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THERAPEUTIC TARGETS FOR SEPSIS

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ABSTRACT

Sepsis is the initial systemic inflammatory response syndrome (SIRS) associated with a confirmed infectious process. It is a spectrum of clinical conditions caused by the immune response of a host to severe infection (e.g., bacterial, fungal, viral, protozoal). To date, treatment for sepsis consists of eradication of infection through early and aggressive treatment with appropriate antibacterial therapy. However, despite advances in the development of powerful antibiotics, sepsis is still life-threatening and the U.S. mortality rate per year remains unchanged from 2006 (approximately 750,000 cases/year). Due to the complex nature of the syndrome, successful treatment may require targeting multiple mechanisms. Thus, the search continues for effective treatment strategies for sepsis and investigators are focusing on identifying novel targets and combinations of targets for therapeutic intervention. This article presents those drug targets that are currently under active investigation for the treatment of sepsis.

INTRODUCTION

Sepsis is defined as the initial systemic inflammatory response syndrome (SIRS) associated with a confirmed infectious process. It is a spectrum of clinical conditions caused by the immune response of a host to severe infection. The cause is usually bacterial infection of the lungs, abdomen or urinary tract, although fungal, viral or protozoal infections may also lead to sepsis. The three stages in a continuum of clinical response to infection are: sepsis, severe sepsis and septic shock. Response is characterized by systemic inflammation and coagulation and leads to cellular and organ dysfunction, which is further complicated by immune suppression. Sepsis may be self-limiting or may progress to severe sepsis, which is characterized by the development of hypoperfusion abnormalities, acute organ dysfunction or hypotension. Severe sepsis can progress to septic shock, which manifests as hypoperfusion, multiple organ failure and persistent hypotension unresponsive to fluid resuscitation. The final stage is referred to as multiple organ dysfunction syndrome, or MODS, and is evidenced by the presence of abnormal organ function in an acutely ill patient in whom homeostasis cannot be maintained without therapeutic intervention (1-5).

Due to inconsistencies in definition criteria, the prevalence of sepsis and septic shock is difficult to quantify. Epidemiological data demonstrate that sepsis is very common in general intensive care units (ICUs) and that the incidence is increasing. Despite advances in the development of powerful antibiotics, sepsis remains life-threatening and the U.S. mortality rate is unchanged from 2006 (approximately 750,000 cases/year). Given the aging of the world population, the incidence of sepsis is expected to increase by approximately 1.5% per year. Moreover, other factors contribute to the increase in incidence, such as changes in technologies used in ICUs and the number of patients treated therein, changes in the choice and use of antibiotics, the emergence of antibiotic resistance, increased numbers of immunocompromised patients, etc. (1, 3, 6-10).

Sepsis is caused by innate and adaptive immune responses of the host to toxin-producing bacterial infection of the bloodstream, where the host response is excessive or poorly regulated and the infection cannot be contained. This may be due to the characteristics of the infectious agent itself, a high burden of infection or the presence of superantigens and other factors endowing virulence. Antibiotic resistance may also contribute to the pathophysiology of sepsis. In the years preceding 1987, Gram-negative aerobic bacilli were documented as the primary source of septic infections. However, the balance has shifted since then in favor of Gram-positive organisms as the cause of the majority of sepsis cases (5, 7, 11, 12).

The pathology of sepsis is extremely complex. The overwhelming inflammatory host reaction in response to the local release of endotoxins and other components of the microbial cell walls in sepsis prompts the release of macrophage-derived cytokines that amplify the inflammatory response. Bacterial toxins bind to cell receptors and activate regulatory proteins (e.g., nuclear factor NF-kappa-B [NF-κB]), inducing the production of proinflammatory cytokines (e.g., TNF-α and interleukin-1 [IL-1]). In addition to their direct effects on organ function, these inflammatory cytokines also induce the release of other proinflammatory cytokines (IL-6, IL-8) and inflammatory mediators (e.g., thromboxanes, leukotrienes, platelet-activating factor [PAF], prostaglandins [PGs], complement). The inflammatory mediators subsequently stimulate the production of inducible nitric oxide synthase (iNOS), which in turn causes the release of NO from endothelial cells, macrophages and vascular smooth muscle cells; they also stimulate increased adhesion molecule expression on endothelial cells and neutrophils. The body will

