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Sepsis as a model of SIRS

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1. ABSTRACT

Sepsis describes a complex clinical syndrome that results from the host inability to regulate the inflammatory response against infection. Despite more than 20 years of extensive study, sepsis and excessive systemic inflammatory response syndrome (SIRS) are still the leading cause of death in intensive care units. The clinical study of sepsis and new therapeutics remains challenging due to the complexity of this disease. Therefore, many animal models have been employed to investigate the pathogenesis of sepsis and to preliminarily test potential therapeutics. However, so far, most therapeutics that have shown promising results in animal models failed in human clinical trials. In this chapter we will present an overview of different experimental animal models of sepsis and compare their advantages and disadvantage. The studies in animal models have greatly improved our understanding about the inflammatory mediators in sepsis. In this chapter we will also highlight the roles of several critical mediators including TNF-α, IL-1β, IL-6, chemokines, substance P, hydrogen sulfide and activated protein C in animal models of sepsis as well as in clinical studies.

2. INTRODUCTION

Sepsis is a systemic inflammatory response to an active infectious process in the host (1). A frequent complication of sepsis associated systemic inflammatory response syndrome (SIRS) is the development of organ system dysfunction, including acute lung injury, shock, renal failure, and multiple organ dysfunction syndrome (MODS). Sepsis and its sequelae represent a continuum of clinical and pathophysiological severity. Some clinically identifiable stages (severe sepsis, sepsis-induced hypotension, septic shock) along this continuum may have independent prognostic implications. The definition of sepsis and sepsis syndromes have been unified considerably by the recommendation of European and American consensus groups (Summarized in Table 1) (1).

Sepsis develops when the initial, appropriate host defense to an infection becomes magnified, and then out of control. Innate immunity generally preserves host integrity in respect to molecular components of invading microbial pathogens. However, if the host response to eradicate the invasive pathogens is exaggerated, it leads to an excessive

Table 1. Definition of sepsis and sepsis associated conditions (1)

Clinical disorder	Definition
SIRS	The systemic inflammatory response to a variety of severe clinical insults. The response is manifested by two or more of the following conditions: Temperature >38 ºC or < 36 ºC Hear rate >90 beats/min Respiratory rate > 20 breaths/min or PaCO2 <32mmHg White blood cell count >12 × 109/l or < 4 × 109/l or >10% immature neutrophils
Sepsis	The systemic response to infection, manifested by two or more of the following conditions: Temperature >38 ºC or < 36 ºC Hear rate >90 beats/min Respiratory rate > 20 breaths/min or PaCO2 <32mmHg White blood cell count >12 × 109/l or < 4 × 109/l or >10% immature neutrophils
Severe sepsis	Sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to oliguria, lactic acidosis, or an acute alteration in mental status.
Septic shock	Sepsis with hypotension despite adequate fluid resuscitation along the presence of perfusion abnormalities; Refractory septic shock is present if hypotension persist for >1 h despite fluid resuscitation and pharmacological support.
Sepsis-induced hypotension	Hypotension: a systemic blood pressure <90 mmHg or a reduction of ≥ 40mm Hg from baseline in the absence of other causes for hypotension
MODS	Presences of altered organ function in an acutely ill patient such that homeostasis can not be maintained without intervention.

release of inflammatory mediators (eg. tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), chemokines, substance P and hydrogen sulfide (H_2S)) and impairment of homeostasis. Clinically, the initiation of sepsis is often insidious and the conditions may include fever, mental alteration, transient hypotension, decreased urine output or inexplicable thrombocytopenia. If untreated, development of respiratory or renal failure, coagulation abnormalities, and unresponsive hypotension may occur.

