.J., Sustained hypermetabolic sepsis in rats: characterization of the model. *J Surg Res*, 35:201-210, 1983.
30. Cavaillon, J.M. and Abib-Conquy, M., Bench-to-bedside review: endotoxin tolerance as a model of leukocyte reprogramming in sepsis. *Crit Care*, 10:233, 2006.
31. Deitch, E.A., Animal models of sepsis and shock: a review and lessons learned. *Shock* 9:1-11, 1998.
32. Garrido, A.G., Figueiredo, L.F.P. and Silva, M.R., Experimental models of sepsis and septic shock: an overview. *Acta Cir Bras*, 19: 82-8, 2004.
33. Sato, T., Isoyama, T., Tanaka, J., Jones, R.T., Cowley, R.A. and Trump, B.F., The pathophysiology of septic shock: changes in hemodynamics in rats following live E coli injection. An application of the thermodilution method for measurement of cardiac output. *Adv Shock Res*, 7:25-42, 1982.
34. Taylor, F.B., Staging of the pathophysiologic responses of the primate microvasculature to *Escherichia coli* and endotoxin: Examination of the elements of the compensated response and their links to the corresponding uncompensated lethal variants. *Crit Care Med*, 29:S78-89, 2001.
35. Espat, N.J., Cendan, J.C., Beierle, E.A., Auffenberg, T.A., Rosenberg, J., Russel, D., Kenney, J.S., Fisher, E., Montegut, W., Lowry, S.F., Copeland, E.M. and Moldawer, L.L., PEG-BP-30 Monotherapy attenuates the cytokine-mediated inflammatory cascade in baboon *Escherichia coli* septic shock. *J Surg Res*, 59:153-8, 1995.
36. de Boer, J.P., Creasey, A.A., Chang, A., Abbink, J.J., Roem, D., Eerenberg, A.J., Hack, C.E. and Taylor, F.B. Jr., Alpha-2-macroglobulin functions as an inhibitor of fibrinolytic, clotting, and neutrophilic proteinases in sepsis: studies using a baboon model. *Infect Immun*, 61(12):5035-5043, 1993.
37. Dehring, D.J., Crocker, S.H., Wismar, B.L., Steinberg, S.M., Lowery, B.D. and Cloutier, C.T., Comparison of live bacteria infusions in a porcine model of acute respiratory failure. *J Surg Res*, 34:151-158, 1983.
38. Lagoam, C.E., De Figueiredo, L.F., Cruz, R.J. Jr., Silva, E. and Rocha, E.S.M., Effects of volume resuscitation on splanchnic

perfusion in canine model of severe sepsis induced by live *Escherichia coli* infusion. *Crit Care*, 8:R221-228, 2004.
39. Oud, L. and Haupt, M.T., Persistent gastric intramucosal ischemia in patients with sepsis following resuscitation from shock. *Chest*, 115:1390-6, 1999.
40. Shaw, J.H.F. and Wolfe, R.R., A conscious septic dog model with hemodynamic and metabolic responses similar to responses of humans. *Surgery*, 95:553-561, 1984.
41. Breuille, D., Voisin, L., Contrepois, M., Arnal, M., Rose, F. and Obled, C., A sustained rat model for studying the long-lasting catabolic state of sepsis. *Infection and immunity*, 67:1079-1085, 1999.
42. Nakatani, T., Sato, T., Marzella, L., Hirai, F., Trump, B.F. and Siegel, J.C., Hepatic and systemic metabolic response to aerobic and anaerobic intra-abdominal abscesses in a highly reproducible chronic rat model. *Circ Shock*, 13:271-294, 1984.
43. Rittirsch, D., Hoesel, L.M. and Ward, P.A., The disconnect between animal models of sepsis and human sepsis. *J Leukoc Biol*, 81:137-143, 2007.
44. Deitch, E.A., Rodent models of intra-abdominal infection. *Shoc*, 24:(Suppl. 1)19-23, 2005.
45. Hubbard, W.J., Choudhry, M., Schwacha, M.G., Kerby, J.D., Rue, L.W. 3rd, Bland, K.I. and Chaudry, I.H., Cecal ligation and puncture. *Shock*, 24:52-57, 2005.
46. Clowes, G.H. Jr, Zuschneid, W., Turner, M., Blackburn, G., Rubin, J., Toala, P. and Green, G., Observations on the pathogenesis of the pneumonitis associated with severe infections in other parts of the body. *Ann Surg*, 167:630-650, 1968.
47. Imamura, M. and Clowes, G.H. Jr, Hepatic blood flow and oxygen consumption in starvation, sepsis and septic shock. *Surg Gynecol Obstet*, 141:27-34, 1975.
48. Baker, C.C., Chaudry, I.H., Gaines, H.O. and Baue, A.E., Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. *Surgery*, 94:331-335, 1983.
49. Ebong, S., Call, D., Nemzek, J., Bolgos, G., Newcomb, D. and Remick, D., Immunopathologic alterations in murine models of sepsis of increasing severity. *Infect Immun*, 67:6603-6610, 1999.
50. Wang, P., Wood, T.J., Ba, Z.F. and Chaudry I.H., Pentoxifylline maintains vascular endothelial cell function during hyperdynamic and hypodynamic sepsis. *Surgery*, 120(2):367-73, 1996.
51. Cuenca, A.G., Delano, M.J., Kelly-Scumpia, K.M., Moldawer, L.L. and Efron, P.A., Cecal ligation and puncture. *Curr Protoc Immunol*, 19:19.13, 2010.
52. Stearns-Kurosawa, D.J., Osuchowski, M.F., Valentine, C., Kurosawa, S. and Remick, D.G., The pathogenesis of sepsis. *Annu Rev Pathol*, 6:19-48, 2011.
53. Ebong, S.J., Call, D.R., Bolgos, G., Newcomb, D.E., Granger, J.I., O'Reilly, M. and Remick, D.G., Immunopathologic responses to nonlethal sepsis. *Shock*, 12:118-126, 1999.
54. Singleton, K.D. and Wischmeyer, P.E., Distance of cecum ligated influences mortality, tumor necrosis factor-alpha and interleukin-6 expression following cecal ligation and puncture in the rat. *European Surgical Research*, 35(6):486-491, 2003.
55. Zanotti-Cavazzoni, S.L., Guglielmi, M., Parrillo, J.E., Walker, T., Dellinger, R.P. and Hollenberg, S.M., Fluid resuscitation influences cardiovascular performance and mortality in a murine model of sepsis. *Intensive Care Medicine*, 35(4):748-754, 2009.
56. Newcomb, D., Bolgos, G., Green, L. and Remick, D.G., Antibiotic treatment influences outcome in murine sepsis: mediators of increased morbidity. *Shock*, 10:110-117, 1998.
57. Holly, M.K., Dear, J.W., Hu, X., Schechter, A.N., Gladwin, M.T., Hewitt, S.M., Yuen, P.S. and Star, R.A., Biomarker and drug-target discovery using proteomics in a new rat model of sepsis-induced acute renal failure. *Kidney Int*, 70(3):496-506, 2006.
58. Ganopolsky, J.G. and Castellino, F.J., A protein C deficiency exacerbates inflammatory and hypotensive responses in mice during polymicrobial sepsis in a cecal ligation and puncture model. *Am. J. Pathol*, 165:1433-1446, 2004.
59. Parker, S.J. and Watkins, P.E., Experimental models of gram-negative sepsis. *Br J Surg*, 88:22-30, 2001.

60. Freise, H., Bruckner, U.B. and Spiegel, H.U., Animal models of sepsis. *J Invest Surg*, 4:195-212, 2001.
61. Zantl, N., Uebe, A., Neumann, B., Wagner, H., Siewert, J.R., Holzmann, B., Heidecke, C.D. and Pfeffer, K., Essential role of gamma interferon in survival of colon ascendens stent peritonitis, a novel murine model of abdominal sepsis. *Infect Immun*, 66:2300-2309, 1998.
62. Lustig, M.K., Bac, V.H., Pavlovic, D., Maier, S., Grundling, M., Grisk, O., Wendt, M., Heidecke, C.D. and Lehmann, C., Colon ascendens stent peritonitis, a model of sepsis adapted to the rat: physiological, microcirculatory, and laboratory changes. *Shock*, 28:59-64, 2007.
63. Maier, S., Traeger, T., Entleutner, M., Westerholt, A., Kleist, B., Huser, N., Holzmann, B., Stier, A., Pfeffer, K. and Heidecke, C.D., Cecal ligation and puncture versus colon ascendens stent peritonitis: two distinct animal models for polymicrobial sepsis. *Shock*, 21:505-511, 2004.
64. Feterowski, C., Mack, M., Weighardt, H., Bartsch, B., Kaiser-Moore, S. and Holzmann, B., Chemokine receptor 2 regulates leukocyte recruitment and IL-10 production during acute polymicrobial sepsis. *Eur J Immunol*, 34:3664-3673, 2004.
65. Dejager, L., Pinheiro I., Dejonckheere, E. and Libert C., Cecal ligation and puncture: The gold standart model for polymicrobial sepsis? *Trends Microbiol*, 19(4):198-208, 2011.
66. King, D.W., Gurry, J.F., Ellis-Pegler, R.B. and Brooke, B.N., A rabbit model of perforated appendicitis with peritonitis. *Br J Surg*, 62(8):642-644, 1975.
67. Browne, M.K. and Leslie, G.B., Animal models of peritonitis. *Surg Gynecol Obstet*, 143:738 -740, 1976.
68. Mathiak, G., Szewczyk, D., Abdullah, F., Ovadia, P., Feuerstein, G. and Rabinovici, R.,An improved clinically relevant sepsis model in the conscious rat. Crit Care Med, 28:1947-52, 2000.
69. Natanson, C., Fink, M.P., Ballantyne, H.K., MacVittie, T.J., Conklin, J.J. and Parrillo, J.E., Gram-negative bacteremia produces both severe systolic and diastolic cardiac dysfunction in a canine model that simulates human septic shock. *J Clin Invest*, 78:259-70, 1986.
70. Stamme, C., Bundschuh, D.S., Hartung, T., Gebert, U., Wollin, L., Nürsing, R., Wendel, A. and Uhlig, S., Temporal sequence of pulmonary and systemic inflammatory response to grade de polymicrobiaal peritonitis in mice. *Infect Immun*, 67:5642-50, 1999.
71. Aranow, J.S., Zhuang, J., Wang, H., Larkin, V., Smith, M. and Fink, M.P., A selective inhibitor of inducible nitric oxide synthase prolongs survival in a rat model of bacterial peritonitis: comparison with two nonselective strategies. *Shock*, 5:116-121, 1996.
72. Martineau, L. and Shek, P., A sustained release bacterial inoculum infusion model of intra-abdominal infection in conscious rats: bacteriology, metabolism, and histopathology. *Shock*, 5:446-454, 1996.
73. Ahrenholz, D.H. and Simmons, R.L., Fibrin in peritonitis. Beneficial and adverse effects of fibrin in experimental *E. coli* peritonitis. *Surgery*, 88:41-7, 1980.
74. Kinasewitz, G.T., Chang, A.C., Peer, G.T., Hinshaw, L.B., Taylor and F.B. Jr, Peritonitis in the baboon: a primate model which simulates human sepsis. *Shock*, 13:100-109, 2000.