in vivo *32*: 1653-1658 (2018)
doi:10.21873/invivo.11428

The Relationship Between Sepsis-induced Immunosuppression and Serum Toll-like Receptor 9 Level

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Abstract. *Background/Aim: Our aim was to determine serum TLR-9 levels in sepsis and evaluate the relationship between sepsis and serum TLR-9 levels. Materials and Methods: The study group consisted of 80 consecutive patients with sepsis and 100 healthy individuals. The demographic characteristics, co-morbidities and hemodynamic data of all patients were recorded. Results: TLR-9 serum levels in sepsis were statistically significantly lower compared to the control group. It was also seen that when the lactate level was >5 mmol/l in patients in the sepsis group, the serum TLR-9 levels were substantially higher. Conclusion: There is a relationship between sepsis-induced immunosuppression and serum TLR-9 levels. The host immunity system can be activated by means of TLR-9-related systems, while hyperlactatemia may play a stimulating role in the re-activation of the immune system.*

Sepsis is a serious clinical problem worldwide (1-3). Severe sepsis and septic shock lead to significant morbidity and mortality in critically-ill patients despite improvements in intensive care and treatment methods (4). Sepsis is characterized by an uncontrolled systemic inflammatory response in the immune system as a result of complex interactions between host and infectious agents (5). Sepsis, serious sepsis, and septic shock represent increasingly severe degrees of systemic inflammatory responses to infection (6). Increased uncontrolled inflammatory response leads to increased mortality from sepsis.

Toll-like receptors (TLRs) have been recognized as a component of the innate immune system. TLRs have an important role in the innate immune system to recognize many pathogens and create a host immune response through production of necrosis factor-α, interleukin (IL)-1, IL6 and other pro-inflammatory cytokines (7, 8). In our study, serum TLR9 levels in sepsis were analyzed in relation to clinical and prognostic parameters.

Patients and Methods

This prospective study was approved by the Ethics Committee of the Istanbul University, Turkey (Approval number 1341). All the procedures followed in the study were in accordance with the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. A total of 180 patients were enrolled in the study. The study group consisted of 80 consecutive patients with diagnosis of sepsis at the intensive care unit. In each case, diagnoses were established with histological examination. The control group comprised 100 healthy individuals. The demographic characteristics, co-morbidities and hemodynamic data of all patients were recorded. Blood gas samples and laboratory data of the cases were collected. Microbial cultures of blood, sputum, a wound swab, abscess, and urine from each patient were performed. Blood samples were collected in EDTA-coated tubes by standard venipuncture method. The serum samples of the participants were stored at −20˚C until analysis. TLR9 levels were determined by ELISA technique (Uscn Life Science Inc, Wuhan, Hubei, PRC; Lot No: L150309211).

Statistical analyses. Statistical analyses, using SPSS software version 21.0 (Armonk, NY, USA, Armonk, NY, USA), included the Chi-square test. Student's *t*-test, one-way ANOVA and

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Key Words: Sepsis, TLR9, immunosuppression, toll-like receptors.

Mann–Whitney *U*-tests were used to compare the demographic data between the groups. Whenever an expected cell value was less than five, Fisher's exact test was used. A *p*-value of less than 0.05 was regarded as statistically significant.

Results

The demographic characteristics, biochemistry and laboratory data are presented in Tables I and II. No demographic differences were noted between the two patient groups. However, the number of patients with chronic renal failure, chronic obstructive pulmonary disease, acute kidney injury and those requiring continuous renal replacement therapy was statistically higher in the sepsis group compared to the control group. Acute kidney injury developed in 36.2% and renal replacement therapy was needed in 31.2% of patients with sepsis.

The mean number of leukocytes, and levels of serum C-reactive protein, lactate, glucose, serum creatinine, blood urea nitrogen, plasma potassium, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, lactate dehydrogenase, total bilirubin and body temperature were all statistically significantly higher in the sepsis group compared to the control group. However, arterial blood gas pH and levels of HCO_3, blood hemoglobin and serum sodium and the number of platelets and the PaO_2/FIO_2 ratio were statistically significantly lower in the group of patients with sepsis. In the study, mean arterial pressure of our sepsis cases was also significantly lower, while heart rate values, inotropic agent and vasoconstrictor drug usage rates were found to be significantly higher than those of the control group. Mortality was found to be 93.8% in the sepsis group.

