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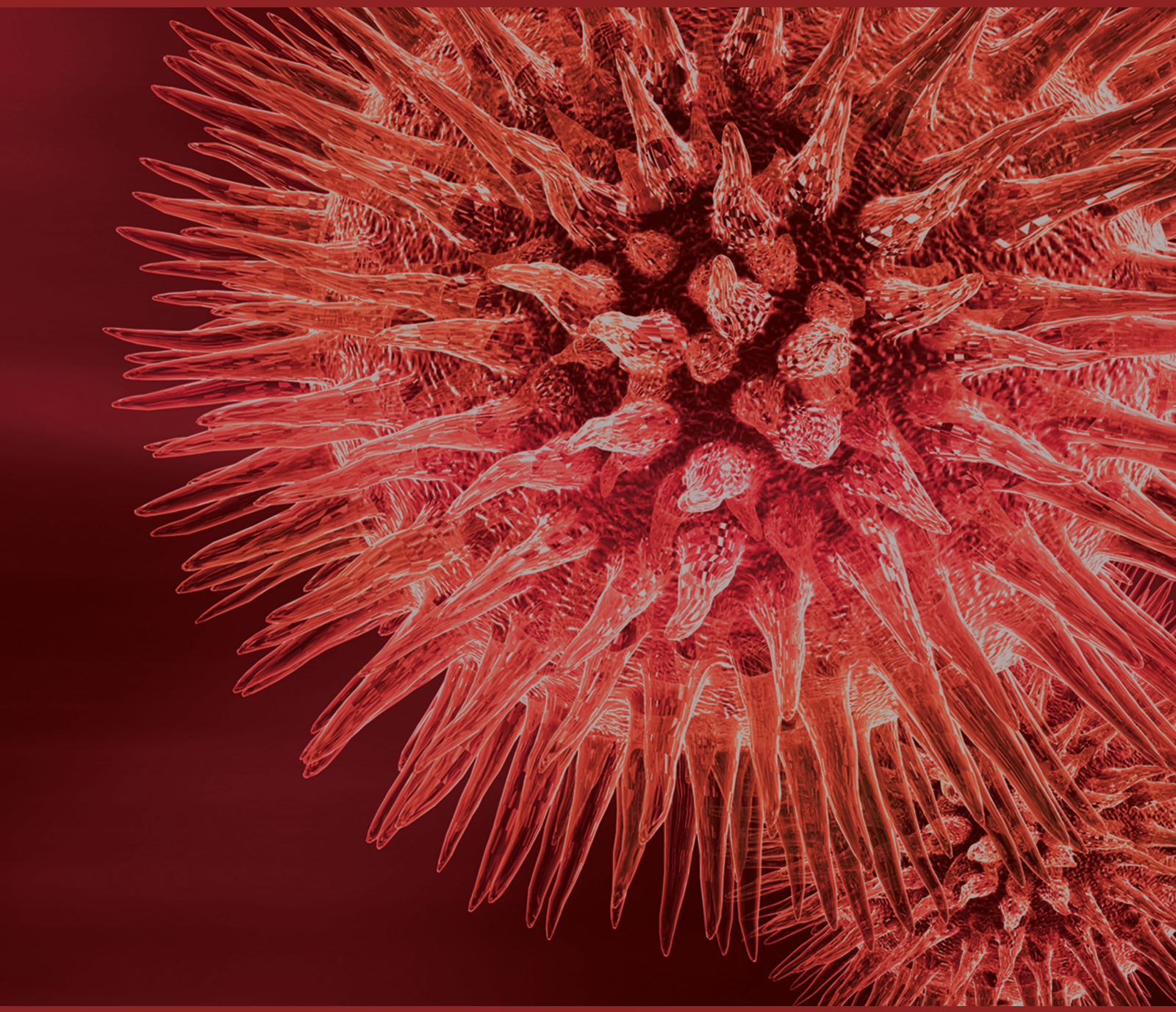
Sepsis: Pathogenesis, Biomarkers, and Treatment

Guest Editors: Baoli Cheng, Andreas H. Hoeft, Malte Book, Qiang Shu, and Stephen M. Pastores



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Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 846935, 2 pages
http://dx.doi.org/10.1155/2015/846935

Editorial

Sepsis: Pathogenesis, Biomarkers, and Treatment

Baoli Cheng,[1] Andreas H. Hoeft,[2] Malte Book,[3] Qiang Shu,[4] and Stephen M. Pastores[5]

[1]*Department of Anesthesiology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Qingchun Road 79, Hangzhou 310003, China*
[2]*Department of Anesthesiology and Intensive Care Medicine, University Hospital Bonn, Bonn, Germany*
[3]*Department of Anesthesiology, Bern University Hospital, 3010 Bern, Switzerland*
[4]*Department of Surgery, Children's Hospital, School of Medicine, Zhejiang University, Zhejiang, China*
[5]*Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

Correspondence should be addressed to Baoli Cheng; tianwai1979@163.com

Received 5 January 2015; Accepted 5 January 2015

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Sepsis is an infection-initiated systemic inflammatory syndrome with an estimated incidence of 18 million cases annually worldwide. Despite advances in intensive care and supportive technology, the mortality rate of sepsis still ranges from 15% to 80%, reminding scientists and clinicians that it remains to be a major clinical challenge. The key to winning the "campaign" to combat sepsis is improved understanding of the epidemiology, pathogenesis, and biomarkers of sepsis and discovery of novel therapies. The present special issue shows several encouraging results and provides comprehensive reviews of the latest advances in this field.

The effector cells from the innate and adaptive immune systems play a crucial role in sepsis. Dendritic cells, in particular, serve as professional antigen presenting cells and are involved in the aberrant immune response to sepsis. In this special issue, X. Fan et al. discuss the effects of sepsis on the amount, surface molecule expression, cytokine secretion, and T-cell activating function of dendritic cells and the underlying mechanisms in their review "Alterations of Dendritic Cells in Sepsis: Featured Role in Immunoparalysis." Recent postmortem studies of patients who died of sepsis showed that depletion of CD4 and CD8 lymphocytes is an important characteristic. Thus, knowledge of these circulating lymphocyte abnormalities is relevant for the understanding of sepsis pathophysiology. R. de Pablo et al., who have previously reported on the alteration of B cells, natural killer cells, and T-cell function in septic patients, summarize their latest findings on the role of blood lymphocytes in sepsis and discuss the different kinetic patterns of lymphocyte subsets and their relationship to outcome in their review "Role of Circulating Lymphocytes in Patients with Sepsis." Both the clinical and basic researches have shown that sepsis-associated immunosuppression is associated with adverse outcomes. A novel heterogeneous population of immature myeloid cells that possess immunosuppressive activities, termed myeloid-derived suppressor cells (MDSCs), has gained much attention in recent sepsis studies. D. Lai et al. discuss the complex functions of MDSCs in the pathogenesis of sepsis. Their review "Myeloid-Derived Suppressor Cells in Sepsis" also proposes that the overall role of MDSCs involves much more than simply being an immunosuppressive cell population. These 3 review articles provide a comprehensive analysis of the major important immune cells in sepsis and highlight potential therapeutic targets. As a group who have investigated the function of the family of defensins in sepsis for nearly 10 years, G.-H. Xie et al. summarize the *in vitro*, *in vivo*, and genetic studies on the effects of defensins as well as the corresponding mechanisms within sepsis. Their review, "Defensins and Sepsis," also points out that the function of defensins reflects both their immunomodulatory and broad-spectrum antimicrobial effects.

Although the international Surviving Sepsis Campaign guidelines have been released for 10 years, sepsis remains a fatal syndrome due to the lack of efficient biomarkers and novel treatments. D. N. Nguyen et al. investigated plasma

cortisol levels in septic patients with delirium and coma and found that cortisol is a potential biomarker of brain dysfunction in their article "Cortisol Is an Associated-Risk Factor of Brain Dysfunction in Patients with Severe Sepsis and Septic Shock." F. Song et al. and P. Madhusudan et al. discuss two important but controversial issues related to the Surviving Sepsis Campaign Guidelines. In a meta-analysis of 12 randomized trials involving 4100 septic patients, "Intensive Insulin Therapy for Septic Patients: A Meta-Analysis of Randomized Controlled Trials," F. Song et al. reported no benefit and a higher incidence of hypoglycemia with intensive insulin therapy compared with conservative glucose management. P. Madhusudan et al. discuss the current debate on the choice, amount, and end points for fluid resuscitation in sepsis in their review "Fluid Resuscitation in Sepsis: Reexamining the Paradigm." K. Xie et al. investigated the therapeutic function of hydrogen gas in a septic animal model for several years, and, in their present review, "Hydrogen Gas Presents a Promising Therapeutic Strategy for Sepsis," they summarize the progress of hydrogen treatment in sepsis. J. Zhou et al. and X. Li et al. explore novel drugs for sepsis from the perspective of the neuroendocrine network in sepsis in their two studies, "Epinephrine Enhances the Response of Macrophages under LPS Stimulation" and "Agmatine Protects against Zymosan-Induced Acute Lung Injury in Mice by Inhibiting NF-κB-Mediated Inflammatory Response."

In this present special issue about the pathogenesis, biomarkers, and treatment of sepsis, the authors provide comprehensive reviews and attractive research perspectives on the mechanisms of sepsis which we hope will inspire researchers investigating novel biomarkers and therapeutic sepsis targets.

Acknowledgment

This study was supported by National Natural Science Foundation of China (no. 81102226).

Baoli Cheng
Andreas H. Hoeft
Malte Book
Qiang Shu
Stephen M. Pastores

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 903720, 10 pages
http://dx.doi.org/10.1155/2015/903720

Review Article

Alterations of Dendritic Cells in Sepsis: Featured Role in Immunoparalysis

Xia Fan, Zheng Liu, He Jin, Jun Yan, and Hua-ping Liang

State Key Laboratory of Trauma, Burns and Combined Injury, Research Institute of Surgery, Daping Hospital, The Third Military Medical University, Chongqing 400042, China

Correspondence should be addressed to Hua-ping Liang; 13638356728@163.com

Received 15 February 2014; Revised 25 May 2014; Accepted 28 July 2014

Academic Editor: Baoli Cheng

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Sepsis, the leading cause of mortality in intensive care unit, is characterized by hyperinflammatory response in the early stage and followed by a period of immunosuppression. This immune disorder is believed to be the potent factor that is tightly associated with high mortality in sepsis. Dendritic cells (DCs) serve as professional antigen-presenting cells that play a vital role in immune response by activating T lymphocytes. During the progression of sepsis, DCs have been reported to take part in the aberrant immune response and be necessary for survival. Therefore, a better understanding of the DCs pathology will be undoubtedly beneficial for resolving the problems occurring in sepsis. This review discusses effects of sepsis on DCs number and function, including surface molecules expression, cytokines secretion, and T cell activation, and the underlying mechanism as well as some potential therapeutic strategies.

1. Introduction

Sepsis is high lethal public disease. In 2012, over 20 million people are affected by sepsis worldwide [1]. The mortality from septic shock and severe sepsis both in Europe and in USA is around 30% and this value is still elevated [1, 2]. Recently, sepsis is defined as the systemic inflammatory response syndrome (SIRS) due to infection [3], which indicated that SIRS and infection are two important factors in determination of sepsis.

When the host receives an infection, both pro- and anti-inflammatory responses are initiated. The inflammatory response is partly mediated by innate immune cells through recognition with invading pathogens or microorganisms [4]. These cells can decide the trend of inflammatory response toward pro- or anti-inflammatory state by producing proinflammatory cytokines (interleukin- (IL-) 1β, tumor necrosis factor- (TNF-) α, and interferon- (IFN-) γ) or anti-inflammatory cytokines (interleukin- (IL-) 10, transforming growth factor- (TGF-) β) [5, 6]. At the early stage of sepsis, there is a large amount of proinflammatory mediators termed as cytokines storm in the host. Therefore, various therapeutic methods have been used to treat sepsis by downregulation of proinflammatory cytokines expression. But in fact it does not bring good news in the clinical setting. There is one possibility that the animal model, such as cecal ligation and puncture (CLP), cannot entirely reflect the real state of septic patients, in which the gender, hormone, age, and other interference factors cannot be neglected [7, 8]. Another possibility is correlated with sepsis progression. Observation from clinical studies showed that about 80% septic patients had a persistence of infectious focus at the day they died [9]. Some other studies also found that the active cytomegalovirus normally existed in the septic patient without resolution [10, 11]. These results indicate that the host immunity exhibits a tolerance status, which makes the patients at an increased risk of subjection to secondary pathogen infection. The immunosuppression is found to be accompanied with immune cells deactivation and apoptosis, impaired antigen-presentation, suppression of proliferation of lymphocytes, and high levels of anti-inflammatory cytokines (IL-10). Moreover, polarization of T helper (Th) cells is toward to the Th2 type that results

in an increase in susceptibility to infection. The aberrant immune response will further lead to multiple organ failure and death.

Among the innate immune cells, dendritic cells (DCs), firstly discovered by Raplph in the early 1970s, are the most potent antigen-presenting cells and central component for linking the innate and adaptive immunity [12–14]. DCs originate from bone marrow $CD34^+$ stem cells and home to all tissues via the blood stream where they developed into immature cells [15]. Immature DCs have high phagocytic properties and readily take up antigen and present the antigen to Th cells. In response to endogenous danger signals or microbial antigens, DCs mature and migrate into the T cell area of lymphoid tissues, where $CD4^+$ T cell will be activated. During the maturation, the phagocytic receptor will be lost, the surface molecules (e.g., MHCI, MHCII, CD80, and CD86) involved DCs migration, and T cells activation will be upregulated [16, 17]. Although many different classification manners have been described, two major subsets of DCs are recognized: myeloid DCs (mDCs) and plasmacytic DCs (pDCs) [18, 19]. The former is derived from bone marrow precursor and the latter is believed to evolve from circulating lymphoid precursor [20, 21]. These two types of DCs have a similar molecular phenotype except for $CD8\alpha^+$, which is present in pDCs but absent in mDCs [22]. Based upon the importance of DCs in immune system and its central role in sepsis [23], this review will focus on the pathology changes of DCs during the evolution of sepsis.

2. The Effect of Sepsis on DCs Numbers

At first, large amounts of studies on animals or patients had featured obvious loss of $CD4^+$ and $CD8^+$ T cells in sepsis [24–27]. Due to the importance of DCs in the immune system, more and more investigators have focused on the change of DC numbers and its role in depletion of T cells. In general, CD11c is believed to be the common marker of murine DC for its steady state. A profound loss in the number of $CD11c^+$ DCs was observed in spleen after sepsis and the time ranging from 12 h to 3 d [28–32]. When the $CD11c^+$ DCs are further divided into $CD8^+CD4^-$, $CD8^-CD4^+$, and $CD8^-CD4^-$, it is found that $CD8^+CD4^-$ and $CD8^-CD4^+$ subsets were lost 36 h after CLP, but the number of $CD8^-CD4^-$ DCs was increased [33]. Thus it could be demonstrated that the reduced number of splenic DCs was mediated by a selective loss of $CD8^+CD4^-$ and $CD8^-CD4^+$ subtypes.

In addition to spleen, sepsis was also found to reduce the percentage of $CD11c^+$ DCs present in local mesenteric nodes beginning 12 h after CLP and reach a 50% decline by 24 h. This phenomenon was also observed in systemic inguinal nodes, but not in popliteal nodes [34]. Moreover, another study was performed on the mice with CLP, which were subsequently intravenously challenged with *Schistosoma mansoni* eggs to develop granulomas. Results showed that there was a significant loss of DC in lung during the granulomatous response [35]. However, it should be noted that gradual reconstitution of DC numbers was found on postsepsis day 28 [30].

In clinical settings, the number of DCs in blood was lower in severe septic or septic shock patients in comparison with healthy controls [36, 37]. For two distinct populations of DCs, mDCs and pDCs, their numbers was markedly reduced in patients with sepsis when compared with controls, and both cell counts recovered slightly until day 28 [38]. But data from another clinical study of twenty-six patients showed that decreased mDC and increased pDC were observed at day 1, and the number of mDCs was not different in survivors and nonsurvivors of septic patients, while pDCs were obviously higher in nonsurvivors [39]. This discrepancy between these two study groups may be due to the different severity of illness. Moreover, reduction of circulating DCs can become a predictive factor for the development of septic complication after pancreatectomy [40]. Besides the adult patients, flow cytometric assay showed that the levels of pDCs and mDCs were also significantly lower in pediatric patients with sepsis [41].

In conclusion, sepsis causes the loss of DCs occurring in various lymphoid and nonlymphoid tissues from septic patients and septic mice. This phenomenon does not result from the inhibition of de novo generation of DCs from progenitors [42, 43], although these monocytic progenitors display characteristics of immunosuppressive properties [44] (Figure 1).

3. The Effect of Sepsis on DCs Function

3.1. Surface Molecular Expression. Upon the stimulation of microbial antigens or danger signals, DCs rapidly mature and migrate through the lymphatic system to lymphoid organs to stimulate T cells mediated immunity response. During this process, DCs will upregulate the presentation of cell surface proteins involved in T cell priming, including MHC, CD40, CD80, and CD86. In the CLP model, no obvious changes of CD40, CD80, and CD86 expression were discovered in $CD11c^+$ splenocytes when compared with control group by 24 h after surgery. Similarly, peritoneal DCs showed CD40 and CD80 did not change in addition to an increase trend in CD86 expression [28]. However, splenic DCs from another study showed that levels of CD40 and CD86 were obviously enhanced by 15 h and 36 h after CLP while MHCI expression was much higher than control at 36 h following CLP. Only slight changes were observed in the expression of CD80 and MHCII [33]. For the DCs in lymph nodes, the percentage of CD40, CD80, CD86, and MHCII did not differ within 24 h between CLP-operated mice and sham-operated mice, but there was a much higher expression of these molecules 36 h after sepsis [33, 34]. In addition, sepsis did not cause the change of CD40 and CD80 in the lung until 7 d after CLP [45]. B and T lymphocyte attenuator (BTLA), a coinhibitory receptor, has been demonstrated to inhibit T cell activation and thus contributed to many diseases [46]. BTLA and its primary ligand, herpes virus entry mediator (HVEM), expressions were found to increase in immature and mature DCs in peritoneum by 24 h after CLP, while $HVEM^+$ DCs were significantly decreased in bone marrow [47].

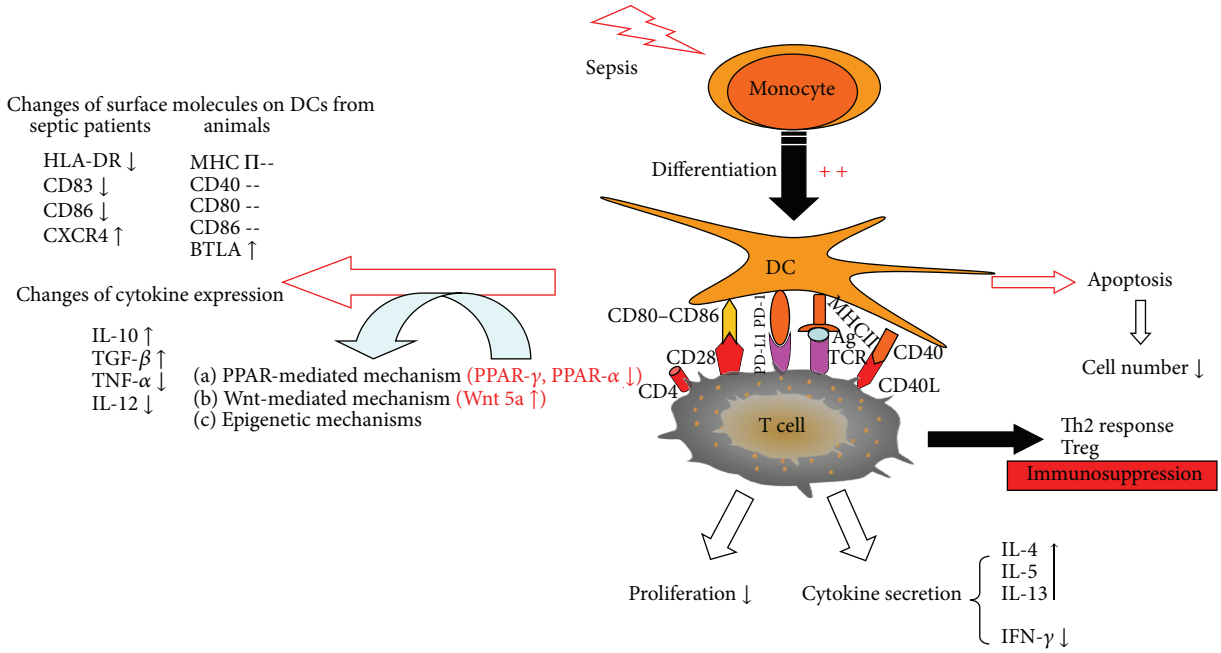

Figure 1: The changes of DCs during sepsis. When suffering from sepsis, DCs will be lost resulting from apoptosis, but differentiation from monocytes is accelerated. The surface molecules associated with DCs function are changed. At the same time, DCs have an aberrant cytokine secretion which results in immune tolerance status. The potential mechanism may be associated with apoptosis, PPARs, Wnt signal, and epigenetic regulation. MHCII: major histocompatibility complex class II, Ag: antigen, TCR: T cell receptor, PD-1: programmed cell death-1, PD-L1: programmed cell death ligand 1, BTLA: B and T lymphocyte attenuator, and PPARs: peroxisome proliferator-activated receptors.

Clinical evidences proposed that the expression of human leukocyte antigen-DR (HLA-DR) is an indicator of immune failure, and with predictive value in clinical practice [48]. A profound decreased expression of HLA-DR on monocytes has been reported in septic patients [49]. But a continuous recovery phenomenon was exhibited in survivors of sepsis within 10 days, whereas there are no changes in nonsurvivors of sepsis [50, 51]. HLA-DR on mDCs in sepsis is three times lower than that in controls (MFI: 174 ± 54 versus 497 ± 128). Similar reduction was seen in pDCs, but with a narrower margin (MFI: 177 ± 66 versus 239 ± 77). At day 28, the expression of HLA-DR on mDCs was recovered but remained lower than that in controls, while HLA-DR on pDCs showed a similar expression pattern to controls [38]. Besides HLA-DR, the percentage of CD83 and CD86 was also reported to be reduced in septic patients, but chemokine receptor CXCR4 was upregulated [39].

3.2. Cytokine Secretion. A large number of studies have reported that septic DCs exhibit an aberrant cytokine secretion pattern, in which levels of proinflammatory cytokines (TNF-α, IL-1β, and IL-12) are significantly depressed and anti-inflammatory cytokines (TGF-β, IL-10) are enhanced [33, 38, 45] (Figure 1). DC-derived IL-12 is believed to be a key host defense cytokine and it is a heterodimeric cytokine composed of an IL-12p40 and IL-12 p35 subunit [30, 52]. Flow cytometric analysis of splenic DCs from LPS-primed mice revealed that the percentage of DCs able to produce IL-12 p40 was dramatically decreased from 1.7% to 0.3% [53]. When DCs were stimulated with TLR2 agonist (Pam3Cys) or TLR4 agonist (LPS) or TLR9 agonist (CpG-DNA), mRNA levels of both *Il12 p40* and *Il12p35* from sepsis splenic DCs were significantly lower than that from sham splenic DCs [30]. Sepsis also resulted in a lower intracellular expression of IL-12 p40 induced by CpG-DNA compared with sham group [33]. In addition, only a small amount of IL-12 p70 was secreted from DC being stimulated with CpG or LPS + CD40L [33]. A similar trend was also seen in lung DCs. The DCs from lungs of postseptic mice with developing granulomas had a lower IL-12 p40 mRNA and IL-12 p70 protein levels compared with controls [35]. Moreover, they also exhibited defective IL-12 synthesis after TLR agonist challenge [45].

IL-10 is a pleiotropic cytokine possessing both anti-inflammatory and immunosuppression properties [54]. In the acute phase of sepsis, endogenous IL-10 production and exogenous administration can reduce the magnitude of the inflammation. Therefore, injection of recombinant adenovirus expressing IL-10, which limits DC maturation and associated T cell activation, could attenuate acute sepsis [55, 56]. However, the upregulation of IL-10 will result in the immunity tolerance that fails to defend the secondary pathogen challenge. 36 h after CLP, DCs from septic mice produced increasing amounts of IL-10 [33]. Upon incubation with TLR agonist, the higher level of IL-10 at both of mRNA and protein level was observed in splenic and lung DCs from postseptic mice in contrast to control [30, 35, 45]. The increased concentration of IL-10 in blood from septic patients is associated with worsened clinical outcome [57]. Furthermore, endogenous IL-10 has been reported to regulate

IL-12 synthesis of DCs in an autocrine manner [58, 59]. DCs from sham mice could increase LPS-induced IL-12 expression in the presence of anti-IL-10 antibody. However, blocking of IL-10 could not rescue the production of IL-12 of postseptic DCs, which suggests that the low production of IL-12 during sepsis is not dependent on IL-10 expression [30].

3.3. T Cell-Stimulatory Capacity. The impact of DCs on T cells proliferation during sepsis was determined in a mixed leucocyte reaction (MLR). IL-2 plays a crucial role in the proliferation of T cells. It was found that the percentage of IL-2-secreting T cells was significantly lower when cultured with DC from septic mice as compared with control mice [33]. This finding was also confirmed when OT-II $CD4^+$ T cells were incubated with DCs in the presence of antigen [60]. However, peritoneal DCs and splenic DCs from CLP mice both showed higher capacity to trigger proliferative response of T cells than those from sham group [28]. In addition, an increased activation of $CD3^+CD4^+$ T cell was also seen in the inguinal nodes and popliteal lymph nodes [34]. For septic patients, immature DCs from patients and health donors had a similar ability to induce T cells proliferation, but mature DCs from patients did not enhance T cell response [43].

Studies on polarization of T cells had showed that OVA peptide-specific $CD4^+$ T cells secreted markedly higher levels of Th2 cytokines such as IL-5, IL-13, and IL-4 but a lower amount of Th1 cytokine IFN-γ when cocultured with postseptic splenic DCs that pulsed with OVA, indicating that Ag-loaded DCs direct T cells toward a Th2-dependent response during severe sepsis [30]. This is consistent with another study in which adoptive transfer of bone-marrow derived DC from septic mice impaired Th1 priming [42]. In addition, the expression of Foxp3 in T cells cocultured with patient or control DCs suggested that $CD1a^+$ DCs from septic patients made the T cells have a stronger regulatory function, because the percentage of naïve T cells expressing Foxp3 when cultured in patient DCs was much higher than that induced by control DCs (93% versus 40%) [61], which suggested that sepsis led to an increase in regulatory T cells (Tregs).

In short, though controversy still exist, DCs will engender apoptotic or anergic T cells after sepsis. These anergic T cells, in turn, may disrupt DCs function.

4. The Potential Mechanisms Involving Changes of DC during Sepsis

4.1. Apoptosis-Dependent Mechanism. Studies by numerous groups have suggested that apoptotic death of immune cells plays a vital role in contributing to the immune hyporesponsiveness and organ injury during sepsis [62–64]. 24 h after CLP, a significant increase of apoptotic and dead DCs was found in mesenteric and inguinal nodes through the staining of annexin V [34]. This result was also confirmed by immunohistochemical staining for active caspase 3, a crucial mediator of apoptosis [29]. However, a high false-positive result may occur, because DCs have phagocytic properties and the positive signal may form the apoptotic debris that is phagocytized by DCs [65, 66]. To further clarify the relationship between apoptosis and the loss of DC, study from the transgenic mice which could overexpress the Bcl-2 reported that overexpression of Bcl-2 could dispel sepsis-induced DCs depletion. Furthermore, $Bim^{-/-}$ mice exhibited remarkably less sepsis-induced loss in the DCs population [67]. Thus these proapoptotic and antiapoptotic proteins play a central role in DC loss during sepsis. In addition to DC loss, uptake of apoptotic DC would make viable DC display tolerogenic state that induces generation of $Foxp3^+$ Treg [68].