Sepsis and its sequelae such as septic shock and multiple organ failure are major medical problems, particularly in elderly, immunocompromised or critically ill patients and are increasingly common causes of morbidity and mortality in medical and surgical intensive care units (2). For instance, in U.S., between 1979 and 2000, the incidence of sepsis was annually increased, from about 164, 000 cases (82.7 per 100, 000 population) to nearly 660, 000 cases (240.4 per 100, 000 population) (3). The increase in the incidence of sepsis may be attributed to the increased use of invasive procedures and immunosuppressive drugs, chemotherapy, transplantation, the emergence of HIV infection, and increasing microbial resistance. Although the total in-hospital mortality rate declined from 27.8% during the period 1979-1984 to 17.9% during the period 1995-2000, the total number of sepsis associated-deaths still increased (3). It has been estimated that the annual costs for treatment of sepsis in US are $16.7 billion (4). Therefore, sepsis remains to be a serious problem in the coming several years.

The pathophysiological development and progression of sepsis is multi-factorial, and may influence several organ systems, including cardiovascular, immunological and endocrine systems. The complexity and diversity of sepsis, and multiplicity of variables encountered in the clinical settings make it difficult to perform clinical studies of sepsis and sepsis treatment. Furthermore, sepsis is a very heterogeneous clinical entity and clinical data are invariably confounded by the effects of age, coexisting diseases, and supportive therapy. Finally, many studies designed to study the pathophysiology of sepsis can only be performed in animals because they require invasive monitoring or employ pharmacological interventions of uncertain benefit. Thus, various animal models have been developed in an attempt to establish reliable systems for investigating pathogenesis and potential therapeutic approach for sepsis and its systemic complications (5-7). These animal models are designed to study different aspects of sepsis including circulatory response, inflammatory response, tissue metabolism, hormonal responses and bacterial clearance etc (5, 6). In this chapter, the different animal models of sepsis are discussed. The inflammatory mediators involved in the pathophsiology of sepsis are reviewed as well.

3. ANIMAL MODELS OF SEPSIS

On the basis of the initiating agent, sepsis models can be divided into five catergories: (1) exogenous administration of a toxin (such as lipopolysaccharide (LPS), endotoxins or zymosan) (2) intravenous infusion of a viable pathogen (such as bacteria), (3) administration of fecal material or live organisms into the peritoneal cavity, (4) placement of infected foreign material into the soft tissue of the extremity, and (5) surgical operations that partially destroy the normal barrier of the gastrointestinal tract . Although so many animal models of sepsis and septic shock have been developed, none of them is perfect to re-establish the clinical sepsis. The advantages and disadvantages of these animal models are summarized in Table 2 (7).

3.1. Exogenous administration of a toxin

LPS is a purified macromolecular glycolipid extracted from the cell walls of gram-negative bacteria, whereas endotoxins contain small amounts of cell wall proteins, lipids, lipoproteins, and polysaccharides in addition to LPS (6, 8). Most of the toxicity of LPS resides in the lipid A moiety. Although various animals, including dog, rats, mice, rabbit, monkeys and baboons, have been studied, endotoxin or LPS models in any species can not reproduce the precise features of gram-negative sepsis in human (9-13). For example, elevated cardiac output and abnormally low systemic vascular resistance usually occur in patients with compensated sepsis. During episodes of septic shock, cardiac output gradually reduces while systemic vascular resistance remains very low. In contrast, bolus injection of large dose of LPS into animals generally results in low cardiac output and normal or elevated systemic resistance. LPS injection causes suppressed gluconeogenesis and subsequent hypoglycemia whereas the opposite occurs in septic patients (6). Furthermore, LPS model can not accurately mimic the cytokine profiles of sepsis (14). Thus, many anti-cytokine clinical trials based

Table 2. Advantages and disadvantages of sepsis animal models (7)