also begin to produce a variety of endogenous anti-inflammatory substances (e.g., endogenous corticosteroids, catecholamines, IL-10, IL-4, PGE_2, soluble TNF receptors, IL-1 receptor antagonist) in an attempt to regulate the inflammatory response. In many cases these anti-inflammatory substances can prevent the initiation of new foci of inflammation. However, they are present in lower levels within tissues and hence are not always capable of preventing new inflammatory events in organs. Thus, severe sepsis will develop when proinflammatory mechanisms escalate beyond the control of anti-inflammatory regulation (1, 4, 11, 13, 14).

The cornerstone of treatment for sepsis is eradication of infection through early and aggressive treatment with appropriate antibacterial therapy involving an agent active against all likely organisms. However, patient survival is not guaranteed, since positive identification of the causative pathogen(s) and focus of infection is required for a successful recovery. Preclinical studies have reported efficacy for various strategies that protect experimental animals against endotoxin or live bacteria. These therapeutic mechanisms include blockade of proinflammatory cytokines (TNF-α, IL-1), administration of anti-inflammatory or counterinflammatory mediators (IL-10, granulocyte colony-stimulating factor [G-CSF]) and inhibition of coagulation or potentiation of endogenous anticoagulant mechanisms. Unfortunately, the promising preclinical results reported have seldom been replicated in the clinic. Results from nearly 70 clinical trials evaluating the efficacy of a range of potential treatments have been disappointing, possibly because the treatments have targeted only a single component of sepsis. Sepsis is a complex disease involving numerous heterogeneous pathological processes and successful treatment may therefore require targeting multiple mechanisms (1, 15-18).

The search for effective treatment strategies for sepsis continues, with research focusing on the identification of novel targets and combinations of targets for drug development. Those targets currently under active investigation are discussed below (Fig. 1). Table I provides a selection of products under active development for each target and Table II includes selected patents.

TARGETS

Bradykinin

Bradykinin (BK) is a nonapeptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) that is a potent vasodilator and mediator of anaphylaxis. It is produced from the decapeptide kallidin (bradykininogen) and mediates the action of kinins on the cardiovascular and renal systems. Kinins are peptide hormones that exert pathophysiological and beneficial physiological effects. Plasma BK is rapidly metabolized (plasma $t_{1/2}$ ~ 17 s) by carboxypeptidase N, generating des-Arg-BK. In addition to being able to induce vasodilatation, vascular permeability and bronchoconstriction, des-Arg-BK is also a potent proinflammatory peptide. The activity of BK is mediated via two G protein-coupled, 7-transmembrane receptors (B_1 and B_2), which are coupled to all known second messengers. The B_2 receptor is constitutively active in healthy tissue, has high affinity for BK, has no affinity for des-Arg-BK and is involved in mediating BK-induced vasodilatory effects. In contrast, the B_1 receptor is only expressed as a result of tissue injury, has higher affinity for des-Arg-BK as compared to BK and is thought to be involved in mediating chronic pain and inflammation. BK has been shown to induce vasoconstriction in response to bacterial infection (i.e., sepsis), which can result in pulmonary endothelial injury. BK is implicated in the pathogenesis of endotoxemic shock, septic shock, multiple organ system failure and adult respiratory distress syndrome (ARDS). Inhibition of bradykinin expression could therefore be beneficial in the treatment of sepsis (19-22).