The mean serum level of TLR9 in the sepsis group was statistically significantly lower than that of the control group (Table II). Although without statistical significance, the serum TLR9 level was lower in the patients who died than in the patients who survived in the sepsis group. Nonetheless, when we grouped the patients in the sepsis group according to their lactate level (≤5 mEq/l and >5 mEq/l), it was observed that the TLR9 level was substantially and statistically higher in the patients with an increased lactate level (Table III). The serum level of TLR9 according to the type of causative pathogen microorganisms in the sepsis group are shown in Table IV. *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* were the organisms most frequently detected by microbial culture. The results of microbial cultures show that some of the sepsis cases were infected with more than one pathogenic microorganism. Although the number of infected individuals was insufficient for statistical evaluation, the serum TLR9 level was found to be highest in the presence of *Corynebacterium striatum* infection and the lowest in *Escherichia coli* infection.

Table I. *Demographic data of our study groups. Data are presented as mean±SD or proportions.*

Characteristic	Sepsis group (n=80)	Control group (n=100)	*p*-Value
Age (years)	64.70±11.51	63.28±11.32	>0.05
Weight (kg)	77.98±11.59	77.39±11.97	>0.05
Height (cm)	167.21±10.01	167.59±8.25	>0.05
BMI (kg/m^2)	27.83±4.49	27.64±4.32	>0.05
Body temperature (˚C)	37.03±0.98	36.49±0.14	<0.001
HR (bpm)			
103.12± 20.39	78.30± 15.77	<0.001	
MAP (mmHg)	65.48±14.97	86.34±12.04	<0.001
CVP (mmHg)	8.62±3.17	8.50±3.17	>0.05
HT (%)	72.5	69	>0.05
NIDDM (%)	15	21	>0.05
IDDM (%)	13.8	8	>0.05
PVD (%)	15	8	>0.05
CRF (%)	10	0	0.002
COPD (%)	37.5	8.1	<0.001
AKI (%)	36.2	0	<0.001
RRT (%)	31.2	0	<0.001
Inotropic agents (%)	70	0	<0.001
Vasoconstrictor drugs (%)	75	0	<0.001

BMI: Body mass index, HR: heart rate, BPM: beat per minute, MAP: mean arterial pressure, CVP: central venous pressure, HT: hypertension, NIDDM: non-insulin-dependent diabetes mellitus, IDDM: insulin-dependent diabetes mellitus, PVD: peripheral vascular disease, COPD: cronic obstructive pulmonary disease, CRF: chronic renal failure, AKI: acute kidney injury, RRT: renal replacement therapy.

Discussion

Today, despite all research, the usage of modern antibiotics and advanced resuscitation methods in intensive-care units, sepsis is still seen at a high frequency and is one of the most important causes of death in hospitals and intensive care units (9-11). The pathogenesis of the sepsis process is tremendously complex and involves a dynamic interplay between the pathogen and the host immune system. In order to reduce sepsis-induced morbidity and mortality, it is essential to understand the molecular mechanisms associated with development of sepsis and sepsis-linked organ injury (7, 8).

The immune system consists of two parts: natural and acquired. The innate immune system is an essential form of host defense against infections. TLRs are recognized as a component of the natural immune system. TLRs are transmembrane proteins. They have a considerable role in host immune system by both creating a host natural immune response against many pathogens and activating the gained immune response (12, 13). The stimulation of TLR receptors by pathogenic microbial substances leads to the activation of many inflammatory cytokines and downstream inflammatory pathways (14, 15). This is the host immune inflammatory response.