Animal models	Advantages	Disadvantages or caveats
Exogenous administration of LPS	1. Endotoxicosis model 2. LPS is convenient to use and doses of LPS are readily measured	1. Single toxin may not mimic responses in human sepsis 2. Variable haemodynamic responses with different doses and infusion rates 3. Intra- and inter-species variability in response to toxin 4. Route of administration, doses of LPS and host species and strains may affect host responses to LPS
Bacterial infection (intravenous infusion of a viable bacteria, administration of fecal material or live organisms into peritoneal cavity)	Intravenous infusion of live organisms may be appropriate to study the blood clearance kinetics of organisms	1. Requires growth and quantification of bacteria prior to administration 2. Significant inter-laboratory variability 3. Large quantity of bacteria used may elicit confounding toxicosis response 4. Host response is dependent on infecting bacterial strain and route of administration 5. Different host responses with infection of different compartments 6. Variable host response dependent on bacterial load and infusion time 7. Genetic background affects host responses to specific pathogens 8. Human therapy potentially withheld could detract from validity of therapeutic agent
CLP	Simple and easy surgical procedure Relatively reproducible No need to prepare and quantitate bacteria CLP model bears an obvious resemblance to clinical conditions	1. Multiple bacterial flora 2. Inter-laboratory variability 3. Human therapy potentially withheld could detract from validity of therapeutic agent 4. Sex and age variability 5. Strain variability 6. Needle size for perforation, the number of punctures and amount of cecum ligated may affect the severity of sepsis 7. Difficult to control bacterial load (amount of stool squeezed into peritoneum) and the magnitude of sepsis challenge 8. Potential of ascribing sepsis therapy success to enhanced abscess formation mechanism
CASP	Induce diffuse peritonitis with persistent systemic infection	1. More challenging surgical procedure and requirement for careful surgical techniques 2. Multiple bacterial flora 3. Human therapy potentially withheld could detract from validity of therapeutic agent 4. Less characterized haemodynamic response 5. Less experience to identify possible confounding variables 6. Mortality rate varies with stents of different diameters 7. Load of stool transferred into peritoneum may be a confounding variable of CASP

on promising results in this model turned out to be unsuccessful (4). In addition, circulatory level of endotoxin is poorly associated with clinical findings, outcome or microbiological condition (4-6). Therefore, LPS injection is currently considered to be a model for endotoxic shock but not for sepsis (4)

3.2. Intravenous infusion of a viable pathogen

Many investigators have utilized intravenous infusion of live bacteria (Such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus), to induce sepsis in many species (5, 6). Although these bacterial infection models do not resemble many important characteristics of clinical sepsis, they can contribute significantly to the understanding of host defense mechanism against infection. They can also be used to observe the blood clearance kinetics of bacteria. In addition, a massive bacteria load is used to overwhelm host defense and to induce sepsis. However, large amount of bacteria commonly administered do not typically colonized and replicate within the host because of rapid lysis by complement. Thus, the transient introduction of high doses of bacteria into an animal may be a potential model of intoxication with endotoxins rather than a model of sepsis. It seems to be more appropriate to choose an experimental host capable of being efficiently infected at relatively low doses with an appropriate pathogen of which the initial colonization progresses to sepsis (15).

3.3. Administration of fecal material or live organisms into the peritoneal cavity

3.3.1. Intraperitoneal implantation of feces

It has been shown that intraperitoneal administration of feces in rats or dogs induces minimal local or systemic responses (16, 17). Implantation of coliform impregnated sponges into the gall bladder or renal pelvis of dogs also causes negligible systemic response (17). Furthermore, many species are resistant to their own fecal materials. In addition, it is difficult to control the dose, species and strains of bacteria implanted into the animals. Therefore, with this animal model, it is unlikely to reproduce the experimental situations of other investigators or even one's own previous findings (5).

3.3.2. Intraperitoneal administration of live bacteria

Perkash *et al.* (18) and Sleeman *et al.* (19) produced a rat model of sepsis by introducing pure bacterial cultures into the peritoneal cavity. However, it has been suggested that this type of procedure is unable to result in significant mortalities (17). Later, Decker at al. described an animal model of severe generalized peritonitis, in which sepsis can be induced by intraperitoneal injection of viable Klebsiella pneumoniae organism mixed in a suspension of mucus (20). This model has been reproduced in mice using large amount of gram-negative aerobic and anaerobic bacteria (17). In addition, intraperitoneal injection of a pure culture of bacteria together with an adjuvant caused the rodents' death with 12 hr after the injection (21). However, the above model represents a model of endotoxin shock rather than a model of peritonitis (21).