HMG-CoA reductase

HMG-CoA reductase is a key enzyme that catalyzes the rate-limiting step in the biosynthetic pathway leading from mevalonate to cholesterol. Isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate play a role in protein prenylation, a crucial step in multiple cellular processes. Protein prenylation (i.e., farnesylation and geranylgeranylation) is a posttranslational modification of proteins involving the addition of isoprenoids. Geranylgeranylation allows the activation of the small GTP-binding proteins Rho and Rac. Activated Rho regulates the activity of nuclear transcription factors such as NF-κB, controls the actin cytoskeleton and induces stress fiber formation. This affects intracellular transport, migration, membrane trafficking, messenger RNA stability and gene transcription. Farnesylation allows the activation of Ras protein. Activated Ras stimulates cytoplasmic signaling pathways such as the mitogen-activated protein kinase (MAPK) pathway that regulates gene transcription and thus the growth, proliferation, differentiation and survival of cells. Statins are inhibitors of HMG-CoA reductase and exert pleiotropic effects independent of cholesterol-lowering actions. These include effects on endothelial function, cell proliferation, inflammatory response, immunological reactions, platelet function and lipid oxidation. Lipopolysaccharide (LPS) is associated with Gram-negative sepsis, where it can activate monocytes and macrophages to release proinflammatory mediators such as TNF-α and NO and the anti-inflammatory mediator IL-10. Studies suggest that statins may reduce iNOS-mediated NO production in endothelial cells; vascular smooth muscle cells appear to be unaffected. Studies using experimental models of sepsis have demonstrated that pretreatment with statins can suppress TNF-α, IL-10 and NO release and reduce markers of organ injury associated with endotoxic shock and LPS-induced sepsis (23-25).

Lactotransferrin

Lactotransferrin (or lactoferrin) is a member of the transferrin family of proteins (serum transferrin, ovotransferrin and melanotransferrin) that binds iron. It is a monomeric, diferric, cationic glycoprotein with a highly conserved three-dimensional structure that may play important roles in iron homeostasis, organ morphogenesis, host defense against infection, inflammation and cancer. Lactotransferrin is found on mucosal surfaces and significant levels are found in human colostrum (7 g/L), mature human milk (1 g/L), tears (3.8 g/L), saliva (20 mg/L), seminal fluid and secondary granules of neutrophils. Normal serum levels in humans range from 0.4 to 2 mg/L as compared to increases to 200 mg/L following its release from neutrophils during sepsis. Lactotransferrin is considered an innate immunity agent and is involved in acquired cellular and humoral immune responses. It exerts broad-spectrum antimicrobial activity