Table II. *Biochemistry and laboratory data of study groups. Data are presented as mean±SD or proportions.*

Characteristic	Sepsis group (n=80)	Control group (n=100)	*p*-Value
Creatinine (mg/dI)	2.04±1.42	0.97±0.34	<0.001
BUN (mg/dl)	62.29±26.99	20.88±5.41	<0.001
Hemoglobin (g/dI)	9.63±1.10	13.26±1.77	<0.001
Leukocytes (n/mm^3)	20478.61±14374.37	7826.60±2272.53	<0.001
Platelets (n/mm^3)	184721.25± 132050.84	306571.97 ±92610.71	<0.001
CRP (mg/dI)	17.43±5.36	0.50±1.03	<0.001
pH	7.35±0.10	7.42±0.31	<0.001
PaO_2/FIO_2	205.28±66.16	384.94±64.94	<0.001
$PaCO_2$ (mmHg)	36.22±12.67	35.00±3.89	>0.05
HCO_3 (mmol/l)	21.76±4.42	24.73±1.24	<0.001
Lactate (mEq/I)	3.78±3.63	1.02±0.43	<0.001
Glucose (mg/dl)	162.87±63.13	113.62±33.71	<0.001
Na^+ (mEq/I)	137.60±7.45	138.94±3.69	>0.05
K^+ (mEq/I)	4.06±0.82	3.81±0.42	0.004
Albumin (g/dl)	2.67±0.58	4.11±0.61	0.004
SGOT (U/dl)	440.16±91.60	29.43±2.23	<0.001
SGPT (U/dl)	212.08±47.86	28.08±2.47	<0.001
Total bilirubin (mg/dl)	3.19±6.50	0.69±0.72	<0.001
LDH (U/l)	480.03±257.14	237.20±124.26	<0.001
TLR9 level (pg/ml)	0.66±1.05	0.74±0.41	<0.001

BUN: Blood urea nitrogen, CRP: C-reactive protein, $PaCO_2$: arterial partial CO_2 pressure, HCO_3: bicarbonate, SGOT: serum glutamic oxaloacetic transaminase, SGPT: serum glutamic pyruvic transaminase, LDH: lactate dehydrogenase, PaO_2/FIO_2: the ratio of partial arterial O_2 pressure and fraction of inspired oxygen.

Table III. *Serum toll-like receptor 9 (TLR9) levels (mean±SD) in patients with sepsis.*

Factor	TLR9 level (pg/ml)	*p*-Value
Lactate level		
>5 mEq/l	0.79±0.36	0.009
≤5 mEq/I	0.62±0.14	
Survived sepsis		
Yes	0.70±0.68	>0.05
No	0.65±0.17	

Table IV. *Serum toll-like receptor 9 (TLR9) level (mean±SD) in the sepsis group according to pathogen.*

Pathogenic microorganisms	Frequency of infection (%)	TLR9 level (pg/ml)
Acinetobacter baumannii	20.6	0.80±1.69
Pseudomonas aeruginosa	20	0.66±0.48
Klebsiella pneumoniae	14.8	0.53±0.42
MRSA	14.2	1.01±1.97
Enterobacter	7.1	1.19±2.83
Escherichia coli	6.3	0.40±0.28
MSSA	4.2	0.64±0.41
Streptococcus pneumoniae	3.5	0.45±0.41
Corynebacterium striatum	2.8	2.61±3.69
VRE	3.2	0.66±0.46
Candida	2.5	0.51±0.38
Serratia marcescens	0.4	0.47
Aspergillus	0.4	1.16

MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: methicillin-sensitive *S. aureus*, VRE: vancomycin-resistant *Enterococcus*.

Additionally, TLR9 is required for optimal activation of bactericidal activity. It carries the pattern of recognition for microbial DNA. TLR9 is essential for the uptake and intracellular killing of the bacteria during infection with *K. pneumoniae* (16). Some studies defined the role of TLR9 as recruiting and activating leukocytes, including dendritic cells and macrophages (17-18). Similar results for *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* have been published in other studies, where TLR9 has critical functions in the generation of the IL12/interferon-γ response (19-21).