The mechanisms by which sepsis caused DC apoptosis are at present not fully explored. A previous study has found that mechanism of apoptosis induced by LPS required activation of acid sphingomyelinase (A-SMase). Inhibition of this enzyme activity and ceramide generation could prevent apoptosis induction [69]. Furthermore, mammalian toll-like receptors (TLR)-dependent pathway is also found to involve in the process of sepsis-induced apoptosis, which was confirmed by several studies: (i) apoptosis of spleen DCs from CLP performed on $TLR4^{-/-}$, $TLR2^{-/-}$, and $TLR2^{-/-}$ $TLR4^{-/-}$ was inhibited [31]. (ii) TNF-α, a production of stimulation of TLRs, could impair mitochondrial integrity and induce apoptosis [70]. (iii) Interferon regulatory factor-1 (IRF-1) whose activation is dependent on intact TLR4 signaling was reported to trigger immune cells apoptosis [71]. However, a recent study showed that LPS-induced activation of nuclear factor of activated T cells (NFAT) via CD14 is necessary for DCs apoptosis, which was independent of TLR4 engagement [72].

4.2. Peroxisome Proliferator-Activated Receptors-Mediated Mechanism. Peroxisome proliferator-activated receptors (PPARs) are a superfamily of ligand-activated nuclear transcription factors and are involved in the regulation of lipid metabolism, glucose homeostasis, and cellular differentiation [73–75]. So far, three subtypes have been identified in human: PPAR-α, $\beta(\delta)$, and γ. Peripheral blood monocytes express high levels of PPAR-α and PPAR-β with low expression of PPAR-γ [76]. During the generation of DCs from monocytes and its maturation, PPAR-γ becomes the abundant subtype while the levels of other two subtypes are below the detection limit [76]. It was found that activation of PPAR-γ significantly increased the surface expression of CD36 and CD86 on LPS- and CD40 ligand-challenged DCs, whereas the synthesis of CD80, CXCL10, and CCL5 was reduced [77]. Moreover, it could depress the production of IL-12 with no effect on expression of IL-1β, TNF-α, IL-6, and IL-10 [77]. Studies also showed that PPAR-γ activation inhibited TNF-α induced DC migration from epithelia and subsequent accumulation in the draining lymph nodes [78]. Adoptive transfer of PPAR-γ-activated Ag-presenting DCs resulted in the impaired production of Th1 and Th2 cytokines, so as to induce $CD4^+$ T cell anergy which fail to expand the secondary clone upon restimulation [79]. More interestingly, PPAR-γ was reported to be restricted to $CD1a^-$ cells in the process of cytokine-induced DC differentiation. PPAR-γ transcriptional activity was higher in $CD1a^-$ cells

but not in CD1a$^+$, indicating that the generation of CD1a$^-$ cells might be associated with PPAR-γ [80]. However, a large number of CD1a$^-$ cells were generated from peripheral blood monocytes of septic patients and the percentage of this type cells reached 68% after 7 d [61]. So it is not difficult to hypothesize whether the changes of DC in progression of sepsis were correlated to PPAR-γ. But there is no paper to clarify the connection between PPAR-γ and DCs in sepsis. Hepatic PPAR-γ mRNA expression and protein levels were reported to decrease at 20 h after CLP [81], but the results from another study showed that PPAR-γ expression of peritoneal cells was elevated significantly at both gene and protein levels 6 h after CLP [82]. Additionally, PPAR-γ expression in peripheral blood mononuclear cells from children patient with septic shock was also decreased but its activity was increased when compared to controls [83]. PPAR-γ activation could also promote T cell apoptosis in sepsis [84, 85]. Besides PPAR-γ, PPAR-α expression was reduced in patients with septic shock which was correlated to severity of illness [86]. Cell surface markers and cytokines production were decreased in PPAR-α knockout mice [86]. These data indicate the absence of PPAR-α is not beneficial for treating sepsis.

4.3. Wnt Signal Pathway-Mediated Mechanism. Wnt family is a highly conserved secreted signaling pathway that regulates developmental and homeostatic processes [87, 88]. Wnt proteins activate canonical or noncanonical signal pathway in a context-dependent manner [89, 90]. The former primarily takes part in cell fate determination and the latter is responsible primarily for cell movement and tissue polarity [91]. Wnt and their receptors are found to be expressed in hematopoietic progenitor cells (HPCs) [92], indicating that Wnt may be involved in HPCs differentiation. There was a remarkable expansion of hematopoietic cells after activation if Wnt canonical pathway. Wnt signaling pathway plays a central role in DCs differentiation in means of promotion on conventional DCs differentiation and inhibition on pDCs differentiation [93]. During the differentiation process of DCs from HPCs in vitro, Wnt signaling was upregulated characterized by accumulation of β-catenin and upregulation of Wnt target gene expression [94]. Activation of Wnt canonical pathway by Wnt 3a could promote the degeneration of CD11c$^+$ DCs and enhance their capacity to stimulate T cells proliferation [94]. However, the activation of noncanonical Wnt pathway by Wnt 5a was shown to inhibit DC differentiation [94]. Wnt 5a-treated DCs had worse ability of capturing antigen. Wnt 5a had no effect on LPS-induced DC maturation but impaired the production IL-12p70 and TNF-α while increasing levels of IL-10. Furthermore, Wnt 5a inhibited the T cell proliferation and fail to prime T cell response [95]. So the two types of signal pathway display an opposite effect and sustain the regulation of DCs differentiation by crosstalking to each other. During sepsis, Wnt 5a concentration in sera of patients was elevated and Wnt 5a was also found to induce macrophage differentiation to a tolerogenic phenotype, which was related to induction of IL-10 and suppression of NF-κB signaling [96, 97]. Therefore, Wnt signal pathway may be a factor that contributes to the dysfunction of DCs during sepsis.

4.4. Epigenetic Mechanisms. Epigenetic regulation refers to external modification on gene activity without any changes in DNA sequence. Epigenetic mechanisms have been involved in the maintenance of various genes expression during embryogenesis and caner [98, 99]. In eukaryotic cells, nucleosome is the basic unit of chromatin, consisting of a short length of DNA wrapped around eight histone protein cores (duplicated in H2A, H2B, H3, and H4) [100, 101]. More and more investigators have discovered that histone modifications, including acetylation, ubiquitylation, methylation, and phosphorylation, are important epigenetic mechanisms of gene expression [101]. It is reported that maintenance of Th1/Th2 memory and gene *Il17* expression are associated with ace acetylation and methylation of histone [102]. Histone methylation, especially for the methylation of histone H3 at lysine-4 (H3K4) and at lysine-27(H3K27), is known as a critical mechanism correlated with transcriptional activation and repression [103, 104]. Methylation at H3K4 mediated by MLL family histone methyltransferase (HMT) complex, in conjunction with several structural proteins including WD40-repeat proteins WDR5, RbBP5, and Ash2L, contributed to transcription activation [102, 105] Methylation at H3K27 is mediated by polycomb repressive complex 2 (PRC2) which contains several core components including EZH2, suppressor of Zeste 12 (SUZ12) and embryonic ectoderm development (EED) [104]. It is correlated with transcription silencing. The production of IL-12 as discussed above, an important cytokine directing Th1 immune response, was dramatically depressed in DCs from both septic patients and mice. To test if the aberrant change of IL-12 is correlated with epigenetic mechanism. Chromatin immunoprecipitation techniques were performed and data show that the reduction of IL-12 is mediated by decreasing the H3K4 trimethylation and increasing H3K27 dimethylation at *Il12p35* and *Il12p40* promoter, which result from the suppression in recruitment of MLL complex (WDR5 and RbBP5) and enhancement in recruitment of PRC2 complex (EED and SUZ12) on promoter, respectively [30]. These results indicate that epigenetic modification may be one potential mechanism of long-term immunoparalysis.

5. Potential Therapeutic Modulation of DC Aberrant Function

Given the central role of DCs in the immune response and survival in sepsis, it seems natural that DCs are the hopeful target for improving the aberrant immune response and prolonging the life during sepsis progression. To date many strategies for correcting the DC impaired function have been discovered, as shown in Table 1.

5.1. Increase the Number of DC. It has been mentioned that the loss of DCs is partly dependent on cell apoptosis, so the methods that can inhibit the apoptosis are thought to be beneficial for sepsis. IL-15 is a pluripotent cytokine that can

Table 1: Potential therapeutic approaches for reversing DC impaired function.

Treatment	Major functions	References
IL-15	It can block sepsis-induced apoptosis of immune cells, increase the abundance of Bcl-2 while decreasing Bim and PUMA, and then increase survival.	[109]
TAT-Bcl-xL TAT-BH4	The two peptides can inhibit sepsis-induced lymphocyte apoptosis and improve survival.	[110]
Fms-like tyrosine kinase-3 ligand (Flt3L)	It can increase the numbers of DCs in spleen and reverse immunoparalysis.	[111, 112]
Anti-C5a antibody	It can prevent $IL12^+$DC cells migration from the peritoneal cavity to peripheral blood and lymph nodes, thus improving survival.	[118]
TLR2-derived peptide	It can promote DC maturation and Th1 adaptive immune response.	[121]
Phospholipase A_2 (PLA_2)	It can promote DC maturation and increase the IFN-γ secretion.	[122]
Silencing of miR-142-3p	It can promote the expression of IL-6 and then reduce endotoxin-mediated mortality.	[123]

not only coordinate the innate and adaptive immune system but also inhibit apoptosis by inducing the antiapoptotic proteins Bcl-2 and Bcl-xl in immune cells [106–108]. After the CLP operation, mice were injected s.c. with IL-15 or vehicle. Results showed that IL-15 administration significantly inhibited the apoptosis of splenic CD4, CD8, NK, and DCs induced by sepsis. During this process, IL-15 treatment increased Bcl-2 protein expression in all cells. The level of circulating IFN-γ was increased after IL-15 treatment, whereas both TNF-α and IL-6 production was decreased. Within the observation of 7 days, CLP mice treated with IL-15 had more than three-time improvement in survival compared with CLP only mice [109]. These data demonstrate that IL-15 may be a novel therapy of sepsis. Based upon the antiapoptotic molecules, TAT-Bcl-xL fusion protein and TAT-BH4 peptide were obtained and they have the ability to prevent sepsis-induced lymphocyte apoptosis, and high level of Bcl-xL improved the survival in sepsis [110]. Besides apoptosis, Fms-like tyrosine kinase-3 ligand (Flt3L) treatment was found to increase the number of $CD11c^+$ DC populations by accelerating its expansion, so as to be able to reverse the endotoxin-induced tolerance [111, 112].

5.2. Change the DC Distribution. C5a is a potent chemoattractant among the complement products and possesses a number of functions including the modulation of cytokine and adhesion molecules expression, causing oxidant burst and granule enzymes [113–115]. C5a was reported to be excessively activated and its high expression was harmful for host during sepsis [116, 117]. After treatment with anti-C5a antibody, the $IL\text{-}12^+$ DCs in peripheral blood and lymphoid nodes were decreased but were increased in peritoneal cavity in which $IL\text{-}12^+$ DCs play a protection role in sepsis. Furthermore, anti-C5a antibody-treated mice had a higher survival rate than that in sham mice [118].

5.3. Promote DC Maturation and Increase Proinflammatory Cytokines Release. This function is the most potent in improving the immunoparalysis status in sepsis. It is known that TLR family play a critical role in the clearance of pathogen by promoting proinflammatory response. However, the activation of TLR during this process requires the interaction with coreceptor CD14 which can amplify the inflammatory signal primed by bacterial pathogen [119, 120]. So CD14 is thought to be a potential target for skewing Th1 response in sepsis. TLR2-derived peptide enhances the DC maturation by upregulation of MHCII, CD80, and CD86 expression. The peptide also increased the release of IL-12 and IFN-γ which are key factors for activating Th1 cell. At the same time, TGF-β release was inhibited. It was indicated that the TLR2-derived peptide promoted a T1 adaptive immune response and improved the status of immunosuppression [121]. In addition, the introduction of phospholipase A_2 (PLA_2) enhanced expression of HLA-DR, CD86, CD80, CD83, and CD40 on DCs. PLA_2 also improved the ability of DCs to secrete IFN-γ when cocultured with allogeneic T cells [122]. Moreover, microRNA is also a potential target of immune modulation. Silencing of miR-142-3p which targets the IL-6 $3'$untranslated region significantly promoted the IL-6 expression and reduced endotoxin-induced mortality [123].

6. Conclusion

DCs are crucial in pathogen recognition and induction of specific immune response to protect host from the invading infection. When sepsis develops, DCs from lymphoid and nonlymphoid tissues are lost, which mostly result from the apoptosis. Several surface molecules associated with DCs maturation are changed, in which the most obvious one is HLA-DR. Upon the stimulation of external antigen or danger signal, IL-12 expression is suppressed while IL-10 production is increased, which results in the polarization of Th cell toward Th2 or Treg. During sepsis Wnt or PPAR or epigenetic-mediated mechanism may be involved (Figure 1). Several therapies that focus on improving DCs function have been shown to be able to mitigate the disease symptom. It is known that septic patients need to undergo two stages: a hyperinflammatory state and the secondary occurrence of immunosuppression. However there is no clinical parameter able to point out what the undergoing mechanism is. Therefore, specific biomarkers responsible for reflecting the immune status need to be discovered in future. Furthermore,

it is imperative to find out the ideal therapeutic target that only directs to one phase without affecting the other one.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by a Grant from National "973" Project (no. 2012CB518102).

References

[1] M. M. Levy, A. Artigas, G. S. Phillips et al., "Outcomes of the Surviving Sepsis Campaign in intensive care units in the USA and Europe: a prospective cohort study," *The Lancet Infectious Diseases*, vol. 12, no. 12, pp. 919–924, 2012.

[2] F. Venet, A. C. Lukaszewicz, D. Payen, R. Hotchkiss, and G. Monneret, "Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies," *Current Opinion in Immunology*, vol. 25, no. 4, pp. 477–483, 2013.

[3] G. Drifte, I. Dunn-Siegrist, P. Tissières, and J. Pugin, "Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome," *Critical Care Medicine*, vol. 41, no. 3, pp. 820–832, 2013.

[4] Y. Y. Luan, N. Dong, M. Xie, X. Z. Xiao, and Y. M. Yao, "The significance and regulatory mechanisms of innate immune cells in the development of sepsis," *Journal of Interferon and Cytokine Research*, vol. 34, no. 1, pp. 2–15, 2014.

[5] A. R. Novotny, D. Reim, V. Assfalg et al., "Mixed antagonist response and sepsis severity-dependent dysbalance of pro- and anti-inflammatory responses at the onset of postoperative sepsis," *Immunobiology*, vol. 217, no. 6, pp. 616–621, 2012.

[6] A. Gautam, S. Dixit, M. Embers et al., "Different patterns of expression and of IL-10 modulation of inflammatory mediators from macrophages of Lyme disease-resistant and -susceptible mice," *PLoS ONE*, vol. 7, no. 9, Article ID e43860, 2012.

[7] J.-L. Vincent, Q. Sun, and M.-J. Dubois, "Clinical trials of immunomodulatory therapies in severe sepsis and septic shock," *Clinical Infectious Diseases*, vol. 34, no. 8, pp. 1084–1093, 2002.

[8] X. Huang, F. Venet, C.-S. Chung, J. Lomas-Neira, and A. Ayala, "Changes in dendritic cell function in the immune response to sepsis. Cell- & tissue-based therapy," *Expert Opinion on Biological Therapy*, vol. 7, no. 7, pp. 929–938, 2007.

[9] C. Torgersen, P. Moser, G. Luckner et al., "Macroscopic postmortem findings in 235 surgical intensive care patients with sepsis," *Anesthesia and Analgesia*, vol. 108, no. 6, pp. 1841–1847, 2009.

[10] C. Landelle, A. Lepape, A. Français et al., "Nosocomial infection after septic shock among intensive care unit patients," *Infection Control and Hospital Epidemiology*, vol. 29, no. 11, pp. 1054–1065, 2008.

[11] A. P. Limaye, K. A. Kirby, G. D. Rubenfeld et al., "Cytomegalovirus reactivation in critically ill immunocompetent patients," *The Journal of the American Medical Association*, vol. 300, no. 4, pp. 413–422, 2008.

[12] R. M. Steinman and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution," *The Journal of Experimental Medicine*, vol. 173, no. 5, pp. 1142–1162, 1973.

[13] R. M. Steinman, G. Kaplan, M. D. Witmer, and Z. A. Cohn, "Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro," *Journal of Experimental Medicine*, vol. 149, no. 1, pp. 1–16, 1979.

[14] J. Banchereau and R. M. Steinman, "Dendritic cells and the control of immunity," *Nature*, vol. 392, no. 6673, pp. 245–252, 1998.

[15] P. Efron and L. L. Moldawer, "Sepsis and the dendritic cell," *Shock*, vol. 20, no. 5, pp. 386–401, 2003.

[16] M. Rescigno, F. Granucci, and P. Ricciardi-Castagnoli, "Dendritic cells at the end of the millennium," *Immunology & Cell Biology*, vol. 77, no. 5, pp. 404–410, 1999.

[17] M. F. Lipscomb and B. J. Masten, "Dendritic cells: Immune regulators in health and disease," *Physiological Reviews*, vol. 82, no. 1, pp. 97–130, 2002.

[18] F. Ishikawa, H. Niiro, T. Iino et al., "The developmental program of human dendritic cells is operated independently of conventional myeloid and lymphoid pathways," *Blood*, vol. 110, no. 10, pp. 3591–3600, 2007.

[19] L. Wu and Y.-J. Liu, "Development of dendritic-cell lineages," *Immunity*, vol. 26, no. 6, pp. 741–750, 2007.

[20] M. G. Manz, D. Traver, T. Miyamoto, I. L. Weissman, and K. Akashi, "Dendritic cell potentials of early lymphoid and myeloid progenitors," *Blood*, vol. 97, no. 11, pp. 3333–3341, 2001.

[21] K. Akashi, D. Traver, T. Miyamoto, and I. L. Weissman, "A clonogenic common myeloid progenitor that gives rise to all myeloid lineages," *Nature*, vol. 404, no. 6774, pp. 193–197, 2000.

[22] J. Banchereau, F. Briere, C. Caux et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.

[23] P. O. Scumpia, P. F. McAuliffe, K. A. O'Malley et al., "$CD11c^+$ dendritic cells are required for survival in murine polymicrobial sepsis," *Journal of Immunology*, vol. 175, no. 5, pp. 3282–3286, 2005.

[24] A. Ayala, C. D. Herdon, D. L. Lehman, C. A. Ayala, and I. H. Chaudry, "Differential induction of apoptosis in lymphoid tissues during sepsis: variation in onset, frequency, and the nature of the mediators," *Blood*, vol. 87, no. 10, pp. 4261–4275, 1996.

[25] A. Ayala, C.-S. Chung, Y. X. Xu, T. A. Evans, K. M. Redmond, and I. H. Chaudry, "Increased inducible apoptosis in $CD4^+$ T lymphocytes during polymicrobial sepsis is mediated by Fas ligand and not endotoxin," *Immunology*, vol. 97, no. 1, pp. 45–55, 1999.

[26] C.-S. Chung, W. Wang, I. H. Chaudry, and A. Ayala, "Increased apoptosis in lamina propria B cells during polymicrobial sepsis is FasL but not endotoxin mediated," *American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 280, no. 5, pp. G812–G818, 2001.

[27] R. S. Hotchkiss, K. C. Chang, P. E. Swanson et al., "Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte," *Nature Immunology*, vol. 1, no. 6, pp. 496–501, 2000.

[28] Y. Ding, C.-S. Chung, S. Newton et al., "Polymicrobial sepsis induces divergent effects on splenic and peritoneal dendritic cell function in mice," *Shock*, vol. 22, no. 2, pp. 137–144, 2004.

[29] K. W. Tinsley, M. H. Grayson, P. E. Swanson et al., "Sepsis induces apoptosis and profound depletion of splenic interdigitating and follicular dendritic cells," *The Journal of Immunology*, vol. 171, no. 2, pp. 909–914, 2003.

[30] H. Wen, Y. Dou, C. M. Hogaboam, and S. L. Kunkel, "Epigenetic regulation of dendritic cell-derived interleukin-12 facilitates immunosuppression after a severe innate immune response," *Blood*, vol. 111, no. 4, pp. 1797–1804, 2008.

[31] F. Pène, E. Courtine, F. Ouaaz et al., "Toll-like receptors 2 and 4 contribute to sepsis-induced depletion of spleen dendritic cells," *Infection and Immunity*, vol. 77, no. 12, pp. 5651–5658, 2009.

[32] E. Courtine, F. Pène, N. Cagnard et al., "Critical role of cRel subunit of NF-κB in sepsis survival," *Infection and Immunity*, vol. 79, no. 5, pp. 1848–1854, 2011.

[33] S. B. Flohé, H. Agrawal, D. Schmitz, M. Gertz, and F. U. Schade, "Dendritic cells during polymicrobial sepsis rapidly mature but fail to initiate a protective Th1-type immune response," *Journal of Leukocyte Biology*, vol. 79, no. 3, pp. 473–481, 2006.

[34] P. A. Efron, A. Martins, D. Minnich et al., "Characterization of the systemic loss of dendritic cells in murine lymph nodes during polymicrobial sepsis," *Journal of Immunology*, vol. 173, no. 5, pp. 3035–3043, 2004.

[35] H. Wen, C. M. Hogaboam, J. Gauldie, and S. L. Kunkel, "Severe sepsis exacerbates cell-mediated immunity in the lung due to an altered dendritic cell cytokine profile," *The American Journal of Pathology*, vol. 168, no. 6, pp. 1940–1950, 2006.

[36] D. Grimaldi, S. Louis, F. Pène et al., "Profound and persistent decrease of circulating dendritic cells is associated with ICU-acquired infection in patients with septic shock," *Intensive Care Medicine*, vol. 37, no. 9, pp. 1438–1446, 2011.

[37] O. Guisset, M.-S. Dilhuydy, R. Thiébaut et al., "Decrease in circulating dendritic cells predicts fatal outcome in septic shock," *Intensive Care Medicine*, vol. 33, no. 1, pp. 148–152, 2007.

[38] H. Poehlmann, J. C. Schefold, H. Zuckermann-Becker, H.-D. Volk, and C. Meisel, "Phenotype changes and impaired function of dendritic cell subsets in patients with sepsis: a prospective observational analysis," *Critical Care*, vol. 13, no. 4, article R119, 2009.

[39] F. Riccardi, G. D. P. Matteo, B. Rovati et al., "Flow cytometric analysis of peripheral blood dendritic cells in patients with severe sepsis," *Cytometry B: Clinical Cytometry*, vol. 80, no. 1, pp. 14–21, 2011.

[40] K. Takahashi, S. Satoi, H. Yanagimoto et al., "Circulating dendritic cells and development of septic complications after pancreatectomy for pancreatic cancer," *Archives of Surgery*, vol. 142, no. 12, pp. 1151–1157, 2007.

[41] K. I. Elsayh, A. M. Zahran, I. L. Mohamad, and S. S. Aly, "Dendritic cells in childhood sepsis," *Journal of Critical Care*, vol. 28, no. 5, pp. 881.e7–881.e13, 2013.

[42] E. Pastille, S. Didovic, D. Brauckmann et al., "Modulation of dendritic cell differentiation in the bone marrow mediates sustained immunosuppression after polymicrobial sepsis," *The Journal of Immunology*, vol. 186, no. 2, pp. 977–986, 2011.

[43] V. Faivre, A.-C. Lukaszewicz, A. Alves, D. Charron, D. Payen, and A. Haziot, "Accelerated in vitro differentiation of blood monocytes into dendritic cells in human sepsis," *Clinical and Experimental Immunology*, vol. 147, no. 3, pp. 426–439, 2007.

[44] L. Brudecki, D. A. Ferguson, C. E. McCall, and M. E. Gazzar, "Myeloid-derived suppressor cells evolve during sepsis and can enhance or attenuate the systemic inflammatory response," *Infection and Immunity*, vol. 80, no. 6, pp. 2026–2034, 2012.

[45] C. F. Benjamim, S. K. Lundy, N. W. Lukacs, C. M. Hogaboam, and S. L. Kunkel, "Reversal of long-term sepsis-induced immunosuppression by dendritic cells," *Blood*, vol. 105, no. 9, pp. 3588–3595, 2005.

[46] E. R. Sherwood and R. S. Hotchkiss, "BTLA as a biomarker and mediator of sepsis-induced immunosuppression," *Critical Care*, vol. 17, no. 6, article 1022, 2013.

[47] N. J. Shubin, C. S. Chung, D. S. Heffernan, L. R. Irwin, S. F. Monaghan, and A. Ayala, "BTLA expression contributes to septic morbidity and mortality by inducing innate inflammatory cell dysfunction," *Journal of Leukocyte Biology*, vol. 92, no. 3, pp. 593–603, 2012.

[48] K. Wolk, W.-D. Döcke, V. Von Baehr, H.-D. Volk, and R. Sabat, "Impaired antigen presentation by human monocytes during endotoxin tolerance," *Blood*, vol. 96, no. 1, pp. 218–223, 2000.

[49] Y. L. Tulzo, C. Pangault, L. Amiot et al., "Monocyte human leukocyte antigen-DR transcriptional downregulation by cortisol during septic shock," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 10, pp. 1144–1151, 2004.

[50] K. Tschaikowsky, M. Hedwig-Geissing, A. Schiele, F. Bremer, M. Schywalsky, and J. Schüttler, "Coincidence of pro- and anti-inflammatory responses in the early phase of severe sepsis: longitudinal study of mononuclear histocompatibility leukocyte antigen-DR expression, procalcitonin, C-reactive protein, and changes in T-cell subsets in septic and postoperative patients," *Critical Care Medicine*, vol. 30, no. 5, pp. 1015–1023, 2002.

[51] G. Monneret, A.-L. Debard, F. Venet et al., "Marked elevation of human circulating $CD4^+CD25^+$ regulatory T cells in sepsis-induced immunoparalysis," *Critical Care Medicine*, vol. 31, no. 7, pp. 2068–2071, 2003.

[52] G. Trinchieri, "Interleukin-12 and the regulation of innate resistance and adaptive immunity," *Nature Reviews Immunology*, vol. 3, no. 2, pp. 133–146, 2003.

[53] M. Wysocka, S. Robertson, H. Riemann et al., "IL-12 suppression during experimental endotoxin tolerance: dendritic cell loss and macrophage hyporesponsiveness," *The Journal of Immunology*, vol. 166, no. 12, pp. 7504–7513, 2001.

[54] A. Oberholzer, C. Oberholzer, and L. L. Moldawer, "Interleukin-10: a complex role in the pathogenesis of sepsis syndromes and its potential as an anti-inflammatory drug," *Critical Care Medicine*, vol. 30, no. 1, pp. S58–S63, 2002.

[55] A. Oberholzer, C. Oberholzer, P. A. Efron et al., "Functional modification of dendritic cells with recombinant adenovirus encoding interleukin 10 for the treatment of sepsis," *Shock*, vol. 23, no. 6, pp. 507–515, 2005.

[56] A. Oberholzer, C. Oberholzer, K. S. Bahjat et al., "Increased survival in sepsis by in vivo adenovirus-induced expression of IL-10 in dendritic cells," *The Journal of Immunology*, vol. 168, no. 7, pp. 3412–3418, 2002.

[57] T.-Y. Chuang, H.-T. Chang, K.-P. Chung et al., "High levels of serum macrophage migration inhibitory factor and interleukin 10 are associated with a rapidly fatal outcome in patients with severe sepsis," *International Journal of Infectious Diseases*, vol. 20, no. 1, pp. 13–17, 2014.