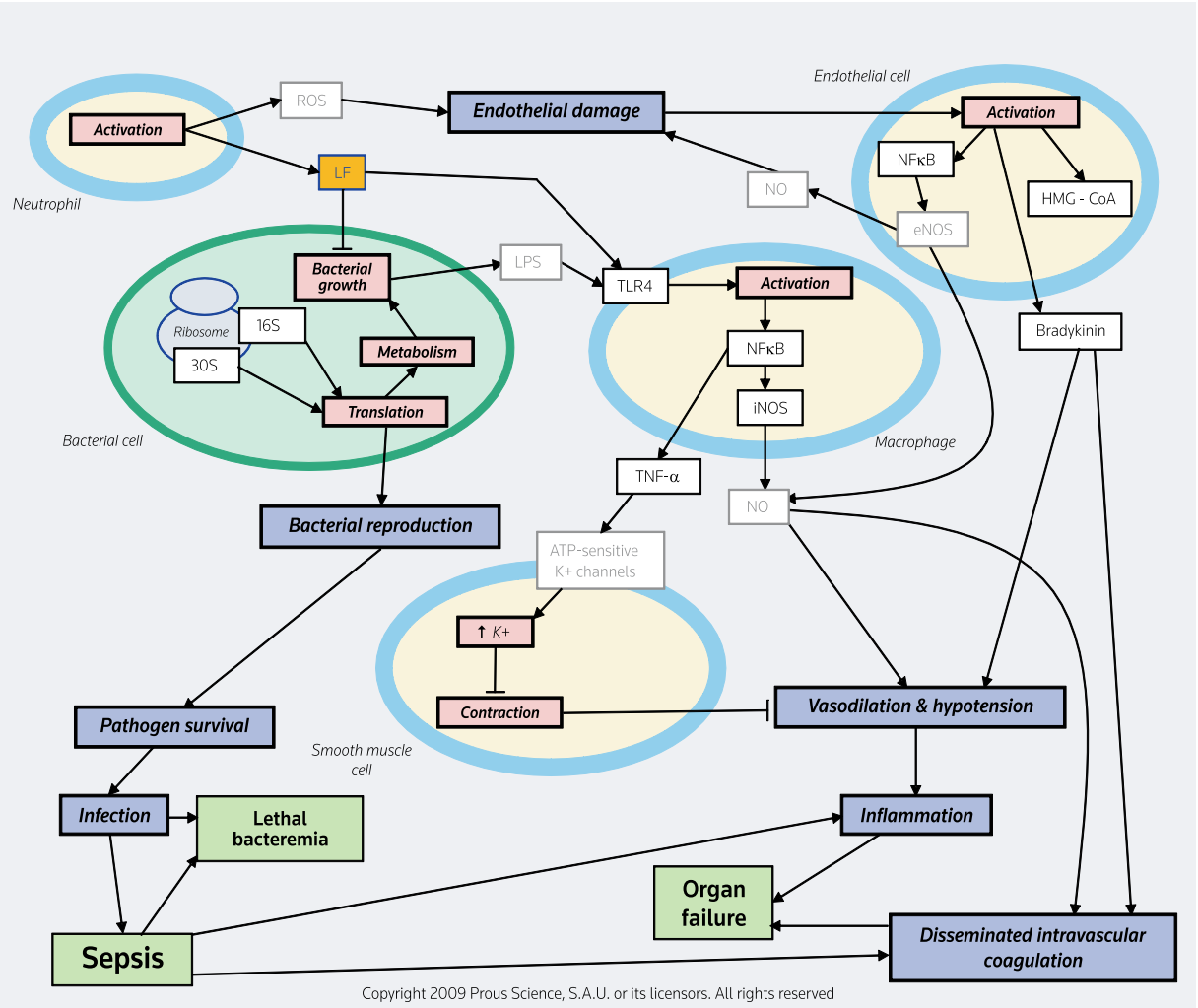

Figure 1. Sepsis targetscape. A diagram showing an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of sepsis and their biological actions. Arrow: positive effect; dash: negative effect. Gray or lighter symbols are targets that are not validated (i.e., targets not associated with a product that is currently under active development for sepsis). Orange+blue symbols represent validated therapeutic recombinant agents. Abbreviations: 16S: 16S ribosomal protein; 30S: 30S ribosomal protein; eNOS: endothelial nitric oxide synthase; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A reductase; iNOS: inducible nitric oxide synthase LF: lactotransferrin; LPS: lipopolysaccharide; NF-κ-B: nuclear factor NF-kappa-B; NO, nitric oxide; ROS: reactive oxygen species; TLR4: Toll-like receptor 4.

against bacteria, fungi, viruses and protozoa, possibly via iron-sequestering activity and a direct interaction with the microbial surface, resulting in cell lysis. It has been shown to upregulate IL-18, which in turn stimulates both $CD4^+$, $CD8^+$ and NK immune cells and it also shifts the immune response from Th2 to Th1. Lactotransferrin may therefore be effective against infections and gastrointestinal injury, and recombinant lactotransferrin may be a promising agent in the prevention of sepsis, especially in neonates (26-28).