There is a hyperinflammatory response during the initial phase of sepsis. During this period, the complement system is activated and TLRs are highly expressed on monocytes and macrophages (22). Termination of this hyperinflammatory response and restoration of the host immune system are mediated by compensatory anti-inflammatory cytokines after control of infection. This compensatory anti-inflammatory response syndrome appears in the majority of patients with sepsis (14). Indeed, the host immune system produces two types of responses against the sepsis. These two immunological responses are consecutive and overlapping phases. If the compensatory anti-inflammatory response is ineffective, the inflammatory reaction can result in destruction of the host cells and became injurious to the body overall. This may influence sepsis-related organ injury or dysfunction and eventual mortality (23-25). On the other hand, deterioration in the regulation of control mechanisms during sepsis may lead to loss of control of inflammation due to excessive activation or extreme suppression of the inflammatory response. For instance, as a result of the long duration of infection or the inability for it to be controlled by antibiotics and other treatment methods, it is possible to switch to the anti-inflammatory state. This is the period of immuno-paralysis or immuno-suppression and is a hallmark of sepsis. These two extreme immune responses in the fight against infection,

inflammatory and anti-inflammatory processes, can ultimately harm the patient (26).

It is known that sepsis affects immune responses leading to immunosuppression. Studies demonstrated that sepsis impairs host immune system homeostasis (27, 28). This is an important pathological mechanism in sepsis and is one of the important breakpoints in sepsis-related death (29, 30). In patients, secondary infection or herpes virus reactivation may develop during the sepsis-associated immunosuppressive stage (31). In our study, infection due to multiple pathogenic microorganisms was detected at a high rate in patients with sepsis.

In a septic-shock study, investigators found that 35% of patients had a down-regulation of *TLR* gene expression compared to their baseline values (32). We are of the opinion that the low levels of serum TLR9 detected in our study were because of down-regulation in these sepsis cases. The decrease in the serum level of TLR9 in the sepsis group in our study can be explained by the fact that the patients had severe sepsis. Again, to stress the point, TLR9 levels in our study were measured only in patients with serious sepsis and septic shock. TLR9 levels may be reduced due to compensatory anti-inflammatory condition in an advanced stage in severe cases of sepsis. Additionally, there was also a statistically significant relationship between these low values and mortality in our study.

Myocardial functions are also affected by various mechanisms in sepsis. Cardiovascular dysfunction is a serious complication in about 40% of patients with sepsis and increases mortality by 20-70%. TLR2 and TLR4 particularly contribute to cardiac dysfunction (33, 34). Nevertheless there is little information on the role of TLR9 in myocardium, but TLR9 expression in the heart has been shown (35). It is also known that TLR9 stimulation leads to nuclear factor κB activation in different tissues (36). In one study, it was found that bacterial DNA increased myocardial cytokine production via TLR9 and caused depletion of cardiomyocyte contractile ability (37).

In addition to hyperinflammation, severe sepsis complicated by hypoperfusion and organ dysfunction due to this cardiovascular dysfunction develops. One of the best indicators in diagnosis of severe sepsis is the level of lactate (38). Our study shows that there is a relationship between serious sepsis progressing to septic shock and increased serum TLR9 level, particularly in those cases where the lactate level increased to >5 mmol/l due to tissue hypo-perfusion. Our conclusion is that there is a decrease in serum TLR9 level in serious sepsis through its down-regulation, while there is an increase in serum TLR9 level, perhaps in connection with TLR9 re-expression, in the cases in which tissue perfusion deteriorates, leading to high values of lactate. Recurrent cycles of hyper- and hypoinflammatory condition may occur in sepsis (39). It is not clear which clinical conditions trigger or cause this change. An increase in serum lactate level may play a crucial role as an immunostimulant or an agent that triggers this change in the TLR9 pathway.

In fact, both inflammatory and anti-inflammatory processes reflect not only uncontrolled systemic response but also survival efforts of the patients with sepsis. Current inflammatory status determination and appropriate management are important points for sepsis treatment. Targeted therapies for inflammatory cytokines in a patient during the hyperinflammatory phase may be performed. However, in clinical trials, these therapies have shown no clinical benefit or, in some cases, have worsened survival (40, 41). Furthermore, compensatory anti-inflammatory response to counter-regulate the host immune response in sepsis may result in immunosuppression, whereas immunostimulants may be more beneficial in these patients (42-44). The immunological state of each patient may differ.