3.3.3. Intraabdominal abscess

Weinstein *et al.* (22) described a rat model of peritonitis. Peritonitis was induced by implanting gelatin capsules containing barium sulfate and rat feces. Barium sulfate is used to increase the toxicity of the implants. This procedure results in generalized peritonitis and causes death within 3 days in most rats. Later, Bartlett *et al.* (23) and Onderdonk *et al.* (24) improved this model by

quantitating the most prevalent bacteria in rat feces and preparing gelatin capsules containing barium sulfate and accurately quantified amount of E. coli, Bacteroides fragilis and other common bacteria. The modified animal model initially induces acute peritonitis, E. coli bacteremia, and high mortality. If animals survive the peritonitis stage, intraabdominal abscesses, in which anaerobic bacteria are the predominant organisms, will occur. This animal model makes it possible to study the roles of microbes in the septic complications following colonic perforation (5).

3.4. Placement of infected foreign material into the soft tissue of the extremity

Hermreck and Thal have established another approach to produce sepsis in large animals (16, 25, 26). Wicks of umbilical tape implanted with animal's own feces are embedded into the soft tissue of the animal's thigh. With this model, animal's leg is greatly swollen and erythematous at 24 hr. The animal model results in a hyperdynamic systemic circulatory response, but no mortality. This model may be used for observing the circulatory response to infection at an extremity. In addition, Walker *et al.* described a dermal abscess model (27). Rats were seeded with Pseudomonas aeruginosa beneath a 20% split-thickness skin graft. The degree of insults and mortality in this model can be adjusted by altering the dimensions of the infection area. This model was later used to investigate heptic gluconeogenesis in septic mice (28).

3.5. Surgical operations that partially destroy the normal barrier of the gastrointestinal tract

Disrupting the normal protective barriers by surgical operations exposes the sterile compartment to pathogens and therefore induces sepsis. Cecal ligation and puncture (CLP) and colon ascendens stent peritonitis (CASP) are the representative examples of this category. Rodents are the main species used in CLP and CASP procedures due to the relatively low cost of performing experiments with large numbers of animals and the availability of genetically modified mouse strains.

3.5.1. The CLP model

The CLP model has been suggested to be the golden standard for sepsis research. It successfully simulates clinical problems like perforated appendicitis and diverticulitis (5, 6). The procedures include midline laparotomy, exteriorization of the cecum, ligation of the cecum distal to the ileocecal valve and puncture of the ligated cecum. This surgical process produces a bowel perforation with leakage of feces into peritoneum and thus causes peritoneal contamination with mixed flora in devitalized tissue. The severity of sepsis, as evaluated by mortality rate, is related to the needle puncture size or the number of punctures. Furthermore, sepsis induced by CLP accurately reflects hemodynamic alterations and clinical developments in human sepsis (14). Also, it has been shown that CLP model is able to reproduce the features of cytokines and chemokines in human sepsis (14). Therefore, the findings obtained from CLP model are usually consistent with the observations found in clinical trials (4-6). However, the resemblance between CLP model and clinical sepsis represents a possible disadvantage, in which it is not easy to manage the extent of the septic challenge caused by CLP (5, 6). In experiments using small animals (mice or rats), increasing the sample size can easily resolve the problem of variability. Variability still remains a dilemma in large animals.

3.5.2. The CASP model

CASP is a murine model of acute polymicrobial septic peritonitis. In the CASP model, a stent with defined diameter is placed into the colon ascendens of mice allowing persistent flow of fecal contents from the colon into the peritoneal cavity to establish infection with intestinal bacteria (29). The process includes laparotomy, insertion and fixation of stent, and fluid resuscitation of animals. The mortality rate, an indicator of the severity of sepsis in CASP, varies when stents with different diameters ranging from 14 to 22 gauge are used. Since the stent can be remove at certain time point, this model may resemble surgical procedures in patients to eradicate the infection focus. Furthermore, CASP is a more demanding surgical procedure. During implantation of the stent, careful surgical techniques are required to maintain patency and avoid abscess formation. In addition, it is to be noted that the CASP model is a relatively new model and that confounding variables might not be fully recognized.