NF-κB

NF-κB is a protein transcription factor and intracellular mediator of the inflammatory cascade. It is involved in the generation of adhesion molecules (ICAM-1, V-CAM 1), iNOS synthase, cyclooxygenase COX-2, cytokines (e.g., IL-1β, IL-2, TNF-α, IL-6, interferon gamma) and chemokines (e.g., IL-8). Other genes that are regulated by NF-κB include those encoding the IL-2 receptor, the IL-12 p40 subunit and c-Myc. NF-κB activation is involved in inflammation and is an important signaling factor for cytokines that appear to participate in several pathological conditions, such as multiple sclerosis, Parkinson's disease, depression, inflammatory bowel disease, sepsis and sepsis-induced organ failure. The NF-κB signaling pathway has been associated with an enhanced inflammatory response and its activation has been significantly correlated with IL-6 and TNF-α production, thus perpetuating the innate immune response. Apoptotic

Table I. *Selected targets and products launched or being actively investigated for sepsis (from Prous Science Integrity®).*

Target	Product	Source	Phase
16S Ribosomal protein	Tobramycin	Lilly	L-1974
30S Ribosomal protein	Tobramycin	Lilly	L-1974
Bradykinin receptors	HM-401	Hansa Medical	Preclinical
HMG-CoA reductase	Simvastatin	University of Chicago	II
Lactotransferrin	rh-Lactoferrin	Agennix	II
NF-κB	CKD-712	Chong Kung Dan	I
Nitric oxide synthase (NOS)	CKD-712	Chong Kung Dan	I
Nitric oxide (NO)	NOX-100	Medinox	I/II
TNF-α	AZD-9773	AstraZeneca/Protherics	II
Toll-like receptor 4 (TLR4, isoform A)	Eritoran tetrasodium	Eisai	III

Table II. *Selected patents for targets being pursued or explored for sepsis (from Prous Science Integrity®).*

Target	Patent	Source	Phase
NF-κB	WO 2007088830	Japan Health Sciences Foundation	Biological testing/Preclinical
	US 2004147435	The Vanderbilt University	Preclinical
Nitric oxide (NO)	WO 2007082208	Oklahoma Medical Research Foundation (OMRF)	Biological testing
TNF-α	JP 2005200324	Daiichi Sankyo	Biological testing
	JP 2005298434	Daiichi Sankyo	Biological testing
	JP 2008260760	Daiichi Sankyo	Biological testing
	WO 2004039806	Ajinomoto	Biological testing
	WO 2006056492	BioXell/Universite Henri Poincare (Nancy-I)	Biological testing
	WO 2007032362	Daiichi Sankyo Co., Ltd.	Biological testing
	WO 2007064997	University of Pittsburgh/Duke University	Biological testing
	WO 2007082208	Oklahoma Medical Research Foundation (OMRF)	Biological testing
	WO 2007088830	Japan Health Sciences Foundation	Biological testing/Preclinical
	WO 2007112015	The Feinstein Institute for Medical Research	Preclinical
	WO 2009005045	Daiichi Sankyo	Biological testing
Toll-like receptor 4 (TLR4, isoform A)	WO 2003087072	Mochida Pharmaceutical	Biological testing/Preclinical

neutrophils are decreased in sepsis and inhibition of NF-κB can restore neutrophil apoptosis to baseline levels. Suppression of NF-κB activation could reduce the acute inflammatory response and organ dysfunction. Thus, targeting NF-κB may be effective in normalizing the escalated immune response seen in sepsis (29-31).