For this reason, while one patient may benefit from targeted therapy, another may suffer as a result of the same therapy. Targeted immune therapies designed according to the patient's current inflammatory condition should be planned. Therefore the immunological status of the patient should be well determined for sepsis-appropriate management and treatment. Ideally, specific biomarkers should be used to help management of sepsis (45). The importance of TLRs in this topic will be determined by future studies.

This study has some limitations; the most important of them is that the current clinic study had a relatively small sample size, it was single-institutional and serum TLR9 levels were analyzed only once. Repetitive segmental determinations would make the evaluations and progress of patients more understandable. We believe that our results are encouraging, but they need to be supported by study in a larger sample and perhaps in different phases of sepsis.

Sepsis is characterized by a hyperinflammatory response in the early stage, but it may be followed by an immunosuppressive phase. This immunosuppression is believed to be the potent factor which is tightly associated with high mortality in sepsis. Modulation of this immunosuppression may help to develop new therapeutic strategies. TLRs have an important role in sepsis pathophysiology. However, the role of TLRs in the development of sepsis and in the creation of tissue damage and sepsis-related organ injury is not clear. Targeting TLRs is an exciting and promising area for inflammation control and sepsis management (46).

Actually, developing sepsis-specific TLR-targeted therapies might not be easy. Blocking a single mediator or activating a single channel will not be enough to intervene in sepsis (47, 48). Further experimental studies are required to understand the underlying mechanism of infection pathogenesis. Animal sepsis models can provide valuable information about the role of TLRs and the underlying mechanism of infection pathogenesis.

A better understanding of TLR biology and the change of the TLR9 level in sepsis may unveil novel therapeutic approaches for sepsis. It may be possible to detect those patients who are on the cusp of septic shock, which would infer higher mortality, and specific alterations in treatment might be considered. Studies have shown that not only microbial products but also some endogenous molecules can stimulate TLRs (49). The host immune system can be activated by means of TLR9- related systems. Hypoperfusion and hyperlactatemia may play an effective stimulatory role in reactivation of the immune system.

Funding

This study was supported by the Istanbul University Scientific Research Projects Unit (Project No: 38207).

Conflicts of Interest

None of the Authors has any conflict of interest to declare.

References

1 Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM and Sibbald WJ: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Commitee. American College of Chest Physicians/Society of Critical Care Medicine. Chest *101*: 1644-1655, 1992.

2 Levy MM, Fink MP and Marshall JC: 2001 SCC/ESICM/ACCP/ATS/SIS International sepsis definitions conferance. Crit Care Med *31*: 1250-1256, 2003.

3 Linde-Zwirble WT and Angus DC: Severe sepsis epidemiology: Sampling, selection, and society. Crit Care *8*: 222-226, 2004.

4 Levy MM, Dellinger RP, Townsend SR, Linde-Zwirble WT, Marshall JC, Bion J, Schorr C, Artigas A, Ramsay G, Beale R, Parker MM, Gerlach H, Reinhart K, Silva E, Harvey M, Regan S, Angus DC; Surviving Sepsis Campaign: The Surviving Sepsis Campaign: Results of an international guideline-based performance improvement program targeting severe sepsis. Crit Care Med *38*: 367-374, 2010.

5 Oberholzer A, Oberholzer C and Moldawer LL: Sepsis syndromes: Understanding the role of innate and acquired immunity. Shock *16*: 83-96, 2001.

6 Rittirsch D, Flieri MA and Ward PA: Harmful molecular mechanisms in sepsis. Nat Rev Immunol *8*: 776-787, 2008.

7 Browne EP: Regulation of B-cell responses by toll-like receptors. Immunology *136*: 370-379, 2012.

8 Latz E, Schoenemeyer A, Visintin A, Fitzgerald KA, Monks BG, Knetter CF, Lien E, Nilsen NJ, Espevik T and Golenbock DT: TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nat Immunol *5*: 190-198, 2004.

9 Dombrovskiy VY, Martin AA, Sunderram J and Paz HL: Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: A trend analysis from 1993 to 2003. Crit Care Med *35*: 1244-1250, 2007.

10 Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock, 2016. Available from: www.survivingsepsis.org

11 Goldstein B, Giroir B and Randolph A: International pediatric sepsis consensus conference: Definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med *6*: 2-8, 2005.