[58] K. W. Moore, R. de Waal Malefyt, R. L. Coffman, and A. O'Garra, "Interleukin-10 and the interleukin-10 receptor," *Annual Review of Immunology*, vol. 19, pp. 683–765, 2001.

[59] L. Zhou, A. A. Nazarian, and S. T. Smale, "Interleukin-10 inhibits interleukin-12 p40 gene transcription by targeting a late event in the activation pathway," *Molecular and Cellular Biology*, vol. 24, no. 6, pp. 2385–2396, 2004.

[60] A. Mohr, J. Polz, E. M. Martin et al., "Sepsis leads to a reduced antigen-specific primary antibody response," *European Journal of Immunology*, vol. 42, no. 2, pp. 341–352, 2012.

[61] V. Faivre, A. C. Lukaszewicz, A. Alves, D. Charron, D. Payen, and A. Haziot, "Human monocytes differentiate into dendritic cells subsets that induce anergic and regulatory T cells in sepsis," *PLoS ONE*, vol. 7, no. 10, Article ID e47209, 2012.

[62] C.-S. Chung, G. Y. Song, J. Lomas, H. H. Simms, I. H. Chaudry, and A. Ayala, "Inhibition of Fas/Fas ligand signaling improves septic survival: differential effects on macrophage apoptotic and functional capacity," *Journal of Leukocyte Biology*, vol. 74, no. 3, pp. 344–351, 2003.

[63] C.-S. Chung, S. Yang, G. Y. Song et al., "Inhibition of Fas signaling prevents hepatic injury and improves organ blood flow during sepsis," *Surgery*, vol. 130, no. 2, pp. 339–345, 2001.

[64] D. E. Wesche-Soldato, J. L. Lomas-Neira, M. Perl, L. Jones, C.-S. Chung, and A. Ayala, "The role and regulation of apoptosis in sepsis," *Journal of Endotoxin Research*, vol. 11, no. 6, pp. 375–382, 2005.

[65] R. S. Hotchkiss, K. W. Tinsley, P. E. Swanson et al., "Sepsis-induced apoptosis causes progressive profound depletion of B and $CD4^+$ T lymphocytes in humans," *The Journal of Immunology*, vol. 166, no. 11, pp. 6952–6963, 2001.

[66] T. J. Moraes and G. P. Downey, "Death of the septic monocyte: is more better?" *Critical Care*, vol. 10, no. 3, article 146, 2006.

[67] O. M. Peck-Palmer, J. Unsinger, K. C. Chang et al., "Modulation of the Bcl-2 family blocks sepsis-induced depletion of dendritic cells and macrophages," *Shock*, vol. 31, no. 4, pp. 359–366, 2009.

[68] R. Kushwah, J. Wu, J. R. Oliver et al., "Uptake of apoptotic DC converts immature DC into tolerogenic DC that induce differentiation of $Foxp3^+$ Treg," *European Journal of Immunology*, vol. 40, no. 4, pp. 1022–1035, 2010.

[69] S. Falcone, C. Perrotta, C. De Palma et al., "Activation of acid sphingomyelinase and its inhibition by the nitric oxide/cyclic guanosine 3′,5′-monophosphate pathway: key events in Escherichia coli-elicited apoptosis of dendritic cells," *Journal of Immunology*, vol. 173, no. 7, pp. 4452–4463, 2004.

[70] J. Zhu, M. Liu, R. H. Kennedy, and S. J. Liu, "TNF-α-induced impairment of mitochondrial integrity and apoptosis mediated by caspase-8 in adult ventricular myocytes," *Cytokine*, vol. 34, no. 1-2, pp. 96–105, 2006.

[71] L. Zhang, J. S. Cardinal, P. Pan et al., "Splenocyte apoptosis and autophagy is mediated by interferon regulatory factor 1 during murine endotoxemia," *Shock*, vol. 37, no. 5, pp. 511–517, 2012.

[72] I. Zanoni, R. Ostuni, G. Capuano et al., "CD14 regulates the dendritic cell life cycle after LPS exposure through NFAT activation," *Nature*, vol. 460, no. 7252, pp. 264–268, 2009.

[73] D. J. Mangelsdorf, C. Thummel, M. Beato et al., "The nuclear receptor super-family: the second decade," *Cell*, vol. 83, no. 6, pp. 835–839, 1995.

[74] C.-H. Lee, P. Olson, and R. M. Evans, "Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors," *Endocrinology*, vol. 144, no. 6, pp. 2201–2207, 2003.

[75] S. Kersten, B. Desvergne, and W. Wahli, "Roles of PPARS in health and disease," *Nature*, vol. 405, no. 6785, pp. 421–424, 2000.

[76] M. A. Jakobsen, R. K. Petersen, K. Kristiansen, M. Lange, and S. T. Lillevang, "Peroxisome proliferator-activated receptor α, δ, γ1 and γ2 expressions are present in human monocyte-derived dendritic cells and modulate dendritic cell maturation by addition of subtype-specific ligands," *Scandinavian Journal of Immunology*, vol. 63, no. 5, pp. 330–337, 2006.

[77] P. Gosset, A. S. Charbonnier, P. Delerive et al., "Peroxisome proliferator-activated receptor gamma activators affect the maturation of human monocyte-derived dendritic cells," *European Journal of Immunology*, vol. 31, no. 10, pp. 2857–2865, 2001.

[78] V. Angeli, H. Hammad, B. Staels, M. Capron, B. N. Lambrecht, and F. Trottein, "Peroxisome proliferator-activated receptor γ inhibits the migration of dendritic cells: consequences for the immune response," *The Journal of Immunology*, vol. 170, no. 10, pp. 5295–5301, 2003.

[79] L. Klotz, I. Dani, F. Edenhofer et al., "Peroxisome proliferator-activated receptor γ control of dendritic cell function contributes to development of $CD4^+$ T cell anergy," *The Journal of Immunology*, vol. 178, no. 4, pp. 2122–2131, 2007.

[80] P. Gogolak, B. Rethi, I. Szatmari et al., "Differentiation of $CD1a^-$ and $CD1a^+$ monocyte-derived dendritic cells is biased by lipid environment and PPARγ," *Blood*, vol. 109, no. 2, pp. 643–652, 2007.

[81] M. Zhou, R. Wu, W. Dong, A. Jacob, and P. Wang, "Endotoxin downregulates peroxisome proliferator-activated receptor-γ via the increase in TNF-α release," *American Journal of Physiology: Regulatory Integrative and Comparative Physiology*, vol. 294, no. 1, pp. R84–R92, 2008.

[82] A. E. Ferreira, F. Sisti, F. Sônego et al., "PPAR-γ/IL-10 axis inhibits MyD88 expression and ameliorates murine polymicrobial sepsis," *The Journal of Immunology*, vol. 192, no. 5, pp. 2357–2365, 2014.

[83] J. M. Kaplan, A. Denenberg, M. Monaco, M. Nowell, H. Wong, and B. Zingarelli, "Changes in peroxisome proliferator-activated receptor-gamma activity in children with septic shock," *Intensive Care Medicine*, vol. 36, no. 1, pp. 123–130, 2010.

[84] M. Soller, A. Tautenhahn, B. Brüne et al., "Peroxisome proliferator-activated receptor γ contributes to T lymphocyte apoptosis during sepsis," *Journal of Leukocyte Biology*, vol. 79, no. 1, pp. 235–243, 2006.

[85] M. V. Schmidt, P. Paulus, A. M. Kuhn et al., "Peroxisome proliferator-activated receptor gamma-induced T cell apoptosis reduces survival during polymicrobial sepsis," *The American Journal of Respiratory and Critical Care Medicine*, vol. 184, no. 1, pp. 64–74, 2011.

[86] S. W. Standage, C. C. Caldwell, B. Zingarelli, and H. R. Wong, "Reduced peroxisome proliferator-activated receptor α expression is associated with decreased survival and increased tissue bacterial load in sepsis," *Shock*, vol. 37, no. 2, pp. 164–169, 2012.

[87] A. Klaus and W. Birchmeier, "Wnt signalling and its impact on development and cancer," *Nature Reviews Cancer*, vol. 8, no. 5, pp. 387–398, 2008.

[88] M. Katoh, "WNT signaling pathway and stem cell signaling network," *Clinical Cancer Research*, vol. 13, no. 14, pp. 4042–4045, 2007.

[89] D. Coudreuse and H. C. Korswagen, "The making of Wnt: new insights into Wnt maturation, sorting and secretion," *Development*, vol. 134, no. 1, pp. 3–12, 2007.

[90] M. D. Gordon and R. Nusse, "Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors," *The Journal of Biological Chemistry*, vol. 281, no. 32, pp. 22429–22433, 2006.

[91] P. Cheng, J. Zhou, and D. Gabrilovich, "Regulation of dendritic cell differentiation and function by Notch and Wnt pathways," *Immunological Reviews*, vol. 234, no. 1, pp. 105–119, 2010.

[92] A. Blumenthal, S. Ehlers, J. Lauber et al., "The Wingless homolog WNT5A and its receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation," *Blood*, vol. 108, no. 3, pp. 965–973, 2006.

[93] S. Malhotra, Y. Baba, K. P. Garrett, F. J. T. Staal, R. Gerstein, and P. W. Kincade, "Contrasting responses of lymphoid progenitors to canonical and noncanonical Wnt signals," *The Journal of Immunology*, vol. 181, no. 6, pp. 3955–3964, 2008.

[94] J. Zhou, P. Cheng, J.-I. Youn, M. J. Cotter, and D. I. Gabrilovich, "Notch and wingless signaling cooperate in regulation of dendritic cell differentiation," *Immunity*, vol. 30, no. 6, pp. 845–859, 2009.

[95] J. Valencia, C. Hernández-López, V. G. Martínez et al., "Wnt5a skews dendritic cell differentiation to an unconventional phenotype with tolerogenic features," *The Journal of Immunology*, vol. 187, no. 8, pp. 4129–4139, 2011.

[96] C. Pereira, D. J. Schaer, E. B. Bachli, M. O. Kurrer, and G. Schoedon, "Wnt5A/CaMKII signaling contributes to the inflammatory response of macrophages and is a target for the antiinflammatory action of activated protein C and interleukin-10," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 3, pp. 504–510, 2008.

[97] C. Bergenfelz, C. Medrek, E. Ekström et al., "Wnt5a induces a tolerogenic phenotype of macrophages in sepsis and breast cancer patients," *Journal of Immunology*, vol. 188, no. 11, pp. 5448–5458, 2012.

[98] R. Margueron, P. Trojer, and D. Reinberg, "The key to development: interpreting the histone code?" *Current Opinion in Genetics and Development*, vol. 15, no. 2, pp. 163–176, 2005.

[99] W. Reik, "Stability and flexibility of epigenetic gene regulation in mammalian development," *Nature*, vol. 447, no. 7143, pp. 425–432, 2007.

[100] R. D. Kornberg and Y. Lorch, "Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome," *Cell*, vol. 98, no. 3, pp. 285–294, 1999.

[101] H. Wen, M. A. Schaller, Y. Dou, C. M. Hogaboam, and S. L. Kunkel, "Dendritic cells at the interface of innate and acquired immunity: the role for epigenetic changes," *Journal of Leukocyte Biology*, vol. 83, no. 3, pp. 439–446, 2008.

[102] K. M. Ansel, I. Djuretic, B. Tanasa, and A. Rao, "Regulation of Th2 differentiation and Il4 locus accessibility," *Annual Review of Immunology*, vol. 24, pp. 607–656, 2006.

[103] Y. Dou, T. A. Milne, A. J. Tackett et al., "Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF," *Cell*, vol. 121, no. 6, pp. 873–885, 2005.

[104] R. Cao and Y. Zhang, "The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3," *Current Opinion in Genetics and Development*, vol. 14, no. 2, pp. 155–164, 2004.

[105] J. Wysocka, T. Swigut, T. A. Milne et al., "WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development," *Cell*, vol. 121, no. 6, pp. 859–872, 2005.

[106] M. K. Kennedy, M. Glaccum, S. N. Brown et al., "Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice," *Journal of Experimental Medicine*, vol. 191, no. 5, pp. 771–780, 2000.

[107] A. Motegi, M. Kinoshita, A. Inatsu, Y. Habu, D. Saitoh, and S. Seki, "IL-15-induced CD8+CD122+ T cells increase antibacterial and anti-tumor immune responses: implications for immune function in aged mice," *Journal of Leukocyte Biology*, vol. 84, no. 4, pp. 1047–1056, 2008.

[108] S. Bulfone-Paus, D. Ungureanu, T. Pohl et al., "Interleukin-15 protects from lethal apoptosis in vivo," *Nature Medicine*, vol. 3, no. 10, pp. 1124–1128, 1997.

[109] S. Inoue, J. Unsinger, C. G. Davis et al., "IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis," *Journal of Immunology*, vol. 184, no. 3, pp. 1401–1409, 2010.

[110] R. S. Hotchkiss, K. W. McConnell, K. Bullok et al., "TAT-BH4 and TAT-Bcl-xL peptides protect against sepsis-induced lymphocyte apoptosis in vivo," *Journal of Immunology*, vol. 176, no. 9, pp. 5471–5477, 2006.

[111] T. E. Toliver-Kinsky, W. Cui, E. D. Murphey, C. Lin, and E. R. Sherwood, "Enhancement of dendritic cell production by Fms-like tyrosine kinase-3 ligand increases the resistance of mice to a burn wound infection," *Journal of Immunology*, vol. 174, no. 1, pp. 404–410, 2005.

[112] M. Wysocka, L. J. Montaner, and C. L. Karp, "Flt3 ligand treatment reverses endotoxin tolerance-related immunoparalysis," *The Journal of Immunology*, vol. 174, no. 11, pp. 7398–7402, 2005.

[113] N. C. Riedemann, R.-F. Guo, T. J. Hollmann et al., "Regulatory role of C5a in LPS-induced IL-6 production by neutrophils during sepsis," *The FASEB Journal*, vol. 18, no. 2, pp. 370–372, 2004.

[114] T. E. Mollnes, O. L. Brekke, M. Fung et al., "Essential role of the C5a receptor in E coli-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation," *Blood*, vol. 100, no. 5, pp. 1869–1877, 2002.

[115] I. M. Goldstein and G. Weissmann, "Generation of C5 derived lysosomal enzyme releasing activity (C5a) by lysates of leukocyte lysosomes," *Journal of Immunology*, vol. 113, no. 5, pp. 1583–1588, 1974.

[116] M. S. Huber-Lang, E. M. Younkin, J. V. Sarma et al., "Complement-induced impairment of innate immunity during sepsis," *The Journal of Immunology*, vol. 169, no. 6, pp. 3223–3231, 2002.

[117] N. C. Riedemann, R.-F. Guo, I. J. Laudes et al., "C5a receptor and thymocyte apoptosis in sepsis," *The FASEB Journal*, vol. 16, no. 8, pp. 887–888, 2002.

[118] N. Ma, C. Xing, H. Xiao et al., "C5a Regulates IL-12$^+$DC Migration to Induce Pathogenic Th1 and Th17 Cells in Sepsis," *PLoS ONE*, vol. 8, no. 7, Article ID e69779, 2013.

[119] C. L. Baumann, I. M. Aspalter, O. Sharif et al., "CD14 is a coreceptor of toll-like receptors 7 and 9," *The Journal of Experimental Medicine*, vol. 207, no. 12, pp. 2689–2701, 2010.

[120] H. K. Lee, S. Dunzendorfer, K. Soldau, and P. S. Tobias, "Double-stranded RNA-mediated TLR3 activation is enhanced by CD14," *Immunity*, vol. 24, no. 2, pp. 153–163, 2006.

[121] A.-C. Raby, B. Holst, E. Le Bouder et al., "Targeting the TLR co-receptor CD14 with TLR2-derived peptides modulates immune responses to pathogens," *Science Translational Medicine*, vol. 5, no. 185, Article ID 185ra64, 2013.

[122] L. Perrin-Cocon, S. Agaugué, F. Coutant et al., "Secretory phospholipase A_2 induces dendritic cell maturation," *European Journal of Immunology*, vol. 34, no. 8, pp. 2293–2302, 2004.

[123] Y. Sun, S. Varambally, C. A. Maher et al., "Targeting of microRNA-142-3p in dendritic cells regulates endotoxin-induced mortality," *Blood*, vol. 117, no. 23, pp. 6172–6183, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 265165, 10 pages
http://dx.doi.org/10.1155/2015/265165

Clinical Study

Incidence and Risk Factors of Postoperative Pulmonary Complications in Noncardiac Chinese Patients: A Multicenter Observational Study in University Hospitals

Yue Jin,[1] Guohao Xie,[1] Haihong Wang,[2] Lielie Jin,[3] Jun Li,[4] Baoli Cheng,[1] Kai Zhang,[1] Andreas Hoeft,[5] and Xiangming Fang[1]

[1]*Department of Anesthesiology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China*
[2]*Intensive Care Unit, The Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China*
[3]*Department of Anesthesiology, The First Affiliated Hospital, Wenzhou Medical College, Wenzhou 325000, China*
[4]*Department of Anesthesiology, The Second Affiliated Hospital, Wenzhou Medical College, Wenzhou 325027, China*
[5]*Department of Anaesthesiology and Intensive Care Medicine, University of Bonn, 53127 Bonn, Germany*

Correspondence should be addressed to Xiangming Fang; xiangming_fang@163.com

Received 9 January 2014; Revised 1 July 2014; Accepted 6 August 2014

Academic Editor: Stephen M. Pastores

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Purpose. To assess the incidence of postoperative pulmonary complications (PPCs) in Chinese inpatients, and to develop a brief predictive risk index. *Methods*. Between August 6, 2012, and August 12, 2012, patients undergoing noncardiac operations in four university hospitals were enrolled. The cohort was divided into two subsamples, cohort 1 to develop a predictive risk index of PPCs and cohort 2 to validate it. *Results*. 1673 patients were enrolled. PPCs were recorded for 163 patients (9.7%), of whom the hospital length of stay (LOS) was longer ($P < 0.001$). The mortality was 1.84% in patients with PPCs and 0.07% in those without. Logistic Regression modeling in cohort 1 identified nine independent risk factors, including smoking, respiratory infection in the last month, preoperative antibiotic use, preoperative saturation of peripheral oxygen, surgery site, blood lost, postoperative blood glucose, albumin, and ventilation. The model was validated within cohort 2 with an area under the receiver operating characteristic curve of 0.90 (95% CI 0.86 to 0.94). *Conclusions*. PPCs are common in noncardiac surgical patients and are associated with prolonged LOS in China. The current study developed a risk index, which can be used to assess individual risk of PPCs and guide individualized perioperative respiratory care.

1. Introduction

More than 230 million major surgical procedures are undertaken each year worldwide [1] and postoperative complications imposed a significant clinical and economic burden to surgical patients as well as the public health systems [2, 3]. Postoperative pulmonary complications (PPCs) are common postoperative complications that occur in 2% to 40% of patients and are associated with increased morbidity, mortality, and length of stay (LOS) [3–9]. In noncardiac patients, PPCs occur more frequently than cardiac complications [10]. Though it came to wide attention in recent years, the literature investigating the incidence and outcome of PPCs in Chinese inpatients remains scarce.

It is known that PPCs have a multifactorial etiology and had been defined broadly, including respiratory tract infection, pneumonia, respiratory failure, atelectasis, pleural effusion, pneumothorax, bronchospasm, and aspiration pneumonitis [11]. Previous studies demonstrated that PPCs were associated with a series of perioperative risk factors, such as age, smoking, chronic obstructive pulmonary disease (COPD), type of surgery, and serum albumin [4, 6, 7, 11–14]. A majority of these risk factors can be intervened and improved [15–17]. Therefore, identifying perioperative risk factors of PPCs is an important step toward improving quality of care in surgical patients, which has been already explored in several studies [11, 12, 18].

Table 1: Definitions of postoperative pulmonary complications.

Respiratory infection
Treatment with antibiotics for a respiratory infection, plus at least one of the following criteria: new or changed sputum, new or changed lung opacities, fever, and leukocyte count >12,000/mm^3
Respiratory failure
Postoperative PaO_2 <60 mmHg on room air, a ratio of PaO_2 to inspired oxygen fraction <300, or SaO_2 <90% and requiring oxygen therapy
Pleural effusion
Chest radiograph demonstrating blunting of the costophrenic angle, evidence of displacement of adjacent anatomical structures, or (in supine position) a hazy opacity in one hemithorax with preserved vascular shadows
Atelectasis
Collapse of the alveoli, lung opacification with shift of the mediastinum, hilum, or hemidiaphragm toward the affected area, and compensatory overinflation in the adjacent nonatelectatic lung
Pneumothorax
A collection of air in the pleural space (the area with no vascular bed surrounding the visceral pleura)
Bronchospasm
Newly detected expiratory wheezing treated with bronchodilators
Aspiration pneumonitis
Acute lung injury after the inhalation of regurgitated gastric contents

PaO_2: partial pressure of oxygen in arterial blood; SaO_2: arterial oxyhemoglobin saturation.

In the United States, Arozullah et al. developed a multifactorial risk index to predict the postoperative pneumonia after major noncardiac surgery [12]. In Canada, McAlister et al. paid attention to the nonthoracic surgery [7]. They found the incidence of PPCs is 8% and identified the preoperative risk factors. Dupont et al. also investigated five independent predictive factors of postoperative pneumonia in France [19]. A recently published risk-prediction equation for PPCs was a significant advance in this field, which identified seven independent risk factors for PPCs by Canet et al. [11]. The researchers further tested this predictive risk score in a large European cohort and found this risk score performed differently between geographic areas [20]. In China, the most populous developing country in the world, however, very limited information about PPCs has been reported so far. Since China is different from the USA and European countries in geographic areas, race, disease spectrum, and social psychological background, investigations assessing the incidence and characteristics of PPCs in Chinese surgical cohorts are indispensable. Hence we conducted the present study to assess the incidence and risk factors of PPCs after noncardiac surgery in China and to develop a risk index of PPCs which is applicable for Chinese inpatients. The results of the present study would help health care providers to understand the existing situation of PPCs in China, to identify high-risk patients from the generic surgical population, and to guide individualized perioperative respiratory care.

2. Materials and Methods

2.1. Study Settings and Patients. We conducted a prospective, multicenter, and observational study of inpatients undergoing noncardiac surgical procedures. This study was performed at 4 university hospitals located in Zhejiang province, China (the First Affiliated Hospital, Zhejiang University School of Medicine, the Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, the First Affiliated Hospital, Wenzhou Medical College, and the Second Affiliated Hospital, Wenzhou Medical College). The inpatients undergoing surgical procedures between August 6, 2012, and August 12, 2012 were enrolled into the study. All patients would receive routine care, and no research-related intervention would be introduced.

2.2. Inclusion and Exclusion Criteria. All noncardiac operations performed under general, spinal, epidural, or regional anesthesia were eligible for inclusion. The exclusion criteria were as follows: (1) younger than 18 years; (2) pregnancy; (3) organ transplantation; (4) procedures performed under local nerve anesthesia; (5) procedures outside the operating room; (6) outpatient procedures (who had an LOS in hospital less than 24 hours); (7) reoperation related to a previous surgical complication; (8) patients with preoperatively intubated trachea.

2.3. Data Collection. Two trained anesthesiologists were assigned at each center to collect the following data: (1) generic information: date of surgery and hospital admission/discharge, age, gender, American society of anesthesiologists (ASA) physical status, height and weight, smoking status, alcohol use, and chronic comorbid disease; (2) preoperative variables: respiratory infection in the last month, antibiotic use, nasogastric tube, saturation of peripheral oxygen (SpO_2), and laboratory results (leucocytes count, neutrophil, hemoglobin, serum creatinine, serum albumin, and fasting blood glucose); (3) intraoperative variables: anaesthetic technique, surgery (type, site, and duration), nasogastric tube, bladder catheter, central venous catheter, blood loss, blood

Table 2: Demographic and clinical characteristics.

	Overall ($n = 1673$)	Cohort 1 ($n = 902$)	Cohort 2 ($n = 771$)	P^*
Male, *n* (%)	770 (46.0)	436 (48.3)	334 (43.3)	0.04
Age, yr	49 (37–60)	48 (36–60)	49 (39–60)	0.69
Education, yr	9 (6–9)	9 (6–9)	9 (9–14.5)	0.31
Smoking status, *n* (%)				0.85
Never smoker	1397 (83.5)	753 (83.5)	644 (83.5)	
Former smoker	222 (13.3)	118 (13.1)	104 (13.5)	
Current smoker	54 (3.2)	31 (3.4)	23 (3.0)	
Drinker, *n* (%)				0.47
Never drinker	1487 (88.9)	804 (89.1)	683 (88.6)	
Former drinker	152 (9.1)	83 (9.2)	69 (8.9)	
Current drinker	34 (2.0)	15 (1.7)	19 (2.5)	
Body mass index, kg/m^2	22.3 (20.3–24.3)	22.1 (20.3–24. 2)	22.4 (20.3–24.3)	0.85
Preoperative SpO_2, %	98 (98-99)	98 (98-99)	98 (98-98)	0.45
Respiratory infection in the last month, *n* (%)	28 (1.7)	16 (1.8)	12 (1.6)	0.85
ASA physical status, *n* (%)				0.43
1	830 (49.6)	464 (51.4)	366 (47.5)	
2	722 (43.2)	380 (42.1)	342 (44.4)	
3	114 (6.8)	54 (6.0)	60 (7.8)	
4	5 (0.3)	3 (0.3)	2 (0.3)	
5	2 (0.1)	1 (0.1)	1 (0.1)	
Emergency surgery, *n* (%)	102 (6.1)	55 (6.1)	47 (6.1)	0.99
Anesthesia, *n* (%)				0.24
General and combined[#]	1251 (74.8)	664 (73.6)	587 (76.1)	
Neuraxial/regional	422 (25.2)	238 (26.4)	184 (23.9)	
Surgical site, *n* (%)				<0.001
Peripheral	1015 (60.7)	620 (68.7)	395 (51.2)	
Abdominal	600 (35.9)	243 (26.9)	357 (46.3)	
Intrathoracic	58 (3.5)	39 (4.3)	19 (2.5)	
Duration of surgery, h				0.63
≤2 h	1238 (74.0)	659 (73.1)	579 (75.1)	
2-3 h	235 (14.0)	132 (14.6)	103 (13.4)	
>3 h	200 (12.0)	111 (12.3)	89 (11.5)	
LOS, d	8 (5–14)	9 (6–14)	8 (5–13)	0.01

Data are median (quartile) unless otherwise specified.
SpO_2: saturation of peripheral oxygen; ASA: American society of anesthesiologists; LOS: length of stay.
[*] Cohort 1 versus cohort 2; [#] this category included general anesthesia alone and general anesthesia combined with regional blockade.

transfusion, pulmonary, and cardiovascular complications; (4) postoperative variables: clinic (SpO_2, mechanical ventilation, vasoactive drugs) and laboratory results within 2 hours after surgery. After operation, surgeon and nurses visited the patients every day and recorded incidence of PPCs. The PPCs were defined in Table 1 [11]. Postoperative mortality was defined as death within 60 days of surgery.

2.4. Outcomes. The primary outcome was the occurrence of PPCs, the postoperative LOS, and the postoperative mortality rate. The secondary outcome is the predictive risk index of PPCs.

2.5. Statistical Analysis. Quantitative data were presented as means and standard deviations (SD) or median and interquartile range (IQR; from 25th to 75th percentiles) as appropriate. Qualitative data were reported as *N* (%). Student's *t*-test or Mann-Whitney *U* test were used for comparison of continuous variables and the chi-squared test or Fisher's exact test for categorical variables to test the relationship between each potential risk factors and PPCs.