16S and 30S ribosomal proteins

Ribosomes are small cytoplasmic particles present in large numbers in all living cells. These structures are responsible for protein synthesis, which is very similar in both eukaryotes and prokaryotes. Bacterial ribosomes are composed of two subunits: the smaller 30S subunit containing 21 proteins and a single 16S RNA molecule, and the larger 50S subunit containing 32 proteins and two RNA molecules (23S and 5S). 16S serves as a scaffold defining the positions of the ribosomal protein with the 3'-end containing the anti-Shine-Dalgarno sequence. This sequence binds upstream to the AUG start codon on the mRNA. 16S interacts with 23S and facilitates binding of 50S and 30S. Many antibiotic agents bind to the 30S and 16S subunits of the bacterial ribosome. This action inhibits translocation of peptidyl-tRNA from the A-site to the P-site and also causes misreading of mRNA, interrupting bacterial protein synthesis necessary for survival and reproduction. The effects of an agent targeting these ribosomal subunits persist even after there is little or none of it detectable in blood. This is due to the potent and irreversible nature of ribosomal binding, thus allowing for a prolonged dosing interval. Thus, both the 16S and 30S subunits are effective targets for the treatment of sepsis (4, 32-34).

TNF-α

TNF-α (also known as cachectin) is a proinflammatory cytokine and a member of the tumor necrosis factor (TNF) family of cytokines that is released by activated macrophages and lymphocytes. It acts via receptors belonging to the TNF family of receptors, among which TNF-R1 and TNF-R2 trigger several signal transduction pathways, resulting in the activation of transcription factors such as NF-κB and c-fos/c-jun. TNF-R1 (also known as CD120a, p55/60) is expressed in

most tissues and is fully activated by both the membrane-bound and soluble trimeric forms of TNF. TNF-R2 (also known as CD120b, p75/80), however, is found only in cells of the immune system and is activated by the membrane-bound form of the TNF homotrimer. Activated factors induce the transcription of antiapoptotic, proliferative, immunomodulatory and inflammatory genes. NF-κB is the major survival factor in preventing TNF-α-induced apoptosis. TNF-α is also a crucial cytokine in the establishment and maintenance of inflammation in multiple autoimmune diseases. Elevated levels of TNF-α are found in a wide range of diseases, including chronic inflammatory conditions such as rheumatoid arthritis, psoriasis, Crohn's disease and sepsis. TNF-α has been shown to decrease sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPases (SERCA2) which upsets Ca^{2+} homeostasis and thus cardiac function, a mechanism thought to be responsible for cardiac dysfunction during sepsis and heart failure. TNF-α is considered to play a pivotal role in many of the pathological processes seen in sepsis, although evidence also suggests that TNF-α as an immunomodulator may be protective in sepsis. Modulation of TNF-α may therefore be effective in sepsis (30, 35-37).

Toll-like receptor 4 (TLR4)

TLR4 is a member of a class (TLR1-10) of single-membrane-spanning, noncatalytic receptors that are pattern recognition receptors (PRRs). They are the key recognition structures of the innate immune system that recognize those molecules shared by pathogens but distinct from host molecules. When activated, they initiate the production of inflammatory cytokines, chemokines, tissue-degrading enzymes and type I interferons. TLR signaling is also involved in activation of the adaptive immune system via upregulation of costimulatory molecules of antigen-presenting cells (APCs). TLRs therefore can link innate and acquired immune responses. Thus, microbial products activate TLRs, resulting in widespread inflammation, which eventually can lead to organ failure, shock and death. TLR4, in particular, is the main receptor for bacterial endotoxin. Studies suggest that TLR4 is constitutively suppressed and that the release of TLR4 suppression may be one of the first processes in sepsis. Blockade of this receptor may therefore be an effective treatment for sepsis (35, 38-40).