12 Opal SM: Phylogenetic and functional relationships between coagulation and the innate immune response. Crit Care Med *28*: 77-80, 2000.

13 Tenover FC: Rapid detection and identification of bacterial pathogens using novel molecular technologies: Infection control and beyond. Clin Infect Dis *44*: 418-423, 2007.

14 Cohen J: The immunopathogenesis of sepsis. Nature *420*: 885-891, 2002.

15 Underhill DM and Ozinsky. A: Toll-like receptors: key mediators of microbe detection. Curr Opin Immunol *14*: 103-110, 2002.

16 Paczosa MK and Mecsas J. Klebsiella pneumoniae: Going on the offense with a strong defense. Microbiol Mol Rev *80(3)*: 629-661, 2016.

17 Albiger B, Dahlberg S, Sandgren A, Wartha F, Beiter K, Katsuragi H, Akira S, Normark S and Henriques-Normark B: Toll-like receptor 9 acts at an early stage in host defence against pneumococcal infection. Cell Microbiol *9*: 633-644, 2007.

18 Bhan U, Lukacs NW, Osterholzer JJ, Newstead MW, Zeng X, Moore TA, McMillan TR, Krieg AM, Akira S and Standiford TJ: TLR9 is required for protective innate immunity in Gram-negative bacterial pneumonia: role of dendritic cells. J Immunol *179*: 3937-3946, 2007.

19 Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K and Akira S: A Toll-like receptor recognizes bacterial DNA: Nature *408*: 740-745, 2000.

20 Mogensen TH, Paludan SR, Kilian M and Ostergaard L: Live *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* activate the inflammatory response through toll-like receptors 2, 4, and 9 in species-specific patterns. J Leukoc Biol *80*: 267-277, 2006.

21 Sjölinder H, Mogensen TH, Kilian M, Jonsson AB and Paludan SR: Important role for toll-like receptor 9 in host defense against meningococcal sepsis. Infect Immun *76*: 5421-5428, 2008.

22 Wang H and Ma S: The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. Am J Emerg Med *26*: 711-715, 2008.

23 Utahaisangsook S, Day NK, Bahna SL, Good RA and Haraguchi S: Innate immunity and its role against infections. Ann Allergy Asthma Immunol *88*: 253-265, 2002.

24 Takeda K, Kaisho T and Akira S: Toll-like receptors. Annu Rev Immunol *21*: 335-376, 2003.

25 Beutler B and Poltorak A: Sepsis and evolution of the innate immune response. Crit Care Med *29*: 2-6, 2001.

26 Sherwood ER and Hotchkiss RS: BTLA as a biomarker and mediator of sepsis-induced immunosuppression. Crit Care *17*: 1022, 2013.

27 Zhang L, Cardinal JS, Bahar R, Evankovich J, Huang H, Nace G, Billiar TR, Rosengart MR, Pan P and Tsung A: İnterferon regulatory factor-1 regulates the autophagic response in LPS-stimulated macrophages through nitric oxide. Mol Med *18*: 201-208, 2012.

28 Zhang L, Yuhang AI and Tsung A: Clinical application: Restoration of immune homeostasis by autophagy as a potential therapeutic target in sepsis. Exp Ther Med *11*: 1159-1167, 2016.