In order to develop a risk index of PPCs, enrolled patients were further divided into two cohorts. Cohort 1 composed of patients from two hospitals and was used to develop the predictive index, while cohort 2 was used to validate the index. In cohort 1, risk factors were firstly analyzed using univariate analysis ($P < 0.05$). Then a multivariate logistic regression was conducted in cohort 1 with the occurrence of PPCs as the dependent factor, incorporating all risk factors on the basis of correlation coefficients between variables lower than 0.4. The forward LR mode was adopted in the process of regression. The adjusted odds ratios (OR) and the confidence intervals (CI) were also calculated. A brief predictive index was then calculated by multiplying the regression (β) by 10 and rounding off to the nearest integer [12]. The brief

Table 3: Incidence of PPCs with LOS according to surgical specialties.

	All patients	PPCs, n (%)	LOS (day)		P
			With PPCs (n = 163)	Without PPCs (n = 1510)	
Total	1673	163 (9.7)	16 (10–23)	8 (5–13)	<0.001
Surgical specialty					
Orthopaedics	303	10 (3.3)	22 (14–31)	11 (6–17)	0.03
Breast	72	2 (2.8)	20 (19–21)	4 (3–8)	<0.001
Gynaecology	208	16 (7.7)	10 (7–19)	6 (4–8)	<0.001
Vascular	33	1 (3.0)	16 (16–16)	9 (7–13)	0.45
Upper gastrointestinal	47	19 (40.4)	18 (16–21)	17 (13–20)	0.68
Lower gastrointestinal	136	16 (11.8)	17 (15–28)	11 (6–15)	<0.001
Hepatobiliary	229	31 (13.5)	24 (16–33)	8 (5–14)	<0.001
Urology	168	7 (4.2)	16 (13–38)	8 (6–11)	0.16
Kidney	56	8 (14.3)	13 (10–17)	10 (7–14)	0.65
Head and neck	147	9 (6.1)	4 (3–7)	7 (5–9)	0.21
Thoracic	49	19 (38.8)	12 (10–15)	14 (9–16)	0.36
Neurosurgery	36	8 (22.2)	22 (12–27)	18 (12–25)	0.40
Endocrinology	147	14 (9.5)	8 (7–14)	7 (6–8)	0.11
Others	41	4 (2.4)	10 (7–26)	7 (5–9)	0.16

Data are median (quartile) unless otherwise specified.
PPCs: postoperative pulmonary complications; LOS: length of stay.

predictive index then validated in cohort 2 to evaluate the model's discriminatory capability, and the area under the receiver operating characteristic (ROC) curve was displayed (c-statistic).

Data were analyzed using SPSS 16.0 (SPSS inc., Chicago, USA). All these tests were two-tailed and statistical significance was considered when a P value was less than 0.05.

3. Results

3.1. Demographic and Clinical Characteristics. Between August 6, 2012, and August 12, 2012, 2001 patients were undergoing noncardiac surgery, of which 328 were excluded according to the inclusion criteria or lost. Consequently, the final sample included in the statistical analysis consisted of 1673 patients, 902 in cohort 1 and 771 in cohort 2 (Figure 1). The basic characteristics of the study subjects are detailed in Table 2.

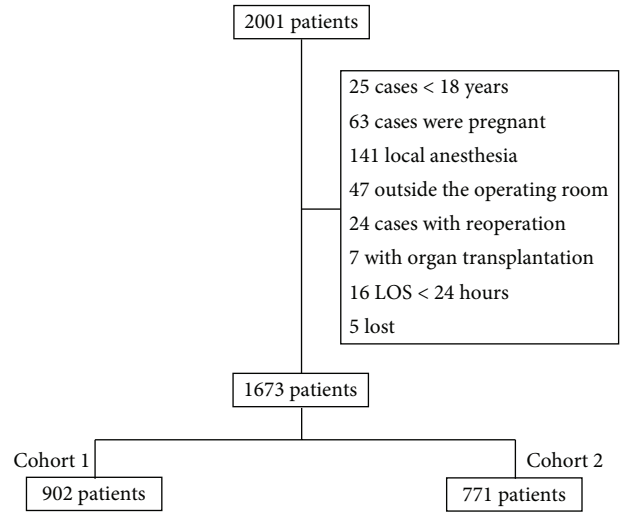

Figure 1: Flow-chart of study population. LOS: the length of stay.

3.2. PPCs, LOS, and Mortality. A total of 163 (9.7% of the 1673 patients) patients were recorded with 237 episodes of PPCs. Postoperative respiratory failure developed in 30 patients (1.8%), bronchospasm in 48 (2.9%), pleural effusion in 7 (0.4%), respiratory infection in 131 (7.8%), atelectasis in 19 (1.1%), cardiopulmonary edema 1 (0.06%), and pneumothorax in 1 (0.06%). Most PPCs occurred after upper gastrointestinal surgery (40.4%), followed by thoracic (38.8%), neurosurgery (22.2%), kidney (14.3%), hepatobiliary (13.5%), and lower gastrointestinal surgeries (11.8%). The median postoperative LOS was longer in patients with PPCs (16 days, IQR 10–23 days) than in those without PPCs (8 days, IQR 5–13 days) ($P < 0.001$), especially in orthopaedics ($P = 0.03$), breast ($P < 0.001$), gynaecology ($P < 0.001$), lower gastrointestinal ($P < 0.001$), and hepatobiliary ($P < 0.001$) surgery. The detailed information on the characteristics of PPCs and LOS was shown in Table 3.

Four (0.24%) patients died in the hospital, 3 (1.84%) of the 163 patients with PPCs and 1 (0.07%) of the 1510 patients without PPCs; the mortality was significantly higher in patients with PPCs than those without ($P < 0.001$).

3.3. Risk Factors and PPCs. The variables having a statistically significant impact on the incidence of PPCs detected from the cohort 1 are shown in Table 4. Then the independent variables were entered into the logistic regression model, except for these have high collinearity with others (intraoperative blood

Table 4: Distribution of results of independent variables in cohort 1.

	Number of patients	Number (%) of patients with PPCs	P
Age, yr			<0.001
<59	670	65 (9.7)	
≥60	232	47 (20.3)	
ASA physical status			<0.001
1	464	20 (4.3)	
2	380	77 (20.3)	
3	54	13 (24.1)	
4	3	1 (33.3)	
5	1	1 (100.0)	
Smokers			0.001
No	753	79 (10.5)	
Yes	149	33 (22.1)	
Respiratory infection in the last month			<0.001
No	886	101 (11.4)	
Yes	16	11 (68.8)	
Diabetes			0.001
No	846	97 (11.5)	
Yes	56	15 (26.8)	
COPD			<0.001
No	890	106 (11.9)	
Yes	12	6 (50.0)	
Cirrhosis			0.014
No	895	109 (12.2)	
Yes	7	3 (42.9)	
Stroke/transient ischaemic attack			0.001
No	887	106 (12.0)	
Yes	15	6 (40.0)	
Preoperative antibiotic use			<0.001
No	298	19 (6.4)	
Yes	604	93 (15.4)	
Preoperative SpO_2, %			<0.001
≥96	845	92 (10.9)	
<96	57	20 (35.1)	
Preoperative anemia			0.007
No	837	97 (11.6)	
Yes	65	15 (23.1)	
Preoperative LOS, d			0.002
<2	126	5 (4.0)	
≥2	776	107 (13.8)	
Surgery site			<0.001
Peripheral	620	36 (5.8)	
Abdominal	243	56 (23.0)	
Intrathoracic	39	20 (51.3)	
Anesthesia			<0.001
Regional	238	10 (4.2)	
General	664	102 (15.4)	
Intraoperative nasogastric tube			<0.001
No	847	85 (10.0)	
Yes	55	27 (49.1)	

Table 4: Continued.

	Number of patients	Number (%) of patients with PPCs	P
Intraoperative bladder catheter			<0.001
No	466	28 (6.0)	
Yes	436	84 (19.3)	
Intraoperative central venous catheter			<0.001
No	821	85 (10.4)	
Yes	81	27 (33.3)	
Intraoperative blood loss, mL			<0.001
<100	636	32 (5.0)	
≥100	266	80 (30.1)	
Intraoperative blood transfusion			<0.001
No	875	102 (11.7)	
Yes	27	10 (37.0)	
Duration of surgery, h			<0.001
≤2 h	659	47 (7.1)	
>2 to 3 h	132	27 (20.5)	
>3 h	111	38 (34.2)	
Postoperative SpO_2, %			<0.001
≥96	891	101 (11.3)	
<96	11	11 (100.0)	
Postoperative leucocyte, 10^9/L			<0.001
<4	6	2 (33.3)	
4–10	665	47 (7.1)	
>10	231	63 (27.3)	
Postoperative anemia			0.009
No	793	90 (11.3)	
Yes	109	22 (20.2)	
Postoperative fasting blood glucose, mmol/L			<0.001
≤6.1	778	50 (6.4)	
>6.1	124	62 (50.0)	
Postoperative creatinine, μmol/L			<0.001
≤115	872	99 (11.4)	
>115	30	13 (43.3)	
Postoperative albumin, g/L			<0.001
<35	244	85 (34.8)	
≥35	658	27 (4.1)	
Postoperative ventilation			<0.001
No	878	97 (11.0)	
Yes	24	15 (62.5)	
Postoperative vasoactive drug			<0.001
No	893	105 (11.8)	
Yes	9	7 (77.8)	

Smoking: defined as patients who smoked up to 1 year before surgery; Anemia: defined as hemoglobin <100 g/L.
PPCs: postoperative pulmonary complications; ASA: American society of anesthesiologists; COPD: chronic obstructive pulmonary disease; SpO_2: saturation of peripheral oxygen; LOS: length of stay.

loss and duration of surgery; intraoperative blood loss and central venous catheter; intraoperative blood loss and blood transfusion; postoperative ventilation and vasoactive drug; pre- and intraoperative nasogastric tube; pre- and postoperative hemoglobin). Multivariable logistic regression indicated that 9 of those potential predictors were present in the final model. The raw and adjusted odds ratios for the nine variables are shown in Table 5, which also shows the brief predictive index derived from the β coefficient for each variable. This nine-variable regression model had good discrimination and calibration values in cohort 1 (c-statistic 0.91, 95% CI, and from 0.89 to 0.94). The ROC curves and the c-statistics for the validation subsamples (cohort 2) are shown in Figure 2. The brief predictive index developed in the present study has

Table 5: Independent predictors of risk factors for PPCs.

	OR (95% CI) $n = 902$	β coefficient	Risk score
Respiratory infection in the last month	7.03 (1.66–29.80)	1.950	20
Smokers	2.37 (1.27–4.42)	0.861	9
Preoperative antibiotic use	0.238 (0.11–0.54)	−1.436	−14
Preoperative SpO_2 <96%	5.56 (2.38–12.98)	1.715	17
Surgery site			
Peripheral	1		
Abdominal	2.88 (1.49–5.59)	1.058	11
Intrathoracic	12.20 (4.61–32.28)	2.501	25
Intraoperative blood loss ≥100 mL	3.00 (1.64–5.50)	1.100	11
Postoperative fasting blood glucose >6.1 mmol/L	2.60 (1.91–3.54)	0.956	10
Postoperative albumin <35 g/L	4.21 (2.24–7.92)	1.438	14
Postoperative ventilation	7.20 (1.96–26.45)	1.975	20

PPCs: postoperative pulmonary complications; SpO_2: saturation of peripheral oxygen.

potential advantage in predicting PPCs in Chinese inpatients (better *c*-statistic than the risk index in the study of Canet et al.) [11]. The most relevant cut point was the score of 13 (sensitivity 90.2%, specificity 79.4%). Table 6 shows the incidence of PPCs by risk index score, the number of patients in each risk class, and the actual incidence of PPCs in cohort 1 and cohort 2.

3.4. Strategies to Reduce PPCs. Opportunities to reduce risk for PPCs occur throughout the perioperative period. Table 7 summarizes perioperative interventions that have been recommended to decrease the risk of PPCs [15, 16, 21, 22].

4. Discussion

This prospective study assessed the 9.7% incidence of PPCs after noncardiac surgery in Chinese university hospitals and found increased hospital LOS by 8 days in patients with PPCs than those without. Furthermore, we identified the perioperative risk factors of PPCs and developed a brief risk index for predicting PPCs in Chinese inpatients.

In the current study, the incidence of PPCs after noncardiac surgery and the hospital LOS in patients with PPCs were comparable to the incidence observed in some previous studies [11, 23–25]. The high incidence of PPCs and the increased hospital LOS indicating PPCs is also an important public health issue demanding nationwide attention in China. Unexpectedly, we found the postoperative mortality in patients with PPCs was lower than several previous results [3, 11]. This may be in association with the higher percentage of ASA class 1 to 2 patients and younger median age. Firstly, the medical resources in China were mainly concentrated in larger hospitals, especially the university hospitals and tertiary hospitals. So the patients always chose to go to large hospitals directly, even if they only have a cold or want to remove a small lipoma on body surface. Secondly, the percentage of emergency surgery in the present study was low, since none of the four studied hospitals had a major trauma center.

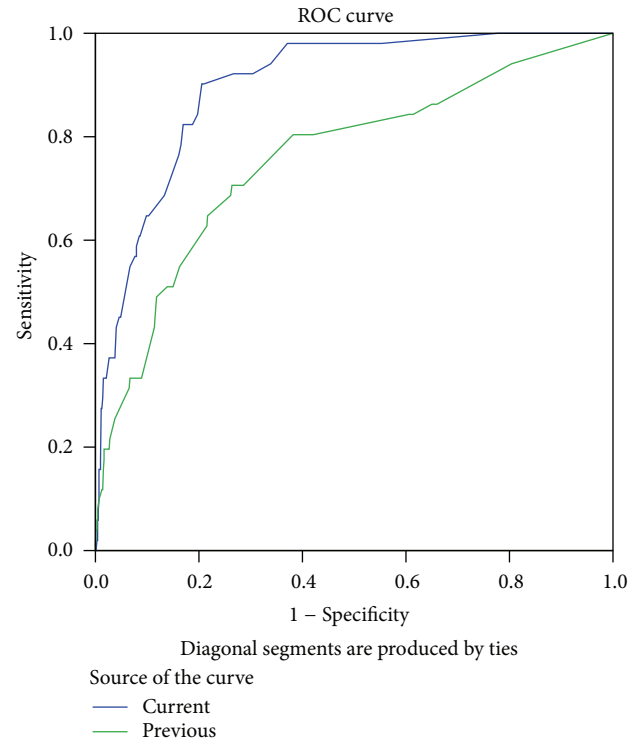

Figure 2: Receiver operating characteristic (ROC) curves drawn for the current model and previous index (Canet's) in cohort 2. *c*-statistic for the area under the ROC curve (AUC) = 0.90 (95% CI, 0.86 to 0.94) drawn from the current index and AUC = 0.76 (95% CI, from 0.68 to 0.83) drawn from the previous index.

Nine independent risk factors were finally selected to participate in the brief predictive index for PPCs, including smoking, respiratory infection in the last month, antibiotic

Table 6: Distribution of PPCs risk index scores in patients.

	Risk class			
	Low risk (<13 points)	Intermediate risk (13–30 points)	High risk (31–42 points)	Extremely high risk (>42 points)
Cohort 1, *n* (%)	672 (74.5)	127 (14.1)	67 (7.4)	36 (4.0)
Cohort 2, *n* (%)	577 (74.8)	110 (14.3)	54 (7.0)	30 (3.9)
Rate of PPCs in cohort 1, *n* (%)	21 (3.1)	22 (17.3)	38 (56.7)	31 (86.1)
Rate of PPCs in cohort 2, *n* (%)	5 (0.9)	17 (15.5)	12 (22.2)	17 (56.7)

PPCs: postoperative pulmonary complications.

Table 7: Interventions to reduce the risk of PPCs.

Preoperative interventions
A careful history taking and physical examination
Encourage cessation of smoking for at least 2 months
Appropriate use of antibiotics and delay surgery if respiratory infection is present
Recommend a regular exercise program (e.g., walking, upper limb exercises, swimming, pool exercises, etc.)
Treat patients with established asthma with inhaled corticosteroids
Treat patients with established COPD with regular bronchodilators
Intraoperative interventions
Substitute less ambitious procedure for upper abdominal or thoracic surgery when possible
Minimize blood loss
Limit duration of surgery to less than 3 hr
Whenever possible, use spinal or epidural anesthesia
Postoperative interventions
Recommend regular lung expansion modalities such as deep breathing exercises
Perform selective decompression of abdominal contents using nasogastric tube if patient is experiencing symptomatic gastric distension
As soon as possible after surgery, have the patient sit up in a chair

PPCs: postoperative pulmonary complications; COPD: chronic obstructive pulmonary disease.

use, SpO_2, surgery site and blood lost, blood glucose, albumin, and ventilation.

Among the preoperative risk factors, smoking, a history of respiratory infection in the last month, SpO_2, and antibiotic use are strong PPC risk factors. Smoking and respiratory infection may lead to local changes in airway reactivity, pulmonary function, and residual impairment of immunity, which could increase the risk of PPCs for several folds [6, 7]. SpO_2 is an easily recorded objective measure, which reflects the respiratory function as well as cardiovascular functional status [11]. Antibiotic use is a protective factor in the current cohort. It seems to be helpful to use prophylactic antibiotic, especially in these high-risk patients.

The intraoperative risk factors were identified in the present study including surgery site and blood loss, which were similar to many previous studies [4, 7, 14, 26]. Thoracic and abdominal surgeries are always performed via a large painful incision associated with obviously changes in lung compliance and functional residual capacity. As a result, the incidence of PPCs in these surgeries is much higher than others. Significant intraoperative blood loss gives rise to haemodynamic instability and relative ischaemia and so causes ischaemia-reperfusion injury, which can lead to organ dysfunction.

Factors in early stage of postoperative may have a better performance in predicting PPCs. We found postoperative mechanical ventilation, albumin, and blood glucose could increase the risk of PPCs. Among them, ventilation is the most important risk factor for PPCs. Researchers found an increased risk of respiratory events in patients with ventilation [26, 27]. It is important to follow the guidelines for the management of ventilation and the prevention of nosocomial infection.

A brief risk score based on these factors was calculated to predict the incidence of PPCs. In Spain, Canet and coworkers also developed a similar risk index of PPCs [11]. Three of these risk factors (preoperative SpO_2, respiratory infection in the last month, and surgery site) were also identified in the current study. What is more, the brief predictive index developed in the present study has potential advantage in predicting PPCs in Chinese inpatients (better *c*-statistic than the risk index of Canet). This disparity may be due to the different geographic areas, ethnic, cultural, socioeconomic, and political differences between China and Spain. In fact,

the risk index developed by Canet also performed differently between the Western Europe sample and the Eastern Europe sample [20]. In general, the current brief predictive index predicted the risk of PPCs well in both the development and validation cohorts. These findings suggest patients with high risk of PPCs should be closely monitored and early intervened if the risks factors could be modified.

Several limitations in our study should be acknowledged. First of all, only four university hospitals participated. This may result in some bias. Further studies targeting general Chinese hospitals with a larger population are still needed. Another limitation is that the 7-day study period was arbitrarily decided. All patients were enrolled in August. Englesbe et al. demonstrated a significant seasonal variation in surgical morbidity and mortality [28]. They found a dramatic worsening of surgical mortality in July, which was attributed to the influx of inexperienced trainees. However, Ehlert and colleagues refuted the "July Phenomenon" in a larger population [29]. In most hospitals in China, July is marked by an influx of intern. Instead of managing patients directly, they only do the paperwork. Therefore, they may have little effect on the present results. Further study is needed to confirm the impact of seasonal variation in surgical morbidity and mortality.

5. Conclusion

The present prospective, multicenter study found there was high incidence of PPCs which increased hospital LOS in noncardiac surgical inpatients in China. We identified nine objective and easily assessed factors associated with the occurrence of PPCs. A simple risk index based on these factors predicted the development of PPCs. This brief predictive index may be useful for clinicians in estimating patients' risk for PPCs and guide individualized perioperative respiratory care.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This research was supported by National Natural Science Foundation of China (no. 81301652) and Zhejiang Provincial Program for the Cultivation of High-level Innovative Health Talents.

References

[1] T. G. Weiser, S. E. Regenbogen, K. D. Thompson et al., "An estimation of the global volume of surgery: a modelling strategy based on available data," *The Lancet*, vol. 372, no. 9633, pp. 139–144, 2008.

[2] A. A. Ghaferi, J. D. Birkmeyer, and J. B. Dimick, "Variation in hospital mortality associated with inpatient surgery," *The New England Journal of Medicine*, vol. 361, no. 14, pp. 1368–1375, 2009.

[3] A. Shander, L. A. Fleisher, P. S. Barie, L. M. Bigatello, R. N. Sladen, and C. B. Watson, "Clinical and economic burden of postoperative pulmonary complications: patient safety summit on definition, risk-reducing interventions, and preventive strategies," *Critical Care Medicine*, vol. 39, no. 9, pp. 2163–2172, 2011.

[4] L. G. G. Serejo, F. P. da Silva-Júnior, J. P. C. Bastos, G. S. de Bruin, R. M. S. Mota, and P. F. C. de Bruin, "Risk factors for pulmonary complications after emergency abdominal surgery," *Respiratory Medicine*, vol. 101, no. 4, pp. 808–813, 2007.

[5] K. J. Haines, E. H. Skinner, and S. Berney, "Association of postoperative pulmonary complications with delayed mobilisation following major abdominal surgery: an observational cohort study," *Physiotherapy*, vol. 99, no. 2, pp. 119–125, 2013.

[6] R. L. Scholes, L. Browning, E. M. Sztendur, and L. Denehy, "Duration of anaesthesia, type of surgery, respiratory comorbidity, predicted VO2max and smoking predict postoperative pulmonary complications after upper abdominal surgery: an observational study," *Australian Journal of Physiotherapy*, vol. 55, no. 3, pp. 191–198, 2009.

[7] F. A. McAlister, K. Bertsch, J. Man, J. Bradley, and M. Jacka, "Incidence of and risk factors for pulmonary complications after nonthoracic surgery," *The American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 5, pp. 514–517, 2005.

[8] S. F. Khuri, W. G. Henderson, R. G. DePalma et al., "Determinants of long-term survival after major surgery and the adverse effect of postoperative complications," *Annals of Surgery*, vol. 242, no. 3, pp. 326–343, 2005.

[9] D. L. Davenport, W. G. Henderson, S. F. Khuri et al., "Preoperative risk factors and surgical complexity are more predictive of costs than postoperative complications: a case study using the National Surgical Quality Improvement Program (NSQIP) database," *Annals of Surgery*, vol. 242, no. 4, pp. 463–471, 2005.

[10] V. A. Lawrence, S. G. Hilsenbeck, C. D. Mulrow, R. Dhanda, J. Sapp, and C. P. Page, "Incidence and hospital stay for cardiac and pulmonary complications after abdominal surgery," *Journal of General Internal Medicine*, vol. 10, no. 12, pp. 671–678, 1995.

[11] J. Canet, L. Gallart, C. Gomar et al., "Prediction of postoperative pulmonary complications in a population-based surgical cohort," *Anesthesiology*, vol. 113, no. 6, pp. 1338–1350, 2010.

[12] A. M. Arozullah, S. F. Khuri, W. G. Henderson, and J. Daley, "Development and validation of a multifactorial risk index for predicting postoperative pneumonia after major noncardiac surgery," *Annals of Internal Medicine*, vol. 135, no. 10, pp. 847–857, 2001.

[13] G. W. Smetana, V. A. Lawrence, and J. E. Cornell, "Preoperative pulmonary risk stratification for noncardiothoracic surgery: Systematic review for the American College of Physicians," *Annals of Internal Medicine*, vol. 144, no. 8, pp. 581–595, 2006.

[14] A. M. Arozullah, J. Daley, W. G. Henderson, and S. F. Khuri, "Multifactorial risk index for predicting postoperative respiratory failure in men after major noncardiac surgery. The National Veterans Administration Surgical Quality Improvement Program," *Annals of Surgery*, vol. 232, no. 2, pp. 242–253, 2000.

[15] A. Qaseem, V. Snow, N. Fitterman et al., "Risk assessment for and strategies to reduce perioperative pulmonary complications for patients undergoing noncardiothoracic surgery: a guideline from the American College of Physicians," *Annals of Internal Medicine*, vol. 144, no. 8, pp. 575–580, 2006.

[16] V. A. Lawrence, J. E. Cornell, and G. W. Smetana, "Strategies to reduce postoperative pulmonary complications after noncardiothoracic surgery: systematic review for the American

College of Physicians," *Annals of Internal Medicine*, vol. 144, no. 8, pp. 596–608, 2006.

[17] D. O. Warner, "Preventing postoperative pulmonary complications: The role of the anesthesiologist," *Anesthesiology*, vol. 92, no. 5, pp. 1467–1472, 2000.

[18] J. Canet and L. Gallart, "Predicting postoperative pulmonary complications in the general population," *Current Opinion in Anaesthesiology*, vol. 26, no. 2, pp. 107–115, 2013.

[19] H. Dupont, P. Montravers, R. Gauzit, B. Veber, J. Pouriat, and C. Martin, "Outcome of postoperative pneumonia in the Eole study," *Intensive Care Medicine*, vol. 29, no. 2, pp. 179–188, 2003.

[20] V. Mazo, S. Sabate, J. Canet et al., "Prospective external validation of a predictive score for postoperative pulmonary complications," *Anesthesiology*, vol. 121, no. 2, pp. 219–231, 2014.

[21] J. M. Torpy, C. Lynm, and R. M. Glass, "JAMA patient page. Lung complications after surgery.," *The Journal of the American Medical Association*, vol. 302, no. 14, p. 1610, 2009.

[22] G. W. Smetana, "Preoperative pulmonary evaluation," *New England Journal of Medicine*, vol. 340, no. 12, pp. 937–944, 1999.

[23] P. G. Noordzij, D. Poldermans, O. Schouten, J. J. Bax, F. A. G. Schreiner, and E. Boersma, "Postoperative mortality in the Netherlands: A population-based analysis of surgery-specific risk in sdults," *Anesthesiology*, vol. 112, no. 5, pp. 1105–1115, 2010.

[24] N. A. Khan, H. Quan, J. M. Bugar, J. B. Lemaire, R. Brant, and W. A. Ghali, "Association of postoperative complications with hospital costs and length of stay in a tertiary care center," *Journal of General Internal Medicine*, vol. 21, no. 2, pp. 177–180, 2006.

[25] D. A. Thompson, M. A. Makary, T. Dorman, and P. J. Pronovost, "Clinical and economic outcomes of hospital acquired pneumonia in intra-abdominal surgery patients," *Annals of Surgery*, vol. 243, no. 4, pp. 547–552, 2006.

[26] J. Chastre and J. Fagon, "Ventilator-associated pneumonia," *The American Journal of Respiratory and Critical Care Medicine*, vol. 165, no. 7, pp. 867–903, 2002.

[27] A. R. Tait, S. Malviya, T. Voepel-Lewis, H. M. Munro, M. Siewert, and U. A. Pandit, "Risk factors for perioperative adverse respiratory events in children with upper respiratory tract infections," *Anesthesiology*, vol. 95, no. 2, pp. 299–306, 2001.

[28] M. J. Englesbe, S. J. Pelletier, J. C. Magee et al., "Seasonal variation in surgical outcomes as measured by the American College of Surgeons-National Surgical Quality Improvement Program (ACS-NSQIP)," *Annals of Surgery*, vol. 246, no. 3, pp. 456–462, 2007.