REFERENCES

1. Prous Science Integrity Disease Briefings: Sepsis and Septic Shock (online publication). Updated 2009.
2. Martin, J.B., Wheeler, A.P. *Approach to the patient with sepsis*. Clin Chest Med 2009, 30(1): 1-16, vii.
3. Okazaki, Y., Matsukawa, A. *Pathophysiology of sepsis and recent patents on the diagnosis, treatment and prophylaxis for sepsis*. Recent Pat Inflamm Allergy Drug Discov 2009, 3(1): 26-32.
4. van der Poll, T., Opal, S.M. *Host-pathogen interactions in sepsis*. Lancet Infect Dis 2008, 8(1): 32-43.
5. O'Brien, J.M. Jr., Ali, N.A., Aberegg, S.K., Abraham, E. *Sepsis*. Am J Med 2007, 120(12): 1012-22.
6. Martin, C.M., Priestap, F., Fisher, H. et al. *A prospective, observational registry of patients with severe sepsis: The Canadian Sepsis Treatment and Response Registry*. Crit Care Med 2009, 37(1): 81-8.
7. Russell, J.A. *Management of sepsis*. N Engl J Med 2006, 355(16): 1699-713.
8. Angus, D.C., Linde-Zwirble, W.T., Lidicker, J., Clermont, G., Carcillo, J., Pinsky, M.R. *Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care*. Crit Care Med 2001, 29(7): 1303-10.
9. Rivers, E., Nguyen, B., Havstad, S. et al. *Early goal-directed therapy in the treatment of severe sepsis and septic shock*. New Engl J Med 2001, 345(19): 1368-77.
10. Angus, D.C., Wax, R.S. *Epidemiology of sepsis: An update*. Crit Care Med 2001, 29(7, Suppl.): S109-16.
11. Hodgin, K.E., Moss, M. *The epidemiology of sepsis*. Curr Pharm Des 2008, 14(19): 1833-9.
12. Tsiotou, A.G., Sakorafas, G.H., Anagnostopoulos, G., Bramis, J. *Septic shock; current pathogenetic concepts from a clinical perspective*. Med Sci Monit 2005, 11(3): RA76-85.
13. Maitra, U., Chang, S., Singh, N., Li, L. *Molecular mechanism underlying the suppression of lipid oxidation during endotoxemia*. Mol Immunol 2009, Epub ahead of print.
14. Cavaillon, J.M., Annane, D. *Compartmentalization of the inflammatory response in sepsis and SIRS*. J Endotoxin Res 2006, 12(3): 151-70.
15. Cohen, J. *Non-antibiotic strategies for sepsis*. Clin Microbiol Infect 2009, 15(4): 302-7.
16. Dyson, A., Singer, M. *Animal models of sepsis: Why does preclinical efficacy fail to translate to the clinical setting?* Crit Care Med 2009, 37(1, Suppl.): S30-7.
17. Claessens, Y.E., Dhainaut, J.F. *Diagnosis and treatment of severe sepsis*. Crit Care (Lond) 2007, 11(Suppl. 5): S2.
18. Dubois, M.J., Vincent, J.L. *Clinically-oriented therapies in sepsis: A review*. J Endotoxin Res 2000, 6(6): 463-9.
19. Asmis, L.M., Asmis, R., Sulzer, I., Furlan, M., Lämmle, B. *Contact system activation in human sepsis - 47kD HK, a marker of sepsis severity?* Swiss Med Wkly 2008, 138(9-10): 142-9.
20. Colman, R.W. *Regulation of angiogenesis by the kallikrein-kinin system*. Curr Pharm Des 2006, 12(21): 2599-607.
21. Leeb-Lundberg, L.M., Marceau, F., Müller-Esterl, W., Pettibone, D.J., Zuraw, B.L. *International Union of Pharmacology. XLV. Classification of the kinin receptor family: From molecular mechanisms to pathophysiological consequences*. Pharmacol Rev 2005, 57(1): 27-77.
22. Herwald, H., Mörgelin, M., Björck, L. *Contact activation by pathogenic bacteria: A virulence mechanism contributing to the pathophysiology of sepsis*. Scand J Infect Dis 2003, 35(9): 604-7.
23. Alvarez de Sotomayor. M., Vega, S., Mingorance, C., Marhuenda, E., Herrera, M.D. *Effects of HMG-CoA reductase inhibition by simvastatin on vascular dysfunction induced by lipopolysaccharide in rats.* Pharmacology 2008, 82(2): 89-96.
25. Souza Neto, J.L., Araújo Filho, I., Rego, A.C. et al. *Effects of simvastatin in abdominal sepsis in rats.* Acta Cir Bras 2006, 21(Suppl. 4): 8-12.
26. Cohen-Wolkowiez, M., Benjamin, D.K. Jr., Capparelli, E. *Immunotherapy in neonatal sepsis: Advances in treatment and prophylaxis*. Curr Opin Pediatr 2009, 21(2): 177-81.
27. Venkatesh, M., Abrams, S. *Can lactoferrin prevent neonatal sepsis and necrotizing enterocolitis?* Expert Rev Anti Infect Ther 2009, 7(5): 515-25.
28. Venkatesh, M.P., Rong, L. *Human recombinant lactoferrin acts synergistically with antimicrobials commonly used in neonatal practice against coagulase-negative staphylococci and Candida albicans causing neonatal sepsis*. J Med Microbiol 2008, 57(Pt. 9): 1113-21.
29. Baek, Y.S., Haas, S., Hackstein, H. et al. *Identification of novel transcriptional regulators involved in macrophage differentiation and activation in U937 cells*. BMC Immunol 2009, 10: 18.