29 Hotchkiss RS and Karl IE: The Pathophysiology and treatment of sepsis. N Engl J Med *348/2*: 138-150, 2003.

30 Bone RC: The pathogenesis of sepsis. Ann Intern Med *115*: 457-469, 1991.
31 Heininger A, Haeberle H, Fischer I, Beck R, Riessen R, Rohde F, Meisner C, Jahn G, Koenigsrainer A, Unertl K and Hamprecht K: Cytomegalovirus reactivation and associated outcome of critically ill patients with severe sepsis. Crit Care *15*: R77, 2011.
32 Piazza O, Pulcrano G, Fiori PL, Tufano R, Lonardo M, Rossano F and Catania MR: Toll-like receptor kinetics in septic shock patients: A preliminary study. Int J Immunopathol Pharmacol *25*: 425-433, 2012.
33 Knuefermann P, Nemoto S, Misra A, Nozaki N, Defreitas G, Goyert SM, Carabello BA, Mann DL and Vallejo JG: CD14-deficient mice are protected against lipopolysaccharide-induced cardiac inflammation and left ventricular dysfunction. Circulation *106*: 2608-2615, 2002.
34 Knuefermann P, Sakata Y, Baker JS, Huang Ch, Sekiguchi K, Hardarson HS, Takeuchi O, Akira S and Vallejo JG: Toll-like receptor 2 mediates *Staphylococcus aureus*-induced myocardial dysfunction and cytokine production in the heart. Circulation *110*: 3693-3698, 2004.
35 Boyd JH, Mathur S, Wang Y, Bateman RM and Walley KR: Toll-like receptor stimulation in cardiomyoctes decreases contractility and initiates an NF-kappaB-dependent inflammatory response. Cardiovasc Res *72*: 384-393, 2006.
36 Yeo SJ, Yoon JG, Hong SC and Yi AK: CpG DNA induces self and cross-hyporesponsiveness of RAW264.7 cells in response to CpG DNA and lipopolysaccharide: alterations in IL-1 receptor-associated kinase expression. J Immunol *170*: 1052-1061, 2003.
37 Knuefermann P, Schwederski M, Velten M, Krings P, Ehrentraut H, Rğdiger M, Boehm O, Fink K, Dreiner U, Grohe C, Hoeft A, Baumgarten G, Koch A, Zacharowski K and Meyer R: Bacterial DNA induces myocardial inflammation and reduces cardiomyocyte contractility: role of toll-like receptor 9. Cardiovasc Res *78*: 26-35, 2008.
38 Mikkelsen ME, Miltiades AN, Gaieski DF, Goyal M, Fuchs BD, Shah CV, Bellamy SL and Christie JD: Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock. Critical Care Medicine *5*: 1670-1677, 2009.
39 Kollef KE, Schramm GE, Wills AR, Reichley RM, Micek ST and Kollef MH: Predictors of 30-day mortality and hospital costs in patients with ventilator-associated pneumonia attributed to potentially antibiotic-resistant gram-negative bacteria. Chest *134*: 281-287, 2008.
40 Echtenacher B, Urbaschek R, Weigl K, Freudenberg MA and Mannel DN: Treatment of experimental sepsis-induced immunoparalysis with TNF: Immunobiology *208*: 381-389, 2003.
41 Mathias B, Szpila BE, Moore FA, Efron PA and Moldawer LL: A review of GM-CSF therapy in sepsis. Medicine *94*: e2044, 2015.
42 Kox WJ, Volk T, Kox SN and Volk HD: Immunomodulatory therapies in sepsis. Intensive Care Med *26(Supp 1)*: 124-128, 2000.
43 Volk HD, Reinke P and Döcke WD: Clinical aspects: From systemic inflammation to 'immunoparalysis'. Chem Immunol *74*: 162-177, 2000.
44 Muenzer JT, Davis CG, Chang K, Schmidt RE, Dunne WM, Coopersmith CM and Hotchkiss RS: Characterization and modulation of the immunosuppressive phase of sepsis. Infect Immun *78(4)*: 1582-1592, 2010.
45 Singer M: Biomarkers in sepsis. Curr Opin Pulm Med *19*: 305-309, 2013.
46 Angus DC: The search for effective therapy for sepsis: Back to the drawing board? JAMA *306*: 2614-2615, 2011.
47 Cohen J, Opal S and Calandra T: Sepsis studies need new direction. Lancet Infect Dis *12*: 503-505, 2012.
48 Tse MT: Trial watch: Sepsis study failure highlights need for trial design rethink. Nat Rev Drug Discov *12*: 334, 2013.
49 Zhang X, Kimura Y, Fang C, Zhou L, Sfyroera G, Lambris JD, Wetsel RA, Miwa T and Song WC: Regulation of toll-like receptor-mediated inflammatory response by complement *in vivo*. Blood *110*: 228-236, 2007.

Received July 5, 2018
Revised July 25, 2018
Accepted August 1, 2018