[29] B. A. Ehlert, J. T. Nelson, C. E. Goettler et al., "Examining the myth of the "july Phenomenon" in surgical patients," *Surgery*, vol. 150, no. 2, pp. 332–338, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 671087, 11 pages
http://dx.doi.org/10.1155/2014/671087

Review Article

Role of Circulating Lymphocytes in Patients with Sepsis

Raul de Pablo,[1,2] Jorge Monserrat,[2] Alfredo Prieto,[2] and Melchor Alvarez-Mon[2,3]

[1] *Intensive Care Unit, University Hospital "Príncipe de Asturias", University of Alcala, Alcala de Henares, 28805 Madrid, Spain*
[2] *Laboratory of Immune System Diseases and Oncology, National Biotechnology Center (CNB-CSIC) Associated Unit, Department of Medicine and Medical Specialties, University of Alcala, 28871 Madrid, Spain*
[3] *Immune System Diseases and Oncology Service, University Hospital "Príncipe de Asturias", University of Alcala, Alcala de Henares, 28805 Madrid, Spain*

Correspondence should be addressed to Raul de Pablo; raul.depablo.uah@gmail.com

Received 23 February 2014; Revised 15 July 2014; Accepted 29 July 2014; Published 28 August 2014

Academic Editor: Baoli Cheng

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Sepsis is a systemic inflammatory response syndrome due to infection. The incidence rate is estimated to be up to 19 million cases worldwide per year and the number of cases is rising. Infection triggers a complex and prolonged host response, in which both the innate and adaptive immune response are involved. The disturbance of immune system cells plays a key role in the induction of abnormal levels of immunoregulatory molecules. Furthermore, the involvement of effector immune system cells also impairs the host response to the infective agents and tissue damage. Recently, postmortem studies of patients who died of sepsis have provided important insights into why septic patients die and showed an extensive depletion of CD4 and CD8 lymphocytes and they found that circulating blood cells showed similar findings. Thus, the knowledge of the characterization of circulating lymphocyte abnormalities is relevant for the understanding of the sepsis pathophysiology. In addition, monitoring the immune response in sepsis, including circulating lymphocyte subsets count, appears to be potential biomarker for predicting the clinical outcome of the patient. This paper analyzes the lymphocyte involvement and dysfunction found in patients with sepsis and new opportunities to prevent sepsis and guide therapeutic intervention have been revealed.

1. Introduction

Sepsis is a systemic inflammatory response that occurs during infection [1]. Septic shock is the leading cause of multiple organ failure and death in intensive care units, and the incidence is increasing worldwide [2–4]. The pathogenesis of sepsis is a result of a complex network of events involving immune-inflammatory and anti-inflammatory processes triggered by the infection agent [5]. This host response is complex and variable, in which both proinflammatory and anti-inflammatory mechanisms can contribute to either clearance of infection and tissue recovery or organ injury. Early and appropriate intervention is critical for improving the patient's outcome, reducing morbidity and mortality [6]. It is generally accepted that the clinical strategy for improving the outcome of sepsis patients includes the advancement in the knowledge of the pathogenesis of this syndrome as well as the identification of biomarkers to establish risk assessment, predicting the development of individual or multiple organ dysfunctions, guiding antimicrobial therapy, and establishing new and individualized treatments.

Sepsis is initiated when the host responds to pathogen insult. The first line of defenses is constituted by the innate immune system response. Several effector cells are involved in this antimicrobial response including different leukocyte populations. Monocyte-macrophage cells and dendritic cells play a key role in the innate immune response. These cells have the ability to phagocytose bacteria and interact with their products through an interaction with their pattern-recognition receptors. These activated phagocytic cells release proinflammatory mediators, such as cytokines, chemokines, lipid mediators, nitric oxide, and oxygen radicals [7–9]. Activated neutrophils also promote clearance of bacteria, and they subsequently contribute to tissue inflammation and injury through respiratory burst, cytotoxicity, degranulation, increased vascular permeability, and organ injury by releasing several proinflammatory mediators, myeloperoxidases, and proteases [10]. Thus, a "cytokine storm" is generated, which is

responsible for triggering the inflammation. But the immune system, including cells of the adaptive immune response, may also harbor humoral and cellular mechanisms that attenuate the potentially harmful effects of the proinflammatory response. However, the release of anti-inflammatory cytokines also appears to be exacerbated, as illustrated by the strong relationship between high levels of these mediators and poor outcome [11]. Indeed, our group and other researchers have described that an early response to continuously elevated anti-inflammatory cytokine serum levels was better predictor of mortality than the classic proinflammatory cytokines in patients with septic shock [12]. It is important to remark that this compensatory anti-inflammatory response syndrome named CARS is a two-wave process that follows SIRS (systemic inflammatory response syndrome) in experimental animals, but in most of patients both events are concomitant, [13] and it is often found when patients are admitted to the ICU [12]. Furthermore, the adaptive immune response has a relevant role to control of bacterial infection [14]. Adaptive immunity is driven by innate immune cells through sensing microorganisms and presenting antigens in the context of major histocompatibility complex class II (MHC class II) and costimulatory molecules. The recent discovery of subsets of lymphocytes that are defined by their limited antigen receptor variability and are restricted to specific tissue may prove a link between immune activation and antibacterial defense during sepsis [15]. "Innate lymphocytes" are defined by their limited antigen receptor variability, and, therefore, these T cells have a memory phenotype in the absence of deliberate immunization [16]. The innate-like lymphocytes include natural killer T cells, gamma delta T cells, and mucosal-associated invariant T (MAIT) cells. MAIT cells are already primed to gastrointestinal flora and work in cooperation with the innate response to stave off infections [15]. Furthermore, T lymphocytes play a critical role in the regulation of antimicrobial phagocytic and cytotoxic activity of the innate immune response cells [17]. Interferon (IFN)-γ and granulocyte macrophage colony stimulating factor (GM-CSF), mainly produced by T lymphocytes, increase this defensive activity but other cytokines such as interleukin (IL)-10 have inhibitory effects [17, 18]. B lymphocyte response also plays an important role in the defensive host response. B cells produce cytokines, present antigens to T lymphocyte, and differentiate into antibody producing cells [19]. Antibodies bounded to bacteria may increase bacteria opsonization and favor phagocytosis [17]. However, abnormal bacterial induced activation of T and B cells may be followed by inflammation and endothelial and tissue damage [20, 21].

Blood lymphocyte dysfunction during sepsis has long been recognized with significant lymphopenia and decreased lymphocyte T CD4+, CD8+, and natural killer (NK) cells [5]. However, recently a renewed interest in lymphocyte dysfunction during sepsis emerged from studies demonstrating that immunosuppression was present not only in peripheral blood cells but also locally in organs in patients who died of sepsis [22].

In this review, we try to highlight the role of the main populations of blood lymphocytes in sepsis and we discuss how different kinetic patterns of lymphocyte subsets are involved and their relationship to the surviving outcome. This knowledge in the future may have important therapeutic implications for patients with septic shock. Furthermore, circulating lymphocyte abnormalities might have also potential prognostic biomarker signification.

2. Lymphopenia and Anergy

B- and T-lymphopenia is a hallmark of sepsis that can be mimicked in human volunteers receiving a bolus of lipopolysaccharide [11]. Extensive lymphocyte apoptosis is seen in animal models of sepsis and in patients with sepsis [23]. In a model of cecal ligation and puncture, prevention of lymphocyte apoptosis with caspase inhibitors results in a marked improvement in animal surviving [24]. Other studies on animals also suggest that immune depression resulting from the loss of lymphocytes may be the key factor in inability to survive sepsis [24–27].

Anergy is a tolerance mechanism in which the lymphocyte is intrinsically functionally inactivated following an antigen encounter, but it remains alive for an extended period of time in a hyporesponsive state [5]. T-cell anergy relates to a decreased proliferation to mitogen stimulation, a shift toward a T_{H2} profile of cytokine secretion, an increased apoptosis, and an increased percentage of CD4+CD25+ regulatory T lymphocytes (Tregs) [28]. The occurrence of a state of lymphocyte anergy has been described in patients with major trauma or burns, associated with mortality rate and with the development of secondary septic complications [5, 29]. Meakins et al. described that surgical patients who had failure of delayed hypersensitivity response had an increased risk of sepsis and related mortality [30]. Therefore, defective T-cell proliferation and secretion of IL-2 and TNF correlated with sepsis mortality [31].

3. T Lymphocytes

T-cell compartment plays a critical role in regulating the effector stage of the immune response. CD3+CD4+ T lymphocytes or T helper (T_H) cells are mainly involved in the regulation of the immune response [32].

It has been recognized that different CD4+ T cells subsets play a critical role in response to microbial challenges. The first subsets recognized were denoted by T_{H1} and T_{H2} cells based on the selective production of 2 cytokines, IFN-γ and IL-4, respectively [33]. T_{H1} cytokines exert a positive feedback on antigen-presenting cells, whereas T_{H2} cytokines promote downregulation of the immune response. This T_{H1} paradigm was reasonably useful for the initial categorization of mechanisms involving elimination of microbial pathogens. Previous works showed a shift from T_{H1} to T_{H2} cytokine profiles following severe aggression such as trauma, burns, cardiac arrest, and infection [14, 34].

It has been proposed that the lack of a shift from T_{H1} to T_{H2} response increases survival among patients with sepsis [35]. Reductions in circulating CD4+ T-lymphocytes and their shift to a T_{H2} phenotype characterize aspects of sepsis-induced immunosuppression [36]. The associations between

complicated clinical course and unfavorable prognosis of septic patients with the decline of peripheral blood CD4+ T-lymphocytes were established in a majority of trauma victims or surgical patients with secondary sepsis [37, 38]. We have described that T lymphopenia found in patients with septic shock persisted during the first week of follow-up in the intensive care unit (ICU) and was independent of the outcome [39]. These findings are consistent with reports demonstrating significant lymphopenia early in the course of disease [40, 41]. However, at the end of the second week of follow-up, we observed that the absolute number of circulating CD3+CD4+ T cells had clearly normalized in surviving patients with septic shock [36].

Nowadays, we know that the opportunities for T_H lymphocytes diversity are far greater than just T_{H1} and T_{H2} profiles. The newly described T_H cell subsets include T_{H17}, T_{H9}, and T_{H22} cells; follicular helper T (T_{FH}) cells; and different types of regulatory T (Treg) cells [42].

T_{H17} has relatively recently been characterized as an IL-17-producing subset of CD4+ T cells. Naive CD4+ T cells, in the presence of IL-6, IL-21, or TGF-beta, can proceed to a T_{H17} phenotype [43, 44]. Their proliferation and differentiation are supported by IL-23 and IL-1 secreted from antigen-presenting cells [43, 45]. Once differentiated, T_{H17} cells are capable of producing not only IL-17, but also IL-21, IL-22, TNF-α, and IFN-γ [46–48], suggesting plasticity of these cells, with an ability to produce different cytokines depending upon environmental stimulus [46, 48]. IL-17 plays a major role in linking adaptive and innate immune responses. IL-17 is a potent proinflammatory cytokine which induces the production of many other proinflammatory cytokines, chemokines, and other inflammation mediators such as prostaglandin E2 and nitric oxide [49]. Information about its role in human sepsis is scarce [14]. T_{H17} cells contribute to host defense against extracellular bacteria, such as *Staphylococcus aureus* and *Klebsiella pneumoniae* as well as fungi [50, 51]. In humans, T_{H17} lymphocyte count on day 1 and after 6 days in survivors with severe sepsis was higher than that in nonsurvivors [52]. Salomao et al. observed an increased proportion of CD4+ lymphocytes producing IL-17 in patients with sepsis [14]. Thus, T_{H17} differentiation appears to contribute significantly to the surviving in patients with severe sepsis, and it represents one exception in the overall downregulation of T-cell immune functions in these patients.

Treg is one of the T-cell subsets that have strong immunosuppressive activity, playing an essential role in controlling both adaptive and innate immune responses. These cells can downregulate effector activities mediated by CD4+ T cells, CD8+ T cells, NK cells, and also dendritic cells and B cells [37, 52–57]. Recently, Wu et al. found that the circulatory Treg lymphocyte counts on day 1 were higher in surviving patients with severe sepsis than those in nonsurviving ones [35]. This finding confirms results found by other authors [58, 59]. The relative increase in circulating Treg might play a role in lymphocyte anergy described after septic shock [60]. This, altogether, strongly suggests that Treg cells not only represent a reliable marker of immunoparalysis in sepsis, but also may play an important role in its pathogenesis.

CD8+ T lymphocytes are effector cytotoxic cells. We have found a decrease of CD8+ T lymphocytes in patients with septic shock at ICU admission [36]. In survivors, CD8+ T lymphocytes showed a further drop on day 3 of followup, followed by a gradual recovery although numbers failed to reach the count recorded in healthy controls. Importantly, CD3+CD8+ T lymphocyte count in survivors was significantly diminished with respect to nonsurvivors on day 3. A drop in circulating CD3+CD8+ T cells has been described by other authors [52, 54, 61–63].

CD45 is essentialin T-cell differentiation and antigen receptor signaling [64]. When inflammatory agents activate noneffector CD45RA+CD45RO− T lymphocytes, such as bacterial infection, the isoform CD45RO is upregulated and CD45RA is downregulated [65]. CD28 is a costimulatory molecule that plays a key role in regulating the activation and surviving of T lymphocytes [66–68]. It has been reported that patients with severe sepsis showed a significant reduction in T lymphocyte CD28 expression [69]. The migration of circulating T lymphocytes to peripheral lymph nodes depends on the expression of the CD62L homing receptor [70]. We observed downregulation of L-Selectin expression on CD3+CD8+ cells in patients with septic shock and it was associated with a better prognosis. When we analyzed the phenotype of the circulating CD3+CD8+ T cells according to the activation criteria in patients with septic shock at ICU admission, all patients showed low count of CD3+CD8+CD45RA+CD45RO− T lymphocytes (naïve cells), and survivors also have low CD3+CD8+CD45RA−CD45RO+ lymphocytes (memory cells) at day 3 of the follow-up, associated with a lower count of CD3+CD8+CD28+ T lymphocytes. Furthermore, survivors also show lower count of CD3+CD8+CD62L+ T lymphocytes. These findings may suggest that the rapid migration of activated CD8+ T cells to peripheral lymph nodes may be a mechanism contributing to patient survival and, therefore, delayed tissue response could determine the failure of the immune system in patients with the worst outcome [36]. It is known that cellular immune responses play a critical role in the defense against infections and strong T-cell responses have been reported in patients who clear infection [71].

As we have described above, the innate-like lymphocytes include natural killer T (NKT) cells, gamma delta T ($\gamma\delta$-T) cells, and MAIT cells. The main characteristic of these cells is their limited antigen receptor variability, and, therefore, these T cells have a memory phenotype in the absence of deliberate immunization [16]. NKT cells are activationally restricted by the MHC class I-like molecule called CD1d [72]. NKT cells are potent producers of proinflammatory mediators such as IFN-γ; they are capable of activating macrophages, NK cells, dendritic cells, and effector T cells and possess cytotoxic effector activity [73]. Altogether, they have been thought to be significant promoters of the dysregulated septic response [74]. Recently, Heffernan et al. have demonstrated that invariant NKT (iNKT) cells, a type of NKT cells that express an invariant Vα24/Jα18 chain and a restricted β chain [72], are increased in sepsis and this is most pronounced in geriatric nonsurviving patients [75]. However, Grimaldi et al.

did not observe any quantitative changes in circulating NKT cells in critically ill patients with severe infections [76].

$\gamma\delta$-T cells are preferentially localized in mucosal organs containing epithelia and are known to regulate macrophages [77]. Circulating $\gamma\delta$-T cells count is reduced in patients with sepsis [78, 79], and this reduction seems to become more intense as the septic process becomes more severe [80]. Thus, these studies suggest the key role of $\gamma\delta$-T cells in the defense against infection and open up the possibility of initial explorations of new therapeutic strategies [80].

MAIT cells have the ability to be activated in the presence of antigen-presenting cells infected with Gram-positive (except streptococcal and enterococcal bacteria), Gram-negative bacteria and yeasts [81]. They display fast activation upon microbial infection and rapidly express effector mechanisms including high amounts of proinflammatory cytokines production such as INF-γ and IL-17. A recent study by Grimaldi et al. showed an early and marked decrease in MAIT cell counts in patients with severe sepsis and a relationship between this reduction throughout the first 4 days of ICU admission and the development of ICU-acquired infections [76]. These findings suggest that MAIT cells are involved in sepsis-induced immunosuppression. Therefore, the understanding of innate-like lymphocytes may be crucial for the development of potential therapies to restore immune system function in patients with sepsis.

4. Natural Killer (NK) Cells

Recently, several works have highlighted a key role of natural killer cells during sepsis [82, 83]. NK cells have effector cytotoxic activities and immunoregulatory functions such as the production of cytokines such as IFN-γ, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [84, 85]. NK cells are also engaged in crosstalks with other immune cells, such as dendritic cells [86], monocytes, macrophages [87], and neutrophils [88]. Furthermore, NK cells are probably directly involved in the antibacterial response of the innate immune system due to their capacity to recognize pathogen-associated molecular patterns [89]. It is possible that all NK cell subsets are not equivalent in their antibacterial activity [90].

In sepsis, severe lymphopenia also affects circulating NK cells [38, 82, 83]. Andaluz-Ojeda et al. have reported that patients with the highest NK cell number had the lowest probability to survive [83]. However, we do not find higher percentages of NK cells in nonsurviving patients with septic shock in agreement with other authors [82, 91, 92]. Sepsis is also associated with an activation of NK cells. CD69 is rapidly induced in NK cells and its role in NK cytotoxic has been demonstrated in humans [93]. An increase in the counts and percentage of the CD3−CD56+CD69+ cells in nonsurvivors at ICU admission and 48 hours later has been shown [82]. We also found a higher percentage of the expression of CD57, a marker of long-lived and highly differentiated effector NK cells, in patients with septic shock who died. Our data demonstrate that surviving patients with septic shock exhibited more NK cells depletion than nonsurviving ones and that these NK cells are early activated and rapidly differentiated in patients with septic shock.

Functions of circulating NK cells in critically ill patients have been poorly studied. Unexpectedly, and in apparent contradiction with murine data [94–100], Forel et al. found that patients with sepsis exhibited decreased production of IFN-γ, especially those who presented with septic shock [92].

5. B Cells

Classically, B lymphocytes are characterized by their ability to differentiate into immunoglobulin secreting plasma cells. However, B cells also play critical immunoregulatory roles as antigen presenting cells and also as cytokine producing cells. Currently, it is accepted that B cells have an important role in both adaptive and innate immune responses [101]. During the immune response against infectious agents, the production of antibodies by antigen activated B lymphocyte clones is critical for the efficient eradication of many agents. B cells may also act as effective antigen presenting cells of the microorganism antigens to T lymphocytes [102]. Furthermore, the interaction of several bacterial products with B cells may also cause their activation and cytokine secretory function [101]. Interestingly, the activation of B cells by microorganisms takes place not only by antigen recognition, but also through the activation of Toll-like receptors (TLR). Dual antigen-specific B-cell receptors (BCR) and TLR engagement can fine-tune functional B-cell responses, directly linking cell-intrinsic innate and adaptive immune responses [103]. Moreover, it has been demonstrated that B cells also release a broad variety of cytokines. Pivotal to B cells is IL-10 production, which inhibits proinflammatory cytokines and restrains the excessive inflammatory responses that occur during autoimmune diseases or that can be caused by unresolved infections [104, 105].

The role of B lymphocytes in the pathogenesis of sepsis has not been established. Recently, it has been proposed that B cells are involved in the early innate immune response during experimental bacterial sepsis [101] and Darton et al. reported that adults who have recovered from an episode of invasive pneumococcal disease demonstrate defective B-cell activation [106]. More recently, Rauch et al. have demonstrated that innate response activator B cells (IRA-B cells) play a critical role in the response to sepsis, as mice lacking B-cell-derived GM-CSF are unable to clear bacteria, elicit exaggerated inflammatory responses, and, more likely, succumb to infection [107]. Moreover, they have already developed an in vitro system to expand IRA-B cells from their precursors and then inject them into the patient to boost their immune response [108]. Thus, B cells appear to play a role in the sepsis immunodisturbance [109] and one may expect that, by restoring their function, the overall immune response could be improved.

We have reported that patients with septic shock suffer from a severe retraction of peripheral blood B lymphocytes [110]. Furthermore, circulating B cells show increased expression of CD95 antigen. As described in T cells [111], the increased expression of CD95 on B cells from patients with septic shock might contribute to the observed reduction

of circulating B cells in these patients. Several studies have shown an inverse correlation between lymphocyte count and survival [23, 111]. We also found higher percentage of CD95 expression on B cells from nonsurvivors than that from survivors [110].

The retraction of circulating B cells affects heterogeneously the different B-cell subsets in patients with septic shock [100]. In patients with septic shock, the numbers of circulating CD19+CD69+ remain normal, but CD19+CD23+ B lymphocytes are clearly decreased. Furthermore, higher percentages of circulating CD19+CD23+ are associated with better clinical outcome of the patients [110]. CD23 is involved in different regulatory functions such as enhancing antigen presentation, improving B-cell differentiation, and growth [112]. Some authors have reported that CD23 is expressed on activated B cells whereas others have suggested that peripheral blood CD23 B cells resemble classic memory cells [112].

CD80 and CD86 are critical molecules in the B-cell antigen presentation function. In murine studies of sepsis, an important role for CD80 and CD86 antigens in the response to sepsis has been established [113, 114]. Our results showed higher percentage of CD86 expression on B cells from patients with septic shock. Furthermore, at ICU admission, nonsurvivors had more elevated percentages of CD19+CD80+ B cells than those found in survivors [110].

6. Peripheral Blood Lymphocytes as Biomarkers in Sepsis

Leukocyte phenotyping might also have a predictive value for the development of immune-supportive or immunostimulatory therapies in the management of septic shock patients [115, 116]. We have also studied the predicting value for the outcome of combining different T- cell, B-cell, and NK cell markers in patients with septic shock. According to cytomics methodology [117], we have found a set of five immunophenotypic variables (CD3+CD8+CD28+, CD3+CD8+CD45RA+CD45RO−, CD19+CD80+, CD56+CD69+, and CD3+CD11A br+CD11B+ lymphocyte subsets) which are able to improve the prediction for outcome in septic shock patients to a sensitivity of 94% and a specificity of 100% [118].

7. Therapeutic Approaches for Restoring Lymphocyte Functions

Despite the extraordinary developments in understanding the immunopathology of sepsis, advances in immunotherapy have been very disappointing. Recognizing the pivotal role of lymphocytes in orchestrating the body's immune response against infection, based on their ability to interact with cells of innate and adaptive immune system, it might be possible to decrease mortality in sepsis by therapies to augment host immune response through restoring lymphocytes function.

Therapeutic strategies for treating lymphocyte alterations in patients with sepsis should include the restoration of lymphocyte count and function or the blockade of inhibitory signals (Figure 1). Currently, the most promising immunotherapeutic agent is purified interleukin (IL)-7. IL-7 is an essential cytokine that affects both T and B cells and induce T lymphocyte development, survival, expansion, and maturation in humans [119]. In experimental models of sepsis, IL-7 treatment increased the production of CD4 and CD8 cells, restored delayed type hypersensitivity responses, blocked lymphocyte apoptosis, reversed the impaired IFN-γ production leading to macrophage activation, increased expression of cell-adhesion molecules leading to improved T cells recruitment to sites of infection, and increased T-cell receptor diversity leading to more potent immunity against pathogens [120]. Furthermore, IL-7 can mediate the crosstalk between T_{H1} and T_{H17} lymphocytes during sepsis such that neutrophil recruitment and bacterial clearance are improved [121].

IL-15 is also a pleiotropic cytokine having promising results in experimental models of sepsis. The administration of IL-15 improved survival in two different models of sepsis and was associated with an increase in lymphocyte survival, decreased apoptosis of NK cells, dendritic cells, and T cells, and increased IFN-γ secretion [122].

Fms-like tyrosine kinase 3 ligand is a cytokine capable of enhancing the sensitivity of antigen-specific B and T-cell responses upon bacterial challenge, and it would be another potential treatment in infectious disease [123].

The development of lymphocyte apoptosis is markedly more increased in septic patients than in critically ill nonseptic controls [23]. Strategies to block programmed cell death in lymphocytes are suggested to be beneficial in sepsis [119]. Also, amplified antiapoptotic signals might be of therapeutic value. A genetically manipulated T-cell resistant to apoptosis and polyclonal for a variety of pathogens could be transfused during immune dysfunction to restore patient immunity. For instance, mice transfected with the human gene *Bcl-2* were protected from death after cecal ligation and puncture [24]. Moreover, the transfer of T cells from *Bcl-2* overexpressing mice into wild type septic mice also improved survival [124]. More recently, cell penetrating peptides (CPPs) have been used to deliver the antiapoptotic Bcl-xL-derived BH4 peptide to prevent injury-induced apoptosis both in vitro and in vivo [125]. Furthermore, administration of ritonavir, a HIV protease inhibitor which is known to prevent apoptosis in vitro, improved survival in mice with sepsis [126].

The development of blocking antibodies to multiple inhibitory receptors involved in sepsis represents another innovative therapeutic strategy. PD-1 (programmed death 1) is a negative costimulatory molecule expressed on immune effector cells. It is upregulated in sepsis and impairs immunity by inducing apoptosis, increasing production of IL-10, preventing T-cell proliferation, and causing T-cell exhaustion. Guignant et al. showed that PD-1 overexpression on circulating T cells from patients with sepsis, correlated with decreased T-cell proliferation, increased secondary nosocomial infections and mortality [127]. In animal models of bacterial and fungal sepsis, blockade of the PD-1 pathway improves survival [128–130]. Additional receptors associated with cell exhaustion such as BTLA, TIM-3, LAG-3, and CTLA-4 may be good potential therapeutic targets [131].

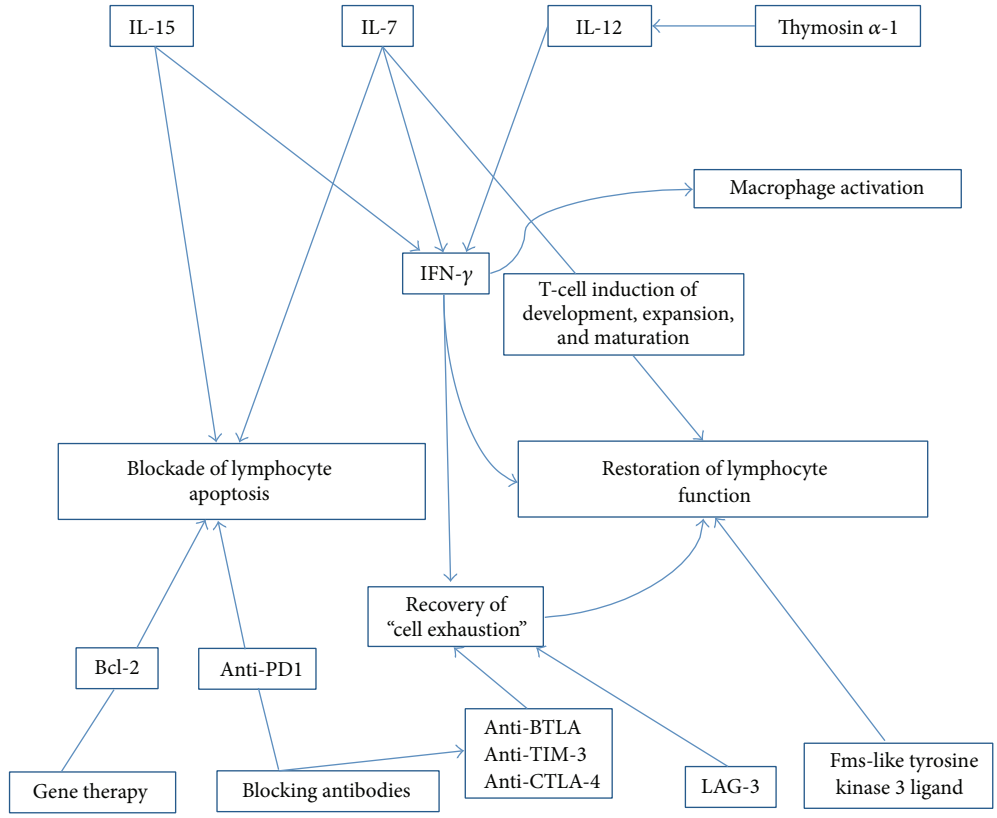

Figure 1: Therapeutics approaches to counteract apoptosis and recovery lymphocyte functions. Based on lymphocyte alterations described in this review, two main therapeutic strategies must be taken into account in patients with sepsis: to block lymphocyte apoptosis for recovery of lymphocyte count or to restore effector lymphocyte functions. Abbreviations: IL: interleukin; IFN: interferon; Bcl-2: B-cell lymphoma 2 gen; PD-1: programmed cell death protein 1; BTLA: B- and T-lymphocyte attenuator; TIM-3: T-cell immunoglobulin and mucin protein 3; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; LAG-3: lymphocyte activation gene 3.