30. Duntas, L.H. *Selenium and inflammation: Underlying anti-inflammatory mechanisms*. Horm Metab Res 2009, 41(6): 443-7.

31. Bopp, C., Bierhaus, A., Hofer, S., Bouchon, A., Nawroth, P.P., Martin, E., Weigand, M.A. *Bench-to-bedside review: The inflammation-perpetuating pattern-recognition receptor RAGE as a therapeutic target in sepsis.* Crit Care 2008, 12(1): 201.

32. Vaiana, A.C., Sanbonmatsu, K.Y. *Stochastic gating and drug-ribosome interactions*. J Mol Biol 2009, 386(3): 648-61.

33. Nishimura, K., Hosaka, T., Tokuyama, S., Okamoto, S., Ochi, K. *Mutations in rsmG, encoding a 16S rRNA methyltransferase, result in low-level streptomycin resistance and antibiotic overproduction in Streptomyces coelicolor A3(2).* J Bacteriol 2007, 189(10): 3876-83.

34. Gromadski, K.B., Rodnina, M.V. *Streptomycin interferes with conformational coupling between codon recognition and GTPase activation on the ribosome*. Nat Struct Mol Biol 2004, 11(4): 316-22.

35. Pappachan, J.V., Coulson, T.G., Child, N.J. et al. *Mortality in adult intensive care patients with severe systemic inflammatory response syndromes is strongly associated with the hypo-immune TNF -238A polymorphism.* Immunogenetics 2009, Epub ahead of print.

36. Kao, Y.H., Chen, Y.C., Cheng, C.C., Lee, T.I., Chen, Y.J., Chen, S.A. *Tumor necrosis factor-alpha decreases sarcoplasmic reticulum Ca^{2+}-ATPase expressions via the promoter methylation in cardiomyocytes.* Crit Care Med 2009, Epub ahead of print.

37. Bhatia, M., He, M., Zhang, H., Moochhala, S. *Sepsis as a model of SIRS.* Front Biosci 2009, 14: 4703-11.

38. Rodriguez, S., Chora, A., Goumnerov, B. et al. *Dysfunctional expansion of hematopoietic stem cells and block of myeloid differentiation in lethal sepsis*. Blood 2009, Epub head of print.

39. Takashima, K., Matsunaga, N., Yoshimatsu, M. et al. *Analysis of binding site for the novel small-molecule TLR4 signal transduction inhibitor TAK-242 and its therapeutic effect on mouse sepsis model*. Br J Pharmacol 2009, 157(7): 1250-62.

40. Leon, C.G., Tory, R., Jia, J., Sivak, O., Wasan, K.M. *Discovery and development of toll-like receptor 4 (TLR4) antagonists: A new paradigm for treating sepsis and other diseases*. Pharm Res 2008, 25(8): 1751-61.