4. INFLAMMATORY MEDIATORS OF SEPSIS IDENTIFIED USING ANIMAL MODELS

4.1. Pro-inflammatory cytokines

Pro-inflammatory cytokines TNF-α and IL-1β are mainly derived from activated macrophages and act via cell membrane bound receptors. TNF-α and IL-1β are produced within the first 30-90 min after LPS administration and lead to further activation of inflammatory cascades including cytokines, lipid mediators, reactive oxygen species and adhesion molecules (30).

IL-6 is a 21-kDa glycoprotein produced by a wide range of cells including monocytes/macrophages, lymphocytes, endothelial cells, fibroblasts and smooth muscle cells in response to stimulation by endotoxin, IL-1β and TNF-α (31). IL-6 possesses various biological functions, such as activation of B- and T-lymphocytes, induction of acute phase protein production in liver, regulation of hematopoiesis and activation of coagulation system (32). Furthermore, administration of IL-6 induces pyrexia (32).

The impact of these pro-inflammatory cytokines in sepsis seems significant because high levels of TNF-α, IL-6 and IL-1β in septic patients are correlated with higher risk for death (33, 34) and injection of TNF-α into experimental animal results in a sepsis-like response (35). However, a series of clinical trials by blocking TNF-α or IL-1β fail to show a dramatic improvement in survival (36). These failures may due to the late enrollment of patients because in practical conditions patients often come to medical attention when the sepsis is already relatively advanced. Blocking these early cytokines at this stage may

be too late for most patients. In animal models of sepsis, blockage of IL-6 could not produce consistent protection (37, 38).

4.2. Chemokines

Neutrophil infiltration is a hallmark of sepsis. Neutrophils play a vital role in host defense by releasing proteolytic enzymes and production of reactive oxygen species to degrade internalized pathogens. However, excessive production of these lytic factors by overwhelming activated neutrophils may correlate with host tissue damage and organ failure during sepsis.

In the last two decades, chemokines, a large family of chemotactic cytokines, have been identified and characterized. Chemokines can control leukocyte migration and infiltration into the tissue. Majority of chemokines are small proteins with molecular weight between 8kd to 12kd. They can be divided into subfamilies according to the relative position of their cysteine residues. The two main subfamilies are CXC and CC chemokines. In the CXC chemokines, the first two cysteines are separated by an amino acid residue. In contrast, in the CC chemokines, the first two cysteines are adjacent to each other.

The biological effect of all the chemokines are mediated by a family of seven-transmembrane-domain G protein-coupled receptors (GPCRs). Nearly 20 different types of chemokine receptors have been described so far. Many chemokine ligands can bind to more than one receptor with high affinity, while many chemokine receptors have more than a ligand. Chemokine receptors are mainly expressed on different types of leukocytes. Besides leukocytes, some chemokine receptors are found to be expressed on endothelial cells, epithelial cells, neurons but their exact biological function is still under investigation.

The most intensively studied chemokine targets are CXCR1, CXCR2 and their ligands probably due to their potent effect on neutrophil migration. Antileukinate, a hexa-peptide, is an inhibitor of binding of CXC chemokines to CXCR2 receptors on human neutrophils. In sepsis, Lin *et al* have reported that an increased survival (from 58% in controls to 89%) even when the inhibitor antileukinate treatment is initiated 24 h after the induction of sepsis (39). Pepducins, GPCR signaling inhibitors that are derived from intracellular loops of CXCR1 and CXCR2, reversed the lethal consequence of sepsis, including disseminated intravascular coagulation and multi organ failure in mice (40). Moreover, mice deficient in CXCR2 or treated with CXCR2-specific antibodies are protected from developing sepsis (41). A recent report from Reutershan *et al* shows that besides CXCR2 on neutrophils, endothelial and epithelial CXCR2 also play an essential role in LPS-induced neutrophil infiltration and lung injury (42). Despite these encouraging findings, Reparixin, a specific interleukin-8 inhibitor *in vitro*, has no effects on inflammation during endotoxemia (43). Further clinical studies have to be done to validate CXCR1, CXCR2 or their ligands as effective therapeutic targets for sepsis in humans.