Table 1: Main lymphocyte types' alterations in sepsis.

Lymphocyte type	Roles	Outcomes in sepsis	References
CD4+ T cells	T_{H1}: positive feedback on antigen presenting cells. T_{H2}: promoting downregulation.	Lack of a shift from T_{H1} to T_{H2} increases survival.	[35]
	T_{H17}: producing IL-17, IL-21, TNF-α, and IFN-γ.	T_{H17} lymphocyte count in survivors was higher.	[52]
	Treg: immunosuppressive activity.	Treg lymphocyte counts higher in surviving patients	[41]
CD8+ T cells	Effector cytotoxic cells.	In survivors, CD8+ T lymphocytes showed a further drop on day 3 of follow-up.	[36]
NK cells	Effector cytotoxic activity and immunoregulatory function.	CD69+, an activation marker, increases in nonsurvivors. CD57+, a marker of long-lived and differentiated NK cells, shows higher percentage in nonsurvivors.	[72]
B cells	Ability to differentiate into immunoglobulin secreting plasma cells.	An inverse correlation between count and survival, but this relation affects heterogeneously the subsets.	[23, 101]

Abbreviations: T_H: T helper; IL: interleukin; TNF: tumor necrosis factor; IFN: interferon.

IFN-γ plays a pivotal role in regulating the adaptive immune response mediated by T-lymphocytes and dendritic cells and in controlling the NK and phagocytic cells of the innate host immune antimicroorganism defense system. Our group showed that surviving patients exhibited significantly higher levels of IFN-γ than the healthy controls during the first 14 days of monitoring [12]. Thus, it could be that the increased circulating IFN-γ levels noted here in survivors might be linked to a better immune response against the microorganisms causing septic shock. Döcke et al. treated patients with sepsis showing low monocyte HLA-DR expression with IFN-γ and observed the recovery of the deficient HLA-DR expression. Clearance of sepsis was achieved in eight out of nine patients. IFN-γ is a critical immunoregulatory cytokine [132].

IL-12 is a cytokine that induces expression of the T_{H1} lymphocyte phenotype [133]. IL-12 increased survival in an animal model of burn injury after a septic challenge. It acts, at least in part, through IFN-gamma [134]. On the other hand, thymosin alpha-1 (Tα1) is a molecule with known immunostimulating properties [135], and it can induce T-cell and dendritic cell maduration as well as increasing IL-12 expression. Wu et al. showed a reduction in 28-day mortalities in patients with severe sepsis with an associated increase in mHLA-DR [136].

In this review, we have described the main changes in circulating lymphocytes from patients with sepsis (Table 1). Do not forget that these cells perform their function in peripheral tissues. Boomer studied postmortem spleen and lung tissue from patients with sepsis who died in ICU and they found that circulating blood cells showed similar findings to those in previous studies [22]. However, the analysis of circulating lymphocytes to be clinically relevant has to be confirmed in a large-size population, with standardized methods, [133] and finally—and most importantly—any targeted therapeutic intervention to restore immune system function must be demonstrated in large randomized studies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was partially funded by Grants from Comunidad de Madrid, Spain, MITIC-CM (S-2010/BMD-2502) and Instituto de Salud Carlos III, Spain, MEC (PI051871, CIBERehd).

References

[1] R. C. Bone, R. A. Balk, F. B. Cerra et al., "Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicin," *Chest*, vol. 101, pp. 1644–1655, 1992.

[2] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, "Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care," *Critical Care Medicine*, vol. 29, no. 7, pp. 1303–1310, 2001.

[3] V. Y. Dombrovskiy, A. A. Martin, J. Sunderram, and H. L. Paz, "Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003," *Critical Care Medicine*, vol. 35, no. 5, pp. 1244–1250, 2007.

[4] G. S. Martin, D. M. Mannino, S. Eaton, and M. Moss, "The epidemiology of sepsis in the United States from 1979 through 2000," *The New England Journal of Medicine*, vol. 348, no. 16, pp. 1546–1554, 2003.

[5] R. S. Hotchkiss and I. E. Karl, "The pathophysiology and treatment of sepsis," *The New England Journal of Medicine*, vol. 348, no. 2, pp. 138–150, 2003.

[6] M. M. Levy, W. L. Macias, J. Vincent et al., "Early changes in organ function predict eventual survival in severe sepsis," *Critical Care Medicine*, vol. 33, no. 10, pp. 2194–2201, 2005.

[7] J. Cohen, "The immunopathogenesis of sepsis," *Nature*, vol. 420, no. 6917, pp. 885–891, 2002.

[8] R. de Pablo, J. Monserrat, A. Prieto, and M. Álvarez-Mon, "Role of circulating soluble chemokines in septic shock," *Medicina Intensiva*, vol. 37, no. 8, pp. 510–518, 2012.

[9] R. De Pablo Sánchez, J. Monserrat Sanz, A. Prieto Martín, E. Reyes Martín, M. Alvarez De Mon Soto, and M. Sanchez Garcia, "The balance between pro-inflammatory and anti-inflammatory citokines in septic states," *Medicina Intensiva*, vol. 29, no. 3, pp. 151–158, 2005.

[10] L. M. Hoesel, T. A. Neff, S. B. Neff et al., "Harmful and protective roles of neutrophils in sepsis," *Shock*, vol. 24, no. 1, pp. 40–47, 2005.

[11] M. Adib-Conquy and J. M. Cavaillon, "Compensatory anti-inflammatory response syndrome," *Thrombosis and Haemostasis*, vol. 101, no. 1, pp. 36–47, 2009.

[12] R. De Pablo, J. Monserrat, E. Reyes et al., "Mortality in patients with septic shock correlates with anti-inflammatory but not proinflammatory immunomodulatory molecules," *Journal of Intensive Care Medicine*, vol. 26, no. 2, pp. 125–132, 2011.

[13] J.-M. Cavaillon, M. Adib-Conquy, I. Cloëz-Tayarani, and C. Fitting, "Immunodepression in sepsis and SIRS assessed by ex vivo cytokine production is not a generalized phenomenon: a review," *Journal of Endotoxin Research*, vol. 7, no. 2, pp. 85–93, 2001.

[14] R. Salomao, M. K. C. Brunialti, M. M. Rapozo, G. L. Baggio-Zappia, C. Galanos, and M. Freudenberg, "Bacterial sensing, cell signaling, and modulation of the immune response during sepsis," *Shock*, vol. 38, no. 3, pp. 227–242, 2012.

[15] J. J. Douglas, J. L. Tsang, and K. R. Walley, "Sepsis and the innate-like response," *Intensive Care Medicine*, vol. 40, no. 2, pp. 249–251, 2014.

[16] A. Bendelac, M. Bonneville, and J. F. Kearney, "Autoreactivity by design: innate B and T lymphocytes," *Nature Reviews Immunology*, vol. 1, no. 3, pp. 177–186, 2001.

[17] J. Monserrat, R. de Pablo, D. Díaz et al., "Cellular and molecular markers of outcome in septic shock, severe sepsis and septic shock," in *Understanding a Serious Killer*, Intech, 2012.

[18] M. Rodriguez-Zapata, I. Salmeron, L. Manzano, O. J. Salmeron, A. Prieto, and M. Alvarez-Mon, "Defective interferon-gamma production by T-lymphocytes from patients with acute brucellosis," *European Journal of Clinical Investigation*, vol. 26, no. 2, pp. 136–140, 1996.

[19] J. H. Anolik, R. J. Looney, F. E. Lund, T. D. Randall, and I. Sanz, "Insights into the heterogeneity of human B cells: diverse functions, roles in autoimmunity, and use as therapeutic targets," *Immunologic Research*, vol. 45, no. 2-3, pp. 144–158, 2009.

[20] A. Albillos, A. De La Hera, E. Reyes et al., "Tumour necrosis factor-alpha expression by activated monocytes and altered T-cell homeostasis in ascitic alcoholic cirrhosis: amelioration with norfloxacin," *Journal of Hepatology*, vol. 40, no. 4, pp. 624–631, 2004.

[21] A. Albillos, A. D. la Hera, M. González et al., "Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement," *Hepatology*, vol. 37, no. 1, pp. 208–217, 2003.

[22] J. S. Boomer, K. To, K. C. Chang et al., "Immunosuppression in patients who die of sepsis and multiple organ failure," *Journal of the American Medical Association*, vol. 306, no. 23, pp. 2594–2605, 2011.

[23] R. S. Hotchkiss, P. E. Swanson, B. D. Freeman et al., "Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction," *Critical Care Medicine*, vol. 27, no. 7, pp. 1230–1251, 1999.

[24] R. S. Hotchkiss, K. W. Tinsley, P. E. Swanson et al., "Prevention of lymphocyte cell death in sepsis improves survival in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 25, pp. 14541–14546, 1999.

[25] C. S. Chung, Y. X. Xu, W. Wang, I. H. Chaudry, and A. Ayala, "Is Fas ligand or endotoxin responsible for mucosal lymphocyte apoptosis in sepsis?" *Archives of Surgery*, vol. 133, no. 11, pp. 1213–1220, 1998.

[26] R. S. Hotchkiss, K. C. Chang, P. E. Swanson et al., "Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte," *Nature Immunology*, vol. 1, no. 6, pp. 496–501, 2000.

[27] C. Oberholzer, A. Oberholzer, F. R. Bahjat et al., "Targeted adenovirus-induced expression of IL-10 decreases thymic apoptosis and improves survival in murine sepsis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 20, pp. 11503–11508, 2001.

[28] F. Venet, A. Lepape, and G. Monneret, "Clinical review: flow cytometry perspectives in the ICU-from diagnosis of infection to monitoring of injury-induced immune dysfunctions," *Critical Care*, vol. 15, no. 5, article 231, 2011.

[29] G. Monneret, F. Venet, A. Pachot, and A. Lepape, "Monitoring immune dysfunctions in the septic patient: a new skin for the old ceremony," *Molecular Medicine*, vol. 14, no. 1-2, pp. 64–78, 2008.

[30] J. L. Meakins, J. B. Pietsch, O. Bubenick et al., "Delayed hypersensitivity: indicator of acquired failure of host defenses in sepsis and trauma," *Annals of Surgery*, vol. 186, no. 3, pp. 241–250, 1977.

[31] C. Heidecke, T. Hensler, H. Weighardt et al., "Selective defects of T lymphocyte function in patients with lethal intraabdominal infection," *The American Journal of Surgery*, vol. 178, no. 4, pp. 288–292, 1999.

[32] J. B. Ochoa and V. Makarenkova, "T lymphocytes," *Critical Care Medicine*, vol. 33, no. 12 supplement, pp. S510–S513, 2005.

[33] T. R. Mosmann and R. L. Coffman, "TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties," *Annual Review of Immunology*, vol. 7, pp. 145–173, 1989.

[34] C. Adrie, M. Adib-Conquy, I. Laurent et al., "Successful cardiopulmonary resuscitation after cardiac arrest as a sepsis-like syndrome," *Circulation*, vol. 106, pp. 562–568, 2002.

[35] H. P. Wu, K. Chung, C. Y. Lin, B. Y. Jiang, D. Y. Chuang, and Y. C. Liu, "Associations of T helper 1, 2, 17 and regulatory T lymphocytes with mortality in severe sepsis," *Inflammation Research*, vol. 62, no. 8, pp. 751–763, 2013.

[36] N. R. Ferguson, H. F. Galley, and N. R. Webster, "T helper cell subset ratios in patients with severe sepsis," *Intensive Care Medicine*, vol. 25, no. 1, pp. 106–109, 1999.

[37] W. G. Cheadle, R. M. Pemberton, D. Robinson, D. H. Livingston, J. L. Rodriguez, and H. C. Polk Jr., "Lymphocyte subset responses to trauma and sepsis," *Journal of Trauma*, vol. 35, no. 6, pp. 844–849, 1993.

[38] C. H. Wakefield, P. D. Carey, S. Foulds, J. R. T. Monson, and P. J. Guillou, "Changes in major histocompatibility complex class II expression in monocytes and T cells of patients developing infection after surgery," *British Journal of Surgery*, vol. 80, no. 2, pp. 205–209, 1993.

[39] J. Monserrat, R. de Pablo, E. Reyes et al., "Clinical relevance of the severe abnormalities of the T cell compartment in septic shock patients," *Critical Care*, vol. 13, no. 1, article R26, 2009.

[40] R. S. Hotchkiss, K. W. Tinsley, P. E. Swanson et al., "Sepsis-induced apoptosis causes progressive profound depletion of B and $CD4^+$ T lymphocytes in humans," *Journal of Immunology*, vol. 166, no. 11, pp. 6952–6963, 2001.

[41] F. Venet, F. Davin, C. Guignant et al., "Early assessment of leukocyte alterations at diagnosis of septic shock," *Shock*, vol. 34, no. 4, pp. 358–363, 2010.

[42] K. Hirahara, A. Poholek, G. Vahedi et al., "Mechanisms underlying helper T-cell plasticity: implications for immune-mediated disease," *Journal of Allergy and Clinical Immunology*, vol. 131, no. 5, pp. 1276–1287, 2013.

[43] E. Bettelli, Y. Carrier, W. Gao et al., "Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells," *Nature*, vol. 441, no. 7090, pp. 235–238, 2006.

[44] P. R. Mangan, L. E. Harrington, D. B. O'Quinn et al., "Transforming growth factor-β induces development of the T_H17 lineage," *Nature*, vol. 441, no. 7090, pp. 231–234, 2006.

[45] M. Veldhoen, R. J. Hocking, C. J. Atkins, R. M. Locksley, and B. Stockinger, "TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells," *Immunity*, vol. 24, no. 2, pp. 179–189, 2006.

[46] C. L. Roark, P. L. Simonian, A. P. Fontenot, W. K. Born, and R. L. O'Brien, "$\gamma\delta$ T cells: an important source of IL-17," *Current Opinion in Immunology*, vol. 20, no. 3, pp. 353–357, 2008.

[47] M. H. Lexberg, A. Taubner, A. Förster et al., "Th memory for interleukin-17 expression is stable in vivo," *European Journal of Immunology*, vol. 38, no. 10, pp. 2654–2664, 2008.

[48] Y. K. Lee, H. Turner, C. L. Maynard et al., "Late developmental plasticity in the T helper 17 lineage," *Immunity*, vol. 30, no. 1, pp. 92–107, 2009.

[49] E. Bettelli, T. Korn, M. Oukka, and V. K. Kuchroo, "Induction and effector functions of TH17 cells," *Nature*, vol. 453, no. 7198, pp. 1051–1057, 2008.

[50] C. S. Ma, G. Y. J. Chew, N. Simpson et al., "Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3," *Journal of Experimental Medicine*, vol. 205, no. 7, pp. 1551–1557, 2008.

[51] P. Ye, F. H. Rodriguez, S. Kanaly et al., "Requirement of interleukin 17 receptor signaling for lung CXC chemokine

and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense," *Journal of Experimental Medicine*, vol. 194, no. 4, pp. 519–527, 2001.

[52] M. Nishijima, "Somatomedin-C as a fetal growth promoting factor and amino acid composition of cord blood in Japanese neonates," *Journal of Perinatal Medicine*, vol. 14, no. 3, pp. 163–169, 1986.

[53] D. B. Hoyt, A. N. Ozkan, J. L. Ninnemann, J. F. Hansbrough, E. Pinney, and S. Wormsley, "Trauma peptide induction of lymphocyte changes predictive of sepsis," *Journal of Surgical Research*, vol. 45, no. 4, pp. 342–348, 1988.

[54] R. Y. Lin, M. E. Astiz, J. C. Saxon, and E. C. Rackow, "Altered leukocyte immunophenotypes in septic shock: Studies of HLA-DR, CD11b, CD14, and IL-2R expression," *Chest*, vol. 104, no. 3, pp. 847–853, 1993.

[55] R. S. Hotchkiss, K. W. Tinsley, P. E. Swanson et al., "Depletion of dendritic cells, but not macrophages, in patients with sepsis," *Journal of Immunology*, vol. 168, no. 5, pp. 2493–2500, 2002.

[56] H. Jonuleit, E. Schmitt, K. Steinbrink, and A. H. Enk, "Dendritic cells as a tool to induce anergic and regulatory T cells," *Trends in Immunology*, vol. 22, no. 7, pp. 394–400, 2001.

[57] L. N. Jiang, Y. M. Yao, and Z. Y. Sheng, "The role of regulatory T cells in the pathogenesis of sepsis and its clinical implication," *Journal of Interferon and Cytokine Research*, vol. 32, no. 8, pp. 341–349, 2012.

[58] F. Hein, F. Massin, A. Cravoisy-Popovic et al., "The relationship between CD4+CD25+CD127- regulatory T cells and inflammatory response and outcome during shock states," *Critical Care*, vol. 14, no. 1, article R19, 2010.

[59] G. Monneret, A. L. Debard, F. Venet et al., "Marked elevation of human circulating CD4+CD25+ regulatory T cells in sepsis-induced immunoparalysis," *Critical Care Medicine*, vol. 31, no. 7, pp. 2068–2071, 2003.

[60] F. Venet, C. Chung, H. Kherouf et al., "Increased circulating regulatory T cells ($CD4^+CD25^+CD127^-$) contribute to lymphocyte anergy in septic shock patients," *Intensive Care Medicine*, vol. 35, no. 4, pp. 678–686, 2009.

[61] M. Holub, Z. Klucková, B. Beneda et al., "Changes in lymphocyte subpopulations and $CD3^+/DR^+$ expression in sepsis," *Clinical Microbiology and Infection*, vol. 6, no. 12, pp. 657–660, 2000.

[62] M. Holub, Z. Klučková, M. Helcl, J. Přihodov, R. Rokyta, and O. Beran, "Lymphocyte subset numbers depend on the bacterial origin of sepsis," *Clinical Microbiology and Infection*, vol. 9, no. 3, pp. 202–211, 2003.

[63] S. Kabisch, K. Gemar, W. Krumholz, F. Salomon, and H. Pralle, "Lymphocyte subpopulations in sepsis-prone patients in a surgical intensive care unit," *Anaesthesist*, vol. 39, no. 9, pp. 439–444, 1990.

[64] T. Mustelin, K. M. Coggeshall, and A. Altman, "Rapid activation of the T-cell tyrosine protein kinase pp56(lck) by the CD45 phosphotyrosine phosphatase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 16, pp. 6302–6306, 1989.

[65] M. L. Hermiston, Z. Xu, and A. Weiss, "CD45: a critical regulator of signaling thresholds in immune cells," *Annual Review of Immunology*, vol. 21, pp. 107–137, 2003.

[66] D. M. Sansom and L. S. K. Walker, "The role of CD28 and cytotoxic T-lymphocyte antigen-4 (CTLA-4) in regulatory T-cell biology," *Immunological Reviews*, vol. 212, pp. 131–148, 2006.

[67] S. Fiorentini, S. Licenziati, G. Alessandri et al., "Cd11b expression identifies $cd8^+cd28^+$ T lymphocytes with phenotype and function of both naive/memory and effector cells," *The Journal of Immunology*, vol. 166, no. 2, pp. 900–907, 2001.

[68] M. Labalette, E. Leteurtre, C. Thumerelle, C. Grutzmacher, B. Tourvieille, and J. Dessalnt, "Peripheral human $CD8^+CD28^+$ T lymphocytes give rise to $CD28^-$ progeny, but IL-4 prevents loss of CD28 expression," *International Immunology*, vol. 11, no. 8, pp. 1327–1336, 1999.

[69] J. Manjuck, D. C. Saha, M. Astiz, L. Eales, and E. C. Rackow, "Decreased response to recall antigens is associated with depressed costimulatory receptor expression in septic critically ill patients," *Journal of Laboratory and Clinical Medicine*, vol. 135, no. 2, pp. 153–160, 2000.

[70] M. L. K. Tang, D. A. Steeber, X. Q. Zhang, and T. F. Tedder, "Intrinsic differences in L-selectin expression levels affect T and B lymphocyte subset-specific recirculation pathways," *Journal of Immunology*, vol. 160, no. 10, pp. 5113–5121, 1998.

[71] P. Sarobe, J.-J. Lasarte, N. García, M. P. Civeira, F. Borrás-Cuesta, and J. Prieto, "Characterization of T-cell responses against immunodominant epitopes from hepatitis C virus E2 and NS4a proteins," *Journal of Viral Hepatitis*, vol. 13, no. 1, pp. 47–55, 2006.

[72] D. I. Godfrey, H. R. MacDonald, M. Kronenberg, M. J. Smyth, and L. Van Kaer, "NKT cells: what's in a name?" *Nature Reviews Immunology*, vol. 4, no. 3, pp. 231–237, 2004.

[73] M. J. Smyth, K. Y. T. Thia, S. E. A. Street et al., "Differential tumor surveillance by natural killer (NK) and NKT cells," *Journal of Experimental Medicine*, vol. 191, no. 4, pp. 661–668, 2000.

[74] B. Leung and H. W. Harris, "NKT cells: the culprits of sepsis?" *Journal of Surgical Research*, vol. 167, no. 1, pp. 87–95, 2011.

[75] D. S. Heffernan, S. F. Monaghan, C. S. Chung, W. G. Cioffi, S. Gravenstein, and A. Ayala, "A divergent response of innate regulatory T-cells to sepsis in humans: circulating invariant natural killer T-cells are preserved," *Human Immunology*, vol. 75, no. 3, pp. 277–282, 2014.

[76] D. Grimaldi, L. Le Bourhis, B. Sauneuf et al., "Specific MAIT cell behaviour among innate-like T lymphocytes in critically ill patients with severe infections," *Intensive Care Medicine*, vol. 40, no. 2, pp. 192–201, 2014.

[77] E. M. Andrew and S. R. Carding, "Murine $\gamma\delta$ T cells in infections: beneficial or deleterious?" *Microbes and Infection*, vol. 7, no. 3, pp. 529–536, 2005.

[78] F. Venet, J. Bohé, A. Debard, J. Bienvenu, A. Lepape, and G. Monneret, "Both percentage of $\gamma\delta$ T lymphocytes and CD3 expression are reduced during septic shock," *Critical Care Medicine*, vol. 33, no. 12, pp. 2836–2840, 2005.

[79] A. Matsushima, H. Ogura, K. Fujita et al., "Early activation of $\gamma\delta$ T lymphocytes in patients with severe systemic inflammatory response syndrome," *Shock*, vol. 22, no. 1, pp. 11–15, 2004.

[80] J. C. Andreu-Ballester, C. Tormo-Calandín, C. Garcia-Ballesteros et al., "Association of $\gamma\delta$ T cells with disease severity and mortality in septic patients," *Clinical and Vaccine Immunology*, vol. 20, no. 5, pp. 738–746, 2013.

[81] . Le Bourhis L, E. Martin, I. Peguillet et al., "Antimicrobial activity of mucosal-associated invariant T cells," *Nature Immunology*, vol. 11, no. 8, pp. 701–708, 2010.

[82] R. de Pablo, J. Monserrat, C. Torrijos, M. Martín, A. Prieto, and M. Alvarez-Mon, "The predictive role of early activation of natural killer cells in septic shock," *Critical Care*, vol. 16, no. 2, article 413, 2012.

[83] D. Andaluz-Ojeda, V. Iglesias, F. Bobillo et al., "Early natural killer cell counts in blood predict mortality in severe sepsis," *Critical Care*, vol. 15, no. 5, Article ID R243, 2011.

[84] C. Romagnani, K. Juelke, M. Falco et al., "CD56brightCD16- killer Ig-like receptor- NK cells display longer telomeres and acquire features of CD56dim NK cells upon activation," *Journal of Immunology*, vol. 178, no. 8, pp. 4947–4955, 2007.

[85] A. Lunemann, J. D. Lunemann, and C. Munz, "Regulatory NK-cell functions in inflammation and autoimmunity," *Molecular Medicine*, vol. 15, no. 9-10, pp. 352–358, 2009.

[86] T. Walzer, M. Dalod, S. H. Robbins, L. Zitvogel, and E. Vivier, "Natural-killer cells and dendritic cells: 'l'union fait la force'," *Blood*, vol. 106, no. 7, pp. 2252–2258, 2005.

[87] F. Bellora, R. Castriconi, A. Dondero et al., "The interaction of human natural killer cells with either unpolarized or polarized macrophages results in different functional outcomes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 50, pp. 21659–21664, 2010.

[88] C. Costantini and M. A. Cassatella, "The defensive alliance between neutrophils and NK cells as a novel arm of innate immunity," *Journal of Leukocyte Biology*, vol. 89, no. 2, pp. 221–233, 2011.

[89] A. Chalifour, P. Jeannin, J. Gauchat et al., "Direct bacterial protein PAMP recognition by human NK cells involves TLRs and triggers α-defensin production," *Blood*, vol. 104, no. 6, pp. 1778–1783, 2004.

[90] F. Souza-Fonseca-Guimaraes, M. Adib-Conquy, and J. Cavaillon, "Natural killer (NK) cells in antibacterial innate immunity: angels or devils?" *Molecular Medicine*, vol. 18, no. 1, pp. 270–285, 2012.

[91] E. J. Giamarellos-Bourboulis, T. Tsaganos, E. Spyridaki et al., "Early changes of CD4-positive lymphocytes and NK cells in patients with severe Gram-negative sepsis," *Critical Care*, vol. 10, no. 6, article R166, 2006.

[92] J. Forel, L. Chiche, G. Thomas et al., "Phenotype and functions of natural killer cells in critically-ill septic patients," *PLoS ONE*, vol. 7, no. 12, Article ID e50446, 2012.

[93] F. Borrego, M. J. Robertson, J. Ritz, J. Peña, and R. Solana, "CD69 is a stimulatory receptor for natural killer cell and its cytotoxic effect is blocked by CD94 inhibitory receptor," *Immunology*, vol. 97, no. 1, pp. 159–165, 1999.

[94] M. Emoto, M. Miyamoto, I. Yoshizawa et al., "Critical role of NK cells rather than Vα14$^+$NKT cells in lipopolysaccharide-induced lethal shock in mice," *Journal of Immunology*, vol. 169, no. 3, pp. 1426–1432, 2002.

[95] W. E. Carson, H. Yu, J. Dierksheide et al., "A fatal cytokine-induced systemic inflammatory response reveals a critical role for NK cells," *Journal of Immunology*, vol. 162, no. 8, pp. 4943–4951, 1999.

[96] A. R. Kerr, L. A. S. Kirkham, A. Kadioglu et al., "Identification of a detrimental role for NK cells in pneumococcal pneumonia and sepsis in immunocompromised hosts," *Microbes and Infection*, vol. 7, no. 5-6, pp. 845–852, 2005.

[97] B. Badgwell, R. Parihar, C. Magro, J. Dierksheide, T. Russo, and W. E. Carson III, "Natural killer cells contribute to the lethality of a murine model of *Escherichi coli* infection," *Surgery*, vol. 132, no. 2, pp. 205–212, 2002.

[98] A. O. Etogo, J. Nunez, C. Y. Lin, T. E. Toliver-Kinsky, and E. R. Sherwood, "NK but not CD1-restricted NKT cells facilitate systemic inflammation during polymicrobial intra-abdominal sepsis," *Journal of Immunology*, vol. 180, no. 9, pp. 6334–6345, 2008.

[99] L. Chiche, J. Forel, G. Thomas et al., "The role of natural killer cells in sepsis," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 986491, 8 pages, 2011.

[100] H. Heremans, C. Dillen, J. van Damme, and A. Billiau, "Essential role for natural killer cells in the lethal lipopolysaccharide-induced Shwartzman-like reaction in mice," *European Journal of Immunology*, vol. 24, no. 5, pp. 1155–1160, 1994.

[101] K. M. Kelly-Scumpia, P. O. Scumpia, J. S. Weinstein et al., "B cells enhance early innate immune responses during bacterial sepsis," *Journal of Experimental Medicine*, vol. 208, no. 8, pp. 1673–1682, 2011.

[102] A. T. Vaughan, A. Roghanian, and M. S. Cragg, "B cells—Masters of the immunoverse," *International Journal of Biochemistry and Cell Biology*, vol. 43, no. 3, pp. 280–285, 2011.

[103] D. J. Rawlings, M. A. Schwartz, S. W. Jackson, and A. Meyer-Bahlburg, "Integration of B cell responses through Toll-like receptors and antigen receptors," *Nature Reviews Immunology*, vol. 12, no. 4, pp. 282–294, 2012.

[104] C. Mauri and A. Bosma, "Immune regulatory function of B cells," *Annual Review of Immunology*, vol. 30, pp. 221–241, 2012.

[105] J. Booth, H. Wilson, S. Jimbo, and G. Mutwiri, "Modulation of B cell responses by Toll-like receptors," *Cell and Tissue Research*, vol. 343, no. 1, pp. 131–140, 2011.

[106] T. C. Darton, J. B. Wing, A. Lees, A. W. Heath, and R. C. Read, "Adult survivors of invasive pneumococcal disease exhibit defective B cell function," *Clinical Infectious Diseases*, vol. 52, no. 9, pp. 1133–1136, 2011.

[107] P. J. Rauch, A. Chudnovskiy, C. S. Robbins et al., "Innate response activator B cells protect against microbial sepsis," *Science*, vol. 335, no. 6068, pp. 597–601, 2012.

[108] C. S. Robbins and F. K. Swirski, "Newly discovered innate response activator B cells: crucial responders against microbial sepsis," *Expert Review of Clinical Immunology*, vol. 8, no. 5, pp. 405–407, 2012.

[109] N. J. Shubin, S. F. Monaghan, and A. Ayala, "Anti-inflammatory mechanisms of sepsis," *Contributions to Microbiology*, vol. 17, pp. 108–124, 2011.

[110] J. Monserrat, R. de Pablo, D. Diaz-Martín et al., "Early alterations of B cells in patients with septic shock," *Critical Care*, vol. 17, no. 3, article R105, 2013.

[111] G. Roth, B. Moser, C. Krenn et al., "Susceptibility to programmed cell death in T-lymphocytes from septic patients: a mechanism for lymphopenia and Th2 predominance," *Biochemical and Biophysical Research Communications*, vol. 308, no. 4, pp. 840–846, 2003.

[112] L. J. Rosenwasser and J. Meng, "Anti-CD23," *Clinical Reviews in Allergy and Immunology*, vol. 29, no. 1, pp. 61–72, 2005.

[113] A. Nolan, H. Kobayashi, B. Naveed et al., "Differential role for CD80 and CD86 in the regulation of the innate immune response in murine polymicrobial sepsis," *PLoS ONE*, vol. 4, no. 8, Article ID e6600, 2009.

[114] A. Nolan, M. Weiden, A. Kelly et al., "CD40 and CD80/86 act synergistically to regulate inflammation and mortality in polymicrobial sepsis," *The American Journal of Respiratory and Critical Care Medicine*, vol. 177, no. 3, pp. 301–308, 2008.

[115] J. E. McDunn and R. S. Hotchkiss, "Leukocyte phenotyping to stratify septic shock patients," *Critical Care*, vol. 13, no. 2, p. 127, 2009.

[116] F. Venet, A. C. Lukaszewicz, D. Payen, R. Hotchkiss, and G. Monneret, "Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant

therapies," *Current Opinion in Immunology*, vol. 25, no. 4, pp. 477–483, 2013.

[117] G. K. Valet and A. Tárnok, "Cytomics in predictive medicine," *Cytometry B: Clinical Cytometry*, vol. 53, no. 1, pp. 1–3, 2003.

[118] J. Monserrat, R. de Pablo, A. Prieto, E. Reyes, and M. Álvarez-Mon, "Using surface molecule expression on lymphocytes to classify septic shock patients," *Critical Care*, vol. 13, no. 3, article 412, 2009.

[119] F. Turrel, C. Guignant, F. Venet, A. Lepape, and G. Monneret, "Innovative therapeutic strategies for restoring lymphocyte functions in septic patients," *Inflammation and Allergy—Drug Targets*, vol. 7, no. 3, pp. 181–186, 2008.

[120] R. S. Hotchkiss, G. Monneret, and D. Payen, "Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach," *The Lancet Infectious Diseases*, vol. 13, no. 3, pp. 260–268, 2013.

[121] K. R. Kasten, P. S. Prakash, J. Unsinger et al., "Interleukin-7 (IL-7) treatment accelerates neutrophil recruitment through $\gamma\delta$ T-cell IL-17 production in a murine model of sepsis," *Infection and Immunity*, vol. 78, no. 11, pp. 4714–4722, 2010.

[122] S. Inoue, J. Unsinger, C. G. Davis et al., "IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis," *Journal of Immunology*, vol. 184, no. 3, pp. 1401–1409, 2010.

[123] B. Pulendran, J. L. Smith, M. Jenkins, M. Schoenborn, E. Maraskovsky, and C. R. Maliszewski, "Prevention of peripheral tolerance by a dendritic cell growth factor: Flt3 ligand as an adjuvant," *Journal of Experimental Medicine*, vol. 188, no. 11, pp. 2075–2082, 1998.

[124] R. S. Hotchkiss, C. M. Coopersmith, and I. E. Karl, "Prevention of lymphocyte apoptosis: a potential treatment of sepsis?" *Clinical Infectious Diseases*, vol. 41, supplement 7, pp. S465–S469, 2005.

[125] J. E. McDunn, J. T. Muenzer, B. Dunne et al., "An anti-apoptotic peptide improves survival in lethal total body irradiation," *Biochemical and Biophysical Research Communications*, vol. 382, no. 4, pp. 657–662, 2009.

[126] J. G. R. Weaver, M. S. Rouse, J. M. Steckelberg, and A. D. Badley, "Improved survival in experimental sepsis with an orally administered inhibitor of apoptosis," *The FASEB Journal*, vol. 18, no. 11, pp. 1185–1191, 2004.

[127] C. Guignant, A. Lepape, X. Huang et al., "Programmed death-1 levels correlate with increased mortality, nosocomial infection and immune dysfunctions in septic shock patients," *Critical Care*, vol. 15, no. 2, article R99, 2011.

[128] X. Huang, F. Venet, Y. L. Wang et al., "PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 15, pp. 6303–6308, 2009.

[129] P. Brahmamdam, S. Inoue, J. Unsinger, K. C. Chang, J. E. McDunn, and R. S. Hotchkiss, "Delayed administration of anti-PD-1 antibody reverses immune dysfunction and improves survival during sepsis," *Journal of Leukocyte Biology*, vol. 88, no. 2, pp. 233–240, 2010.

[130] Y. Zhang, Y. Zhou, J. Lou et al., "PD-L1 blockade improves survival in experimental sepsis by inhibiting lymphocyte apoptosis and reversing monocyte dysfunction," *Critical Care*, vol. 14, no. 6, article R220, 2010.

[131] J. S. Boomer, J. M. Green, and R. S. Hotchkiss, "The changing immune system in sepsis: is individualized immunomodulatory therapy the answer?" *Virulence*, vol. 5, no. 1, pp. 45–56, 2014.

[132] W. Döcke, F. Randow, U. Syrbe et al., "Monocyte deactivation in septic patients: restoration by IFN-γ treatment," *Nature Medicine*, vol. 3, no. 6, pp. 678–681, 1997.

[133] D. Payen, G. Monneret, and R. Hotchkiss, "Immunotherapy—a potential new way forward in the treatment of sepsis," *Critical Care*, vol. 17, no. 1, article 118, 2013.

[134] C. O'Suilleabhain, S. T. O'Sullivan, J. L. Kelly, J. Lederer, J. A. Mannick, and M. L. Rodrick, "Interleukin-12 treatment restores normal resistance to bacterial challenge after burn injury," *Surgery*, vol. 120, no. 2, pp. 290–296, 1996.

[135] A. Serafino, P. Pierimarchi, F. Pica et al., "Thymosin α1 as a stimulatory agent of innate cell-mediated immune response," *Annals of the New York Academy of Sciences*, vol. 1270, no. 1, pp. 13–20, 2012.

[136] J. Wu, L. Zhou, J. Liu et al., "The efficacy of thymosin alpha 1 for severe sepsis (ETASS): a multicenter, single-blind, randomized and controlled trial," *Critical Care*, vol. 17, no. 1, article R8, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 583736, 10 pages
http://dx.doi.org/10.1155/2014/583736

Research Article

Agmatine Protects against Zymosan-Induced Acute Lung Injury in Mice by Inhibiting NF-κB-Mediated Inflammatory Response

Xuanfei Li, Zheng Liu, He Jin, Xia Fan, Xue Yang, Wanqi Tang, Jun Yan, and Huaping Liang

State Key Laboratory of Trauma, Burns and Combined Injury, Research Institute of Surgery, Daping Hospital, The Third Military Medical University, Chongqing 400042, China

Correspondence should be addressed to Huaping Liang; 13638356728@163.com

Received 16 February 2014; Revised 8 July 2014; Accepted 29 July 2014; Published 27 August 2014

Academic Editor: Baoli Cheng

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Acute lung injury (ALI) is characterized by overwhelming lung inflammation and anti-inflammation treatment is proposed to be a therapeutic strategy for ALI. Agmatine, a cationic polyamine formed by decarboxylation of L-arginine, is an endogenous neuromodulator that plays protective roles in diverse central nervous system (CNS) disorders. Consistent with its neuromodulatory and neuroprotective properties, agmatine has been reported to have beneficial effects on depression, anxiety, hypoxic ischemia, Parkinson's disease, and gastric disorder. In this study, we tested the effect of agmatine on the lung inflammation induced by Zymosan (ZYM) challenge in mice. We found that agmatine treatment relieved ZYM-induced acute lung injury, as evidenced by the reduced histological scores, wet/dry weight ratio, and myeloperoxidase activity in the lung tissue. This was accompanied by reduced levels of TNF-α, IL-1β, and IL-6 in lung and bronchoalveolar lavage fluid and decreased iNOS expression in lung. Furthermore, agmatine inhibited the phosphorylation and degradation of IκB and subsequently blocked the activation of nuclear factor (NF)-κB induced by Zymosan. Taken together, our results showed that agmatine treatment inhibited NF-κB signaling in lungs and protected mice against ALI induced by Zymosan, suggesting agmatine may be a potential safe and effective approach for the treatment of ALI.

1. Introduction

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), which is the severest form of injury, are the leading causes of morbidity and mortality in critically ill patients [1]. ALI is characterized by the development of hypoxemia, damage to the alveolar capillary membrane barrier, pulmonary edema, and the resultant respiratory failure [2]. Current therapeutic strategy includes protective ventilation and supportive fluid conservative [3]. Although extensive studies about the pathogenesis of ALI have been conducted, the mortality of ALI remains very high [4]. Thus, it is critical to explore the innovative therapies and effective medications for ALI.

Among the animal models established for the investigation of mechanism involved in ALI, intraperitoneal injection of Zymosan (ZYM) is one of the most commonly used models of ALI [5]. Zymosan is a substance derived from the cell wall of the yeast *Saccharomyces cerevisiae*. When injected into animals, ZYM induces inflammation by a series of mechanisms. Reports show that the onset of ZYM-induced inflammatory response in mouse lung is associated with the gas exchange barrier and that it culminates with maximal neutrophil accumulation, exudate formation, and proinflammatory cytokines production [6, 7]. ZYM is recognized by toll-like receptor 2 (TLR-2) on immune cells (e.g., neutrophils), which subsequently trigger signal cascade for nuclear factor-κB (NF-κB) activation [8]. NF-κB activation is required for maximal expression of many proinflammatory cytokines and chemokines and iNOS involved in the pathogenesis of acute lung injury [9].

Agmatine, a biogenic amine formed by arginine decarboxylation, is widely but unevenly distributed in mammalian tissues. Agmatine has been reported to have various biological actions. It attenuates morphine withdrawal syndromes, inhibits inducible nitric oxide synthase (NOS), and

contributes to polyamine homeostasis [10]. Additionally it is known to exert antidepressant, anxiolytic, antitumor cell proliferative and anticonvulsive effects [11]. However, the precise working mechanisms of agmatine are not yet fully understood. The aim of our study was to investigate the protective effects of agmatine on Zymosan-induced acute lung injury and to assess its relative mechanisms.

2. Materials and Methods

2.1. Reagents. Zymosan and agmatine were obtained from Sigma-Aldrich. Antibodies against iNOS, NF-κB p65, IκB-α, and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and those against phosphor (p)-IκB and NF-κB p65 were from Cell Signaling Tech (Danvers, MA). Mouse TNF-α, IL-1β, and IL-6 enzyme-lined immunosorbent assay (ELISA) kits were purchased from Boster Biotechnology (Wuhan, China). All suspensions were freshly made before use.

2.2. Animals and Treatments. Male C57Bcl/6 mice (weighing 18–22 g) were used in this study. Animal procedures were approved by the Ethics Committee for Animal Experimentation of Third Military Medical University. An inflammation-associated lung injury model was established by aseptic intraperitoneally (IP) injection of ZYM (30 mg/mL suspended in normal Saline (NS)) into mice, at a dose of 600 mg/kg of body weight, as previously described [12]. The same volume of NS was injected through the same route as the sham control.

2.3. Histologic Examination. Lungs were harvested for observing morphologic alterations at 24 hrs after ZYM or NS administration. The subjects were fixed with 10% formalin for 8 hrs at room temperature, embedded in paraffin, and sectioned at 5 μm thickness. After deparaffinization and rehydration, the sections were sequentially stained with hematoxylin and eosin. Histologic changes were evaluated by two independent pathologists, who had no knowledge of the treatment regimen received by each respective animal. The degree of lung injury was scored on a subjective scale ranging from 0 to 3; 0 = absence, 1 = mild, 2 = moderate, and 3 = severe. The ranging scale was used for each of histologic features: congestion, edema, inflammation, and hemorrhage. The final score will be the adding of the single evaluation.

2.4. Wet/Dry Weight Ratio. To quantify the magnitude of pulmonary edema, we evaluated lung wet/dry (W/D) weight ratio at 24 hrs after NS or ZYM administration. The harvested wet lung was weighed and then placed in an oven for 24 hrs at 80°C and weighed when it was dried. The ratio of wet lung to dry lung was calculated [13].

2.5. BALF Collection. At 24 hrs after administration of ZYM or NS, BALF collection was performed by the methods described previously [14]. The mice were anesthetized with pentobarbital, tracheas were cannulated after exsanguination, and lungs were gently washed with 2 mL of PBS. The amount of exudate was calculated by subtracting the volume injected (2 mL) from the total volume recovered. BALF samples were centrifuged at 500 g at 4°C for 12 mins, and the supernatant was stored at −70°C for subsequent analysis of protein and cytokine levels.

2.6. Measurement of Lung MPO Activity. Myeloperoxidase (MPO) activity was measured as an indicator of neutrophil infiltration into the lung tissue as previously described [15]. At 24 hrs after ZYM or NS injection, all animals ($n = 8$ for each group) were sacrificed with pentobarbital. Lungs were obtained and perfused with cold PBS to remove all blood, and homogenated lung supernatants were prepared to detect the activity of MPO. MPO activity was defined by the change in absorbance measured by spectrophotometer at 590 nm and expressed in unit per gram weight of wet tissue. The activity of MPO was measured by using commercial kits purchased from Boster Biotechnology (Wuhan, China).

2.7. Measurement of Cytokine Production. At 6 hrs after ZYM or NS injection, the cytokines levels in BALF and lung tissue were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (mouse TNF-α, IL-1β, and IL-6 ELISA kits are from Boster Biotechnology, Wuhan, China). The optical density (OD) was measured on an ELISA plate scanner. All experiments were performed according to the manufacturers' instructions.

2.8. Immunohistochemistry. Immunohistochemistry was performed as previously described [16]. At 24 hrs after ZY or NS injection, the lung tissues were fixed in 10% PBS-buffered formalin, and 5 μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was blocked with 0.3% (volume/volume [v/v]) hydrogen peroxide in 60% (v/v) methanol for 30 mins. The sections were permeabilized with 0.1% (v/v) PBS-buffered Triton X-100 for 20 mins. Incubate the section in 3% (v/v) normal goat serum in PBS for 20 mins to minimize the nonspecific adsorption. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 mins with avidin and biotin (BD Biosciences, CA, USA). The sections were then incubated overnight with rabbit anti-iNOS mAb (Santa Cruz, CA, USA, 1 : 500 in PBS, v/v) or with control solutions. A biotin-conjugated specific secondary anti-immunoglobulin G and avidin-biotin peroxidase complex were used to detect the specific labeling. To verify the binding specificity for iNOS, some sections were also incubated with primary antibody only (no secondary antibody) or with secondary antibody only (no primary antibody). In these situations, no positive staining was found in the sections indicating that the immunoreactions were positive in all the experiments carried out.

2.9. Western Blot Analysis. The lung tissues were harvested, after mice were killed, and homogenized immediately. Cytoplasmic proteins were extracted from the lungs using Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology, Jiangsu, China) according to the manufacturer's protocol.

For extraction of nucleoprotein, lung tissue were homogenized and lysed in the lysis buffer (10 mM Hepes pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 2% NP-40, and 1 mM PMSF) for 30 min. After that, the lysis buffer was centrifugated at 1,2000 ×g for 15 minutes. Then the supernatant was collected as cytoplasmic protein. Precipitation was washed twice and lysed in the lysis buffer containing Triton X-100 as nucleoprotein. Protein concentrations were determined by BCA protein assay kit. The protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were transferred to a polyvinylidene difluoride membrane. The membrane was incubated overnight with antibodies against iNOS (1 : 400), IκB-α (1 : 800), p-IκB-α (1 : 1000), β-actin (1 : 1000), NF-κB p65 (1 : 800), and LaminB1 (1 : 1000). The membrane was then incubated with the secondary antibodies (anti-rabbit or anti-mouse IgG peroxidase conjugated, 1 : 10000). The blots were visualized with ECL-Plus reagent (Sigma).

2.10. NF-κB DNA Binding Activity Assay. At 6 hrs after ZY or NS injection, nuclear extracts of homogenated lung tissue were prepared. The DNA binding activity of NF-κB in lung tissues was quantified using the TransAM NF-κB p65 transcription factor assay kit (Active Motif, Carlsbad, CA). According to the manufacturer's instructions, all standards and samples were run in duplicate.

2.11. Statistical Analysis. Data were expressed as mean ± SEM. Differences between groups were examined for statistical significance using one-way analysis of variance with Student's t-test. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Agmatine Relieves Zymosan-Induced Lung Injury in Mice. Lung injury was characterized by alveolar thickening, infiltration of neutrophils into the lung interstitium, and alveolar space as well as alveolar hemorrhage. As shown in Figure 1(a), the mice in the control group or AGM-treated alone group showed no significant morphologic damages, indicating that intraperitoneal administration with Saline did not induce additional inflammation response in this protocol. However, ZYM-challenged mice appeared to have significant neutrophil infiltration into lung interstitium, alveolar wall thickening, and alveolar hemorrhage. Interestingly, agmatine treatment reduced infiltrated inflammatory cells and improved lung architecture in ZY-challenged mice. A scoring system was used to grade the degree of lung injury by evaluating congestion, edema, inflammation, and hemorrhage. Lung histologic scores significantly increased in ZY-challenged mice ($P < 0.05$) but were reduced by agmatine treatment ($P < 0.05$) (Figure 1(b)).

3.2. Agmatine Downregulates Zymosan-Induced TNF-α, IL-1β, and IL-6 in Lung and BALF. To test whether agmatine modulates the inflammatory process through the regulation of secretion of proinflammatory cytokines, we detected the levels of TNF-α, IL-1β, and IL-6 in lung and BALF. Six hours after ZYM or Saline injection, the lung and BALF were obtained and measured by ELISA analysis. In the Zymosan group, the concentration of TNF-α, IL-1β, and IL-6 in lung (1521.5 ± 128.4, 718.5 ± 67.2, and 917.4 ± 89.5, resp.) and BALF (157.5 ± 13.4, 124.7 ± 23.9, and 197.1 ± 24.3, resp.) increased significantly compared with that of the sham group in lung (421.1 ± 38.4, 114.8 ± 16.4, and 135.2 ± 17.4, resp.) and in BALF (8.1 ± 1.2, 6.5 ± 2.1, and 13.5 ± 4.2, resp.) (all $P < 0.05$). However, in the ZYM + AGM group, the levels of TNF-α, IL-1β, and IL-6 in lung (926.2 ± 89.4, 495.5 ± 54.2, and 424.3 ± 74.7, resp.) and BALF (56.7 ± 17.5, 35.02 ± 4.7, and 70.5 ± 33.6, resp.) were significantly lower compared with that of the Zymosan group (all $P < 0.05$) (Figure 2).

3.3. Effects of Agmatine on Zymosan-Induced Wet/Dry Weight Ratio, Protein in BALF, and MPO Activity. The pathogenesis of ALI involves increased permeability of the alveolar-capillary membrane, accumulation of protein-rich fluid in the airspaces, pulmonary edema, and pulmonary infiltration of neutrophils. In our study, twenty-four hour after Zymosan challenge, the lung tissues were obtained to employ lung weight and dry ratio (W/D), protein concentration in BALF, and MPO activity. In the Zymosan group, the W/D ratio (6.2 ± 0.6) was significantly increased compared with that of the Saline group (2.9 ± 0.4; $P < 0.05$). However, the ratio was significantly decreased in the ZYM + AGM group (4.1 ± 0.5; $P < 0.05$) compared with that of the Zymosan group (Figure 3(a)). Besides, the protein concentration in bronchoalveolar lavage fluid (BALF) was also increased in Zymosan group (0.71 ± 0.22) compared with that of the Saline group (0.06 ± 0.01; $P < 0.05$), whereas its level in ZYM + AGM group (0.29 ± 0.11) was significantly lower than that of the Zymosan group ($P < 0.05$) (Figure 3(b)). MPO activity, a biochemical marker of neutrophil infiltration, rose to 25.2 ± 1.8 in the lung of the Zymosan group compared with that of the Saline group (5.2 ± 1.4; $P < 0.05$). Treatment with agmatine resulted in a significant reduction in the lung MPO activity of the ZYM + AGM group (11.0 ± 3.4; $P < 0.05$) compared with that of the Zymosan group (Figure 3(c)).

3.4. Agmatine Reduces iNOS Expression in Lung. To understand the iNOS expression in lung, the lung tissues obtained at 24 h after Zymosan administration were detected by immunohistochemistry (Figure 4(a)) and Western blot analysis (Figure 4(b)). A significant increase of iNOS expression in ZYM group was detected compared to that in Saline group by evaluating gray level ratio of iNOS/β-actin (0.78 ± 0.12 versus 0.05 ± 0.03, $P < 0.05$). However, agmatine treatment significantly attenuated iNOS expression in the lung compared to that in Saline group (0.23 ± 0.12 versus 0.78 ± 0.12, $P < 0.05$).

3.5. Agmatine Inhibits Zymosan-Induced NF-κB Activation and DNA Binding Activity in Lung. Nuclear factor (NF)-κB signaling plays a central role in the initiation and regulation of cellular inflammatory response to bacterial stimuli. Thus, to investigate the mechanisms in which agmatine enhanced

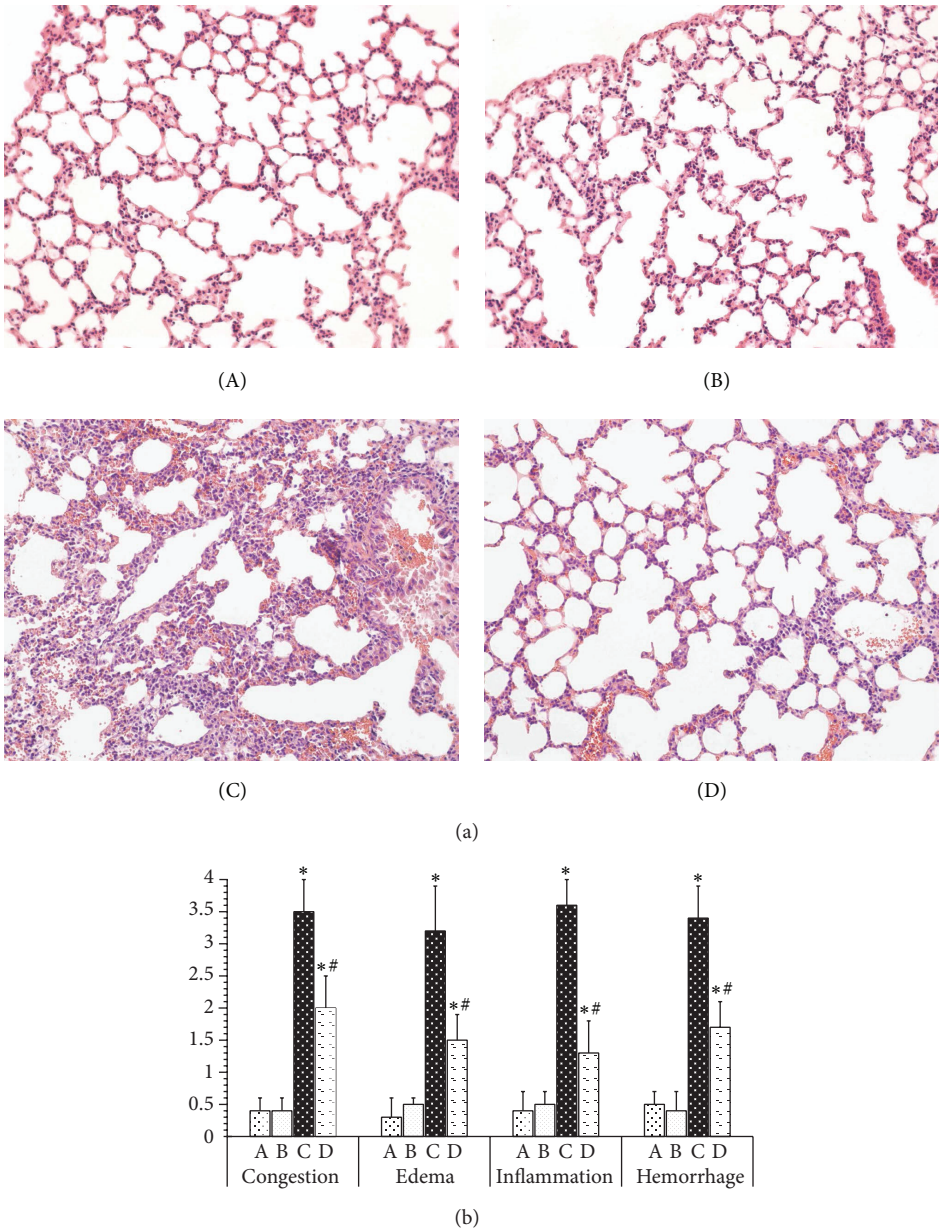

FIGURE 1: Agmatine relieves Zymosan-induced lung injury in mice. (a) Representative micrographs of H&E staining. Lung tissues in NS group (A), agmatine group (B), Zymosan group (C), and Zymosan + agmatine group (D) were measured. (b) The degree of lung injury was scored by evaluating each of histologic features: congestion, edema, inflammation, and hemorrhage. $^{*}P < 0.05$ compared with NS group. $^{\#}P < 0.05$ compared with Zymosan group.

lung inflammation resolution, we assessed the effects of agmatine on the degradation of IκB-α and activation of NF-κB by Western blot analysis, and further we detected its effect on DNA binding activity. The mice treated with ZYM exhibited significant degradation of IκB-α in lungs, whereas agmatine treatment at the dose of 200 mg/kg prevented the IκB-α degradation. In contrast, ZYM challenge induced the nuclear translocation of NF-κB p65 subunit, compared with basal group. Agmatine treatment inhibited the nuclear translocation of NF-κB p65 (Figure 5(a)). Furthermore, the Zymosan administration significantly increased NF-κB p65 DNA binding activity in ZYM group compared with that in Saline group (2.70 ± 0.25 versus 1.10 ± 0.12, $P < 0.05$), whereas its activity in ZYM + AGM group was inhibited compared with that in ZYM group (1.82 ± 0.21 versus 2.70 ± 0.25, $P < 0.05$) (Figure 5(b)).

4. Conclusion

Zymosan has been well recognized in the pathogenesis of ALI. Experimental administration of Zymosan, both systemically and intratracheally, has been used to induce neutrophil

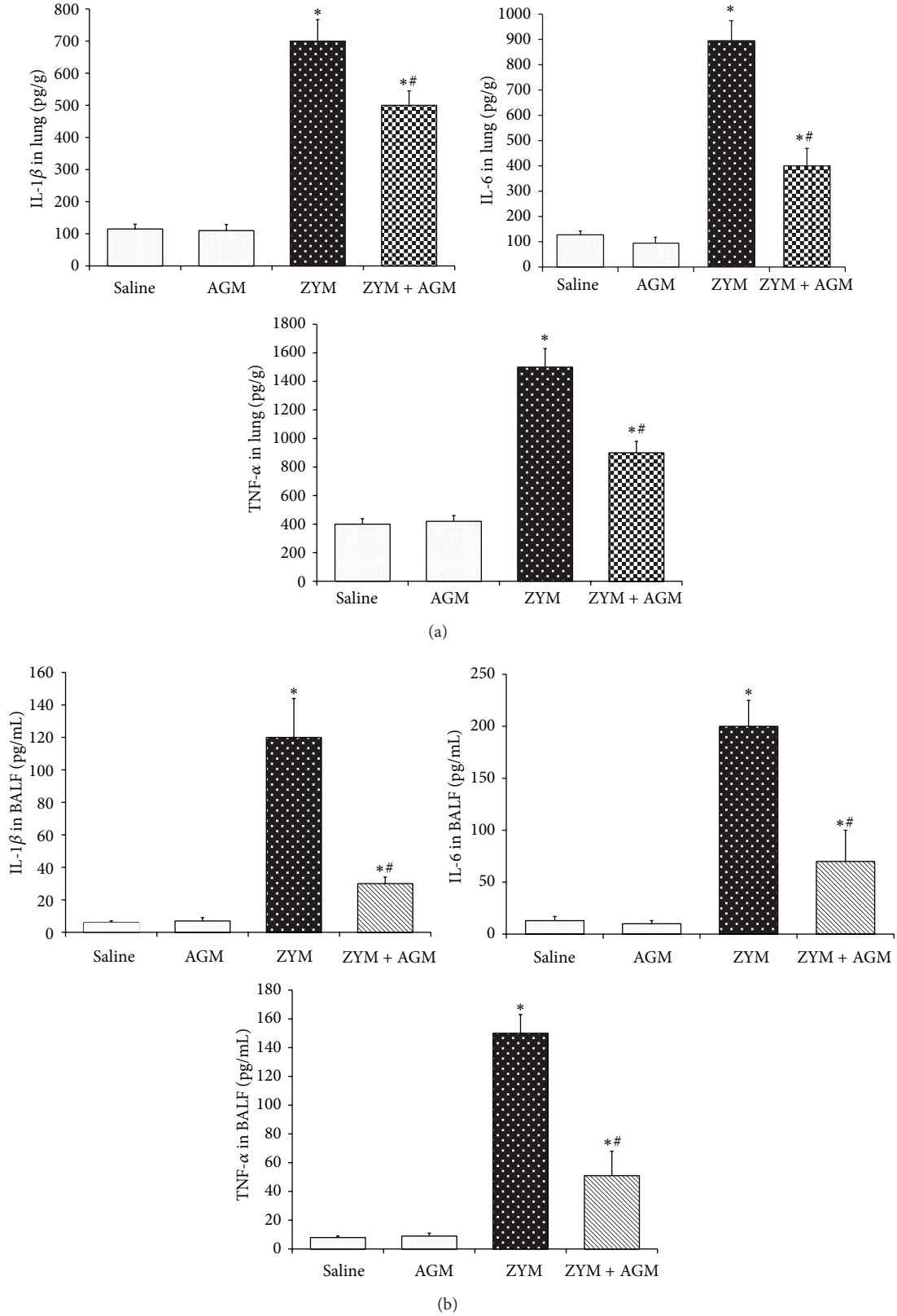

Figure 2: Agmatine downregulates Zymosan-induced TNF-α, IL-1β, and IL-6 in lung and BALF. At 6 hr after ZYM or Saline injection, the cytokines levels in lung and BALF tissue were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits. $^{*}P < 0.05$ compared with NS group. $^{\#}P < 0.05$ compared with Zymosan group.

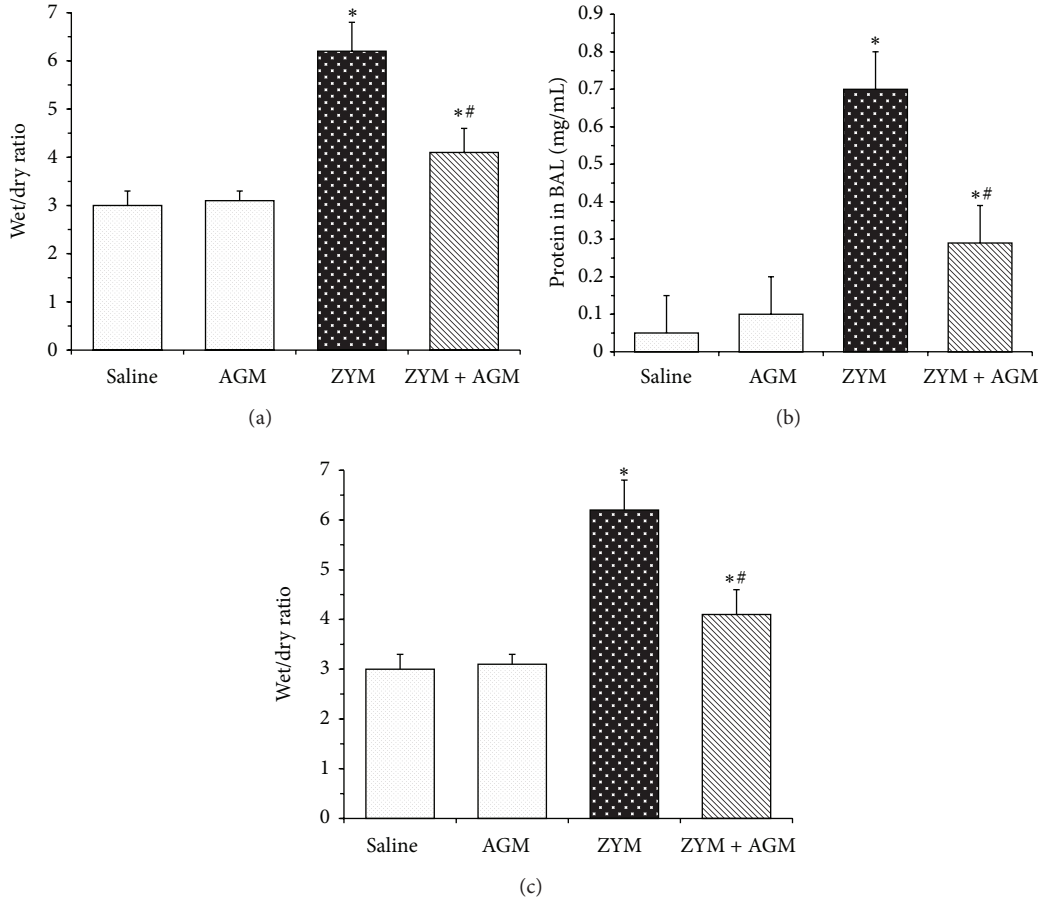

Figure 3: Effects of agmatine on Zymosan-induced wet/dry weight ratio, protein in BALF, and MPO activity. 24 hour after Zymosan challenge, the lung tissues were obtained to detect lung weight and dry ratio (W/D) (a), protein concentration in BALF (b), and MPO activity (c). $^{*}P < 0.05$ compared with NS group. $^{\#}P < 0.05$ compared with Zymosan group.

infiltration and develop pulmonary inflammation in animal models [17, 18]. In the present study, we demonstrated that agmatine protected against ZYM-induced lung inflammation and lung injury in mice. Agmatine treatment inhibited the neutrophil infiltration and lung endothelial permeability in lung of ZYM-challenged mice. Histopathological examination showed that ZYM-induced congestion, edema, inflammation, and hemorrhage in lung were relieved by agmatine. In addition, ZYM induced upregulations of proinflammatory mediators and cytokines in BALF and lung were partially inhibited by agmatine. Furthermore, agmatine treatment inhibited the degradation of IκB-α and subsequent activation and DNA binding activity of NF-κB in lung. These results demonstrate that agmatine exerts a protective effect on ALI via inhibiting NF-κB-mediated inflammatory response.

ALI is characterized by excessive neutrophil infiltration, release of proinflammatory mediators, and loss of vascular barrier integrity [19]. During airway inflammation, neutrophils are the first cells to be recruited and are the predominant cause of tissue damage [20]. Activated neutrophils induce extensive lung inflammation and the destruction of basement membrane and increase the permeability of alveolar capillary membrane [21]. Besides, neutrophils can release damaging mediators, such as cytokines and oxidants, leading to the injury of epithelial-vascular barrier [22]. In the present study, Zymosan-induced mice group caused excessive production of MPO, an enzyme located mainly in the primary granules of neutrophils. Agmatine treatment significantly reduced neutrophil infiltration by determining MPO activity in lung. Furthermore, we detected less protein concentration in BAL and lung W/D weight in mice treated with agmatine, showing the protective effect of agmatine against lung endothelial permeability injury.

Except for neutrophils and the other inflammatory cells, the release of proinflammatory mediators has been reported to be involved in inflammatory cascade [23]. Among them, TNF-α, IL-1β, and IL-6 were considered the most important inflammatory mediators in innate immune response. It has been reported that resident alveolar macrophages release TNF-α and IL-1β in early phase of ALI in response to ZYM stimulation, resulting in the subsequent inflammatory cascade and tissue injury [24]. It is reported that TNF-α elevates intracellular reactive oxygen species which causes mitochondrial damage or ion exchange dysfunction across

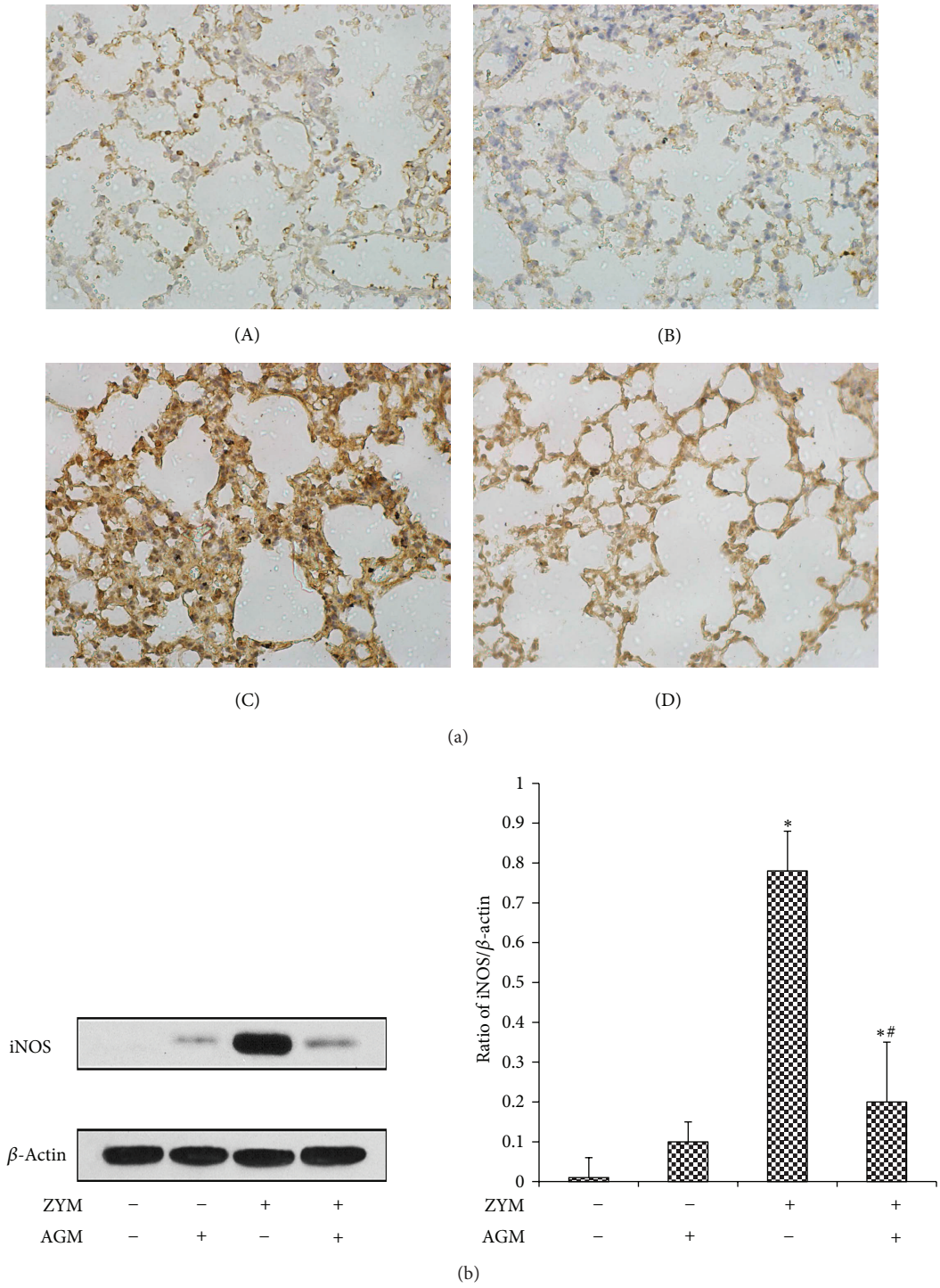

FIGURE 4: Agmatine decreases iNOS expression in lung. (a) Lung samples were obtained from NS group (A), agmatine group (B), Zymosan group (C), and Zymosan + agmatine group (D) for immunohistochemical staining. (b) Lungs were harvested for observing iNOS expression at 24 hrs after ZYM or NS administration by Western blot. $^{*}P < 0.05$ compared with NS group. $^{\#}P < 0.05$ compared with Zymosan group.

the cell membrane [25, 26]. Besides, inhibition of TNF-α was proved to be protective in an animal model of ALI [27]. IL-1β also enhances the recruitment of inflammatory cells into airspaces and alters vascular permeability leading to fluid transport and subsequent lung edema formation [28]. During inflammation, elevated TNF-α and IL-1β are associated with a poor ALI prognosis [29]. In the present study, the expressions of TNF-α, IL-1β, and IL-6 in lung and BALF were markedly induced by ZYM challenge, which was blocked by the treatment of agmatine. Our results confirmed the link

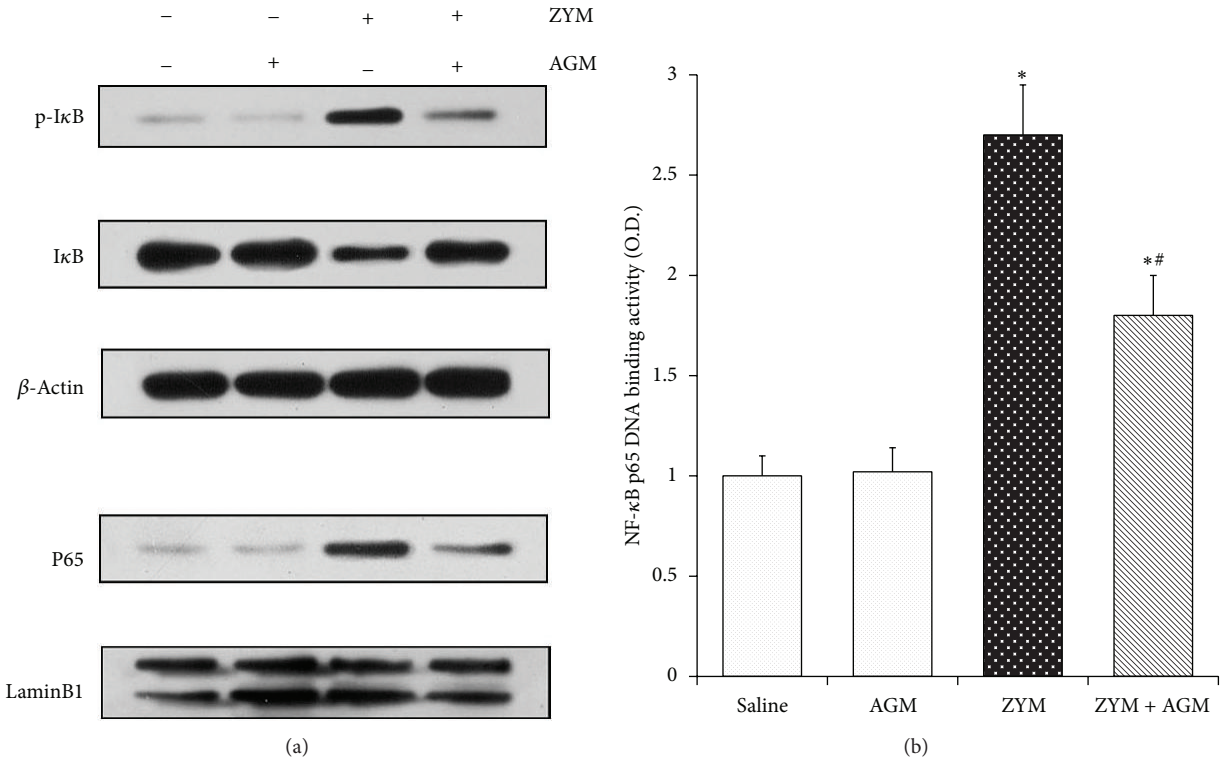

Figure 5: Agmatine inhibits Zymosan-induced NF-κB activation and DNA binding activity in lung. 6 h after ZYM injection with or without agmatine treatments, mice were exsanguinated and their lungs were removed. (a) Western blot was performed to detect p-IκB-α and IκB-α in cytoplasm and NF-κB p65 in nucleus. Expressions of β-actin and LaminB1 were shown as loading controls. (b) DNA binding activity of NF-κB p65 was examined by a TransAM p65 transcription factor ELISA kit. Each bar represents the mean ± SD of 8 mice. $^{*}P < 0.05$ compared with NS group. $^{\#}P < 0.05$ compared with Zymosan group.

between the inflammatory cytokine levels and the extent of lung inflammation, demonstrating the anti-inflammatory effects of agmatine.

Inflammatory activity is mediated by the endogenous free radical NO, which is produced at high levels upon the induction of NO synthase by inflammatory stimulus [30]. In an experimental murine model, ZY administration increases iNOS expression and activity that exacerbates nonseptic shock and leads to cellular and tissue damage including lung injury if unchecked. However, iNOS inhibitors suppress airway inflammation in mice by downregulating proinflammation and chemokine expression that are detrimental to the lung [31]. iNOS-deficient mice also undergo less lung injury after ZY challenge [32]. In the present study, we demonstrated that agmatine treatment significantly reduced iNOS expression and its activity in the lung tissue, and this phenomenon significantly inhibited the inflammatory response. Moreover, NF-κB is a potent regulator of iNOS expression [33], and our results show that agmatine treatment inhibited NF-κB activity as well as iNOS expression in lung. Based on these findings, we speculated that agmatine probably inhibited iNOS expression and activity by blocking NF-κB activation.

It has been reported that NF-κB, a transcription factor, plays a pivotal role in the pathogenesis of immune and inflammatory responses [34]. In experimental animal models of ALI, NF-κB activation is increased [35]. Pharmacological inhibition of NF-κB pathway decreases the production of proinflammatory mediators and protects against endotoxin-induced ALI in animals [36]. Therefore, NF-κB pathway is involved in the pathogenesis of ALI and is known as an important target for anti-inflammatory molecules [37]. Our present study displayed that ZYM obviously enhanced the phosphorylation and degradation of IκB-α. However, agmatine treatment dramatically regulated that this trend, which suggested that suppression of IκB-α activity may be the main reason of agmatine lessening ZYM-initiated pulmonary inflammation in mice. These results suggested that inhibition of NF-κB signaling plays a role in the protective effects of agmatine on ALI.

Agmatine is a cationic polyamine under physiological conditions and thus is thought not to be able to permeate biological membranes, although agmatine can cross the blood-brain barrier when administered peripherally in high doses. In mammalian tissues, agmatine binds to several receptors, including imidazoline, α2-adrenergic, and NMDA glutamate receptor. Sameer et al. reported that agmatine attenuated the acquisition of ethanol conditioned place preference by imidazoline (I_1 or I_2) receptors [38]. Bhalla et al. found that agmatine potentiated oxycodone antinociception in

mice via an imidazoline I_2 receptor-mediated mechanism [39]. Besides, agmatine was found to be protective against glutamate-induced necrotic neuronal cell death through NMDA receptor blockade by interacting with a site located within the NMDA channel pore [40]. Therefore, we speculated that agmatine may protect against zymosan-induced acute lung injury by interacting with its endogenous receptors, which will be tested and verified in the next round of experiments.

In summary, we have demonstrated the protective effects of agmatine on ZYM-induced ALI. It is evidenced by alleviating lung inflammation, reducing neutrophil infiltration, decreasing vascular leakage, and proinflammatory cytokine release, inhibiting NF-κB activation and DNA binding activity in ZYM-challenged mice. These results suggest agmatine may be considered as an effective and safe drug for the potential treatment of ALI.

Conflict of Interests

The authors declare that no conflict of interests exists.

Acknowledgments

This work was supported by a Grant from National "973" Project (no. 2012CB518102).

References

[1] J. Aman, M. van der Heijden, A. van Lingen et al., "Plasma protein levels are markers of pulmonary vascular permeability and degree of lung injury in critically ill patients with or at risk for acute lung injury/acute respiratory distress syndrome," *Critical Care Medicine*, vol. 39, no. 1, pp. 89–97, 2011.

[2] M. A. Matthay, G. A. Zimmerman, C. Esmon et al., "Future research directions in acute lung injury: summary of a National Heart, Lung, and Blood Institute Working Group," *The American Journal of Respiratory and Critical Care Medicine*, vol. 167, no. 7, pp. 1027–1035, 2003.

[3] G. Matute-Bello, G. Downey, B. B. Moore et al., "An official american thoracic society workshop report: features and measurements of experimental acute lung injury in animals," *American Journal of Respiratory Cell and Molecular Biology*, vol. 44, no. 5, pp. 725–738, 2011.

[4] G. D. Rubenfeld, E. Caldwell, E. Peabody et al., "Incidence and outcomes of acute lung injury," *New England Journal of Medicine*, vol. 353, no. 16, pp. 1685–1693, 2005.

[5] C. Metz and W. J. Sibbald, "Anti-inflammatory therapy for acute lung injury: a review of animal and clinical studies," *Chest*, vol. 100, no. 4, pp. 1110–1119, 1991.

[6] M. P. Young, H. L. Manning, D. L. Wilson et al., "Ventilation of patients with acute lung injury and acute respiratory distress syndrome: has new evidence changed clinical practice?" *Critical Care Medicine*, vol. 32, no. 6, pp. 1260–1265, 2004.

[7] H. V. Chaves, R. D. A. Ribeiro, A. M. B. de Souza et al., "Experimental model of zymosan-induced arthritis in the rat temporomandibular joint: role of nitric oxide and neutrophils," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 707985, 11 pages, 2011.

[8] Y. Adachi and N. Ohno, "Contribution of dectin-1 to the recognition of fungal cell wall products and the activation of innate immune response," *Nihon Ishinkin Gakkai Zasshi*, vol. 47, no. 3, pp. 185–194, 2006.

[9] S. Mehta, "The effects of nitric oxide in acute lung injury," *Vascular Pharmacology*, vol. 43, no. 6, pp. 390–403, 2005.

[10] S. Hong, J. E. Lee, C. Y. Kim, and G. J. Je, "Agmatine protects retinal ganglion cells from hypoxia-induced apoptosis in transformed rat retinal ganglion cell line," *BMC Neuroscience*, vol. 8, article 81, 2007.

[11] A. Halaris and J. Plietz, "Agmatine: metabolic pathway and spectrum of activity in brain," *CNS Drugs*, vol. 21, no. 11, pp. 885–900, 2007.

[12] J. Mu, K. Xie, L. Hou et al., "Subanesthetic dose of isoflurane protects against zymosan-induced generalized inflammation and its associated acute lung injury in mice," *Shock*, vol. 34, no. 2, pp. 183–189, 2010.

[13] J. Chen, Y. Li, L. Wang et al., "Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats," *Stroke*, vol. 32, no. 4, pp. 1005–1011, 2001.

[14] J. B. Clark and W. J. Nicklas, "The metabolism of rat brain mitochondria. Preparation and characterization," *The Journal of Biological Chemistry*, vol. 245, no. 18, pp. 4724–4731, 1970.

[15] J. Li, W. Liu, S. Ding et al., "Hyperbaric oxygen preconditioning induces tolerance against brain ischemia-reperfusion injury by upregulation of antioxidant enzymes in rats," *Brain Research*, vol. 1210, pp. 223–229, 2008.

[16] J. W. Soper and P. L. Pedersen, "Isolation of an oligomycin-sensitive ATPase complex from rat liver mitochondria," *Methods in Enzymology*, vol. 55, pp. 328–333, 1979.

[17] R. F. Oppeltz, M. Rani, Q. Zhang, and M. G. Schwacha, "Burn-induced alterations in toll-like receptor-mediated responses by bronchoalveolar lavage cells," *Cytokine*, vol. 55, no. 3, pp. 396–401, 2011.

[18] T. Stevens, K. Ekholm, M. Gränse et al., "AZD9668: pharmacological characterization of a novel oral inhibitor of neutrophil elastase," *Journal of Pharmacology and Experimental Therapeutics*, vol. 339, no. 1, pp. 313–320, 2011.

[19] D. El Kebira, P. Gjorstrup, and J. G. Filepa, "Resolvin E1 promotes phagocytosis-induced neutrophil apoptosis and accelerates resolution of pulmonary inflammation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 37, pp. 14983–14988, 2012.

[20] S. Matsuo, W. Yang, M. Aziz, A. Jacob, and P. Wang, "Cyclic arginine-glycine-aspartate attenuates acute lung injury in mice after intestinal ischemia/reperfusion," *Critical Care*, vol. 17, no. 1, article R19, 2013.

[21] X. Huang and Y. Y. Zhao, "Transgenic expression of FoxM1 promotes endothelial repair following lung injury induced by polymicrobial sepsis in mice," *PLoS ONE*, vol. 7