CCR1 is another attractive target. Targeted disruption of a common receptor for MIP-1α and RANTES, the CCR1 receptor, has been found to be associated with protection against CLP-induced sepsis lethality by promoting cytokine production and enhancing macrophage activity (44). Although CCR1 deficiency had no effect on the inflammatory cell recruitment to the peritoneal cavity (44), it has been reported that blockage of MIP-1α by antibodies attenuate myeloperoxidase (MPO) activity in lung in CLP mice (45). In a recent study, we have shown that treatment with a small molecule CCR1 antagonist BX 471 protects mice against CLP-induced sepsis (46). Because BX471 has a high affinity for human CCR1 as well, this antagonist might be useful to evaluate the therapeutic potential of blocking CCR1 during sepsis in human in the future study.

4.3. Substance P

Substance P is an 11 amino acid neuropeptide (RPKPQQFFGLM-NH2) that is released from nerve endings in many tissues. It is derived from the product of pre-protachykinin-A (PPT-A) gene and expressed almost exclusively in the central and peripheral nervous systems (47). Substance P binds preferentially to neurokinin1 receptors (NK1R) on effector cells and works as a mediator of pain as well as inflammation.

High substance P levels have been identified as late predictive indicators of lethal outcome in patients with postoperative sepsis (48). Our group has shown that deletion of the PPT-A gene (encoding substance P and neurokinin A) attenuates inflammatory cells infiltration and protects against tissue damage in mouse model of sepsis (49). Treatment with NK1R (substance P receptor) antagonist also have a beneficial effect on lung inflammation by reducing leukocyte infiltration in the same mouse model of sepsis (50). Our recent study have shown that substance P may exert a direct stimulatory effect on the expression of chemokines and chemokine receptors in mouse neutrophils through a NK-1R mediated NF-kappaB dependent pathway, indicating a possible role of substance P in neutrophil infiltration and activation in sepsis (51). In clinical conditions, high substance P levels in plasma have been identified as late predictive indicators of lethal outcome in patients with postoperative sepsis (48).

4.4. Gaseous mediators

Nitric oxide (NO), carbon monoxide (CO) and most recently, hydrogen sulphide (H_2S) synthesized from L-arginine by NO synthase, from heme by heme oxygenase and from L-cysteine by either cystathionine beta-synthase (CBS) or cystathionine gamma-lyase (CSE) respectively, are the known neurotransmitters and are also involved in the regulation of vascular tone. NO is produced by three different NO synthases (NOS) .Two of these isoforms, NOS1 and NOS3 produce low levels of NO and are constitutively active. NO production by NOS1 and NOS3 regulates glutamate-mediated neurotransmission and cGMP-mediated vasorelaxation, respectively. Inducible NOS (NOS2) is transcribed in response to several stimuli including lipopolysaccharide (LPS), pro-inflammatory cytokines such as interleukin 1 beta (IL-1β), interferon

gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) and in response to pathogens (52). Carbon monoxide (CO) can exert potent anti-inflammatory effects in animal and cell culture models of sepsis, despite well-known lethal effects at high concentration. Endogenous biological CO arises from the enzymatic degradation of haem, mainly from haemoglobin turnover, catalysed by haem oxygenases (HO) (53). The inducible form of HO, haem oxygenase 1 (HO-1) engages in endogenous cellular defence against oxidative stress. HO-1 confers cytoprotection in many animal models of organ and tissue injury following sepsis. At low concentration, CO follows the cytoprotective potential of HO-1 induction in these models. CO confers protection against endotoxin shock by inhibiting the production of pro-inflammatory cytokines such as TNF-alpha, by modulating p38 mitogen activated protein kinase. CO protection against vascular injury may involve both anti-inflammatory and antiproliferative effects. The protection afforded by CO against liver failure and inflammatory lung injury are associated with the modulation of inducible NOS. Recent *in vitro* studies indicate that CO inhibits proinflammatory signalling by differentially inhibiting the trafficking of toll-like receptors (TLRs). Additional candidate mechanisms in anti-inflammatory effects of CO include the increased expression of heat shock proteins and the tumour suppressor protein, caveolin 1. H_2S stimulates ATP-sensitive potassium channels (K_{ATP}) in the vascular smooth muscle cells, hence vascular tone. In addition, H_2S may react with reactive oxygen and/or nitrogen species limiting their toxic effects but also, attenuating their physiological functions, like nitric oxide does. In contrast to NO and CO, H_2S does not stimulate soluble guanylate cyclase. Endogenous vascular H_2S increases in rats models of septic shock and endotoxic shock (54). In LPS-induced endotoxic shock model, H_2S exhibits pro-inflammatory activity and administration of PAG, an inhibitor of the H_2S-synthesizing enzyme, attenuates tissue injury (55, 56). Similarly, in CLP-induced sepsis model, the effect of inhibition of H_2S formation and administration of NaHS, a H_2S donor, suggests that H_2S plays a pro-inflammatory role in regulating the severity of sepsis and associated organ injury (57). Further study shows that H_2S up-regulates the production of pro-inflammatory cytokines and chemokines and exacerbates the systemic inflammation in sepsis through a mechanism involving NF-kappaB activation (58). H_2S may also act as a regulator of adhesion molecules and chemokine receptor CXCR2 during sepsis (59). Interestingly, in sepsis, H_2S may up-regulate the production of substance P as well, which activate NK-R1 and lead to acute lung injury (60). A possible interaction between H_2S and substance P in sepsis need to be addressed in future studies.

4.5. Activated protein C

The normal homeostatic balance is impaired during sepsis. Pro-coagulant mechanism is activated: expression of tissue factor on mononuclear cells and endothelial cells is enhanced where it activates a series of proteolytic cascades that lead to the production of thrombin from prothrombin, which in turn generates fibrin from fibrinogen. On the other hand, anti-coagulant mechanism is impaired: levels of the plasminogen-activator inhibitor-1 are increased during sepsis, which leads to reduced generation of plasmin from plasminogen, which in turn reduces the fibrin breakdown (30). The net result is enhanced formation of fibrin clots in the microvasculature, leading to impaired tissue perfusion and organ failure.

Activated protein C (APC) is converted from protein C when thrombin complexes with throbomudulin. APC with its cofactor protein S acts as proteolytic inhibitor of the clotting factors Va and VIIa. Moreover, APC increases the fibrinolytic response by inhibiting the activity of plasminogen-activator inhibitor-1 (61). APC also have anti-inflammatory effects by reducing TNF-α, IL-1β and IL-6 production from monocytes and reducing adhesive interactions between neutrophils and endothelial cells (61). Levels of APC are dramatically down-regulated in sepsis which is correlated poor outcome. These studies have finally lead to the development of a recombinant human form of endogenous APC (drotrecogin alfa) that was approved by the Food and Drug Administration for severe sepsis in adults who have a high risk of death.

5. SUMMARY AND PERSPECTIVE

Despite the frustration of many translations from animal models to clinical setting, animal models of sepsis will continue to play an essential role in identifying new inflammatory mediators and testing new potential therapeutics. A better understanding of how to apply different animal models in different conditions may help us to avoid pitfalls. Among the critical inflammatory mediators identified in animal models, some have shown promising clinical relevance. With the development of new specific inhibitors and the increase of our knowledge about the pathogenesis of sepsis, new clinically effective therapeutics may be discovered in the future studies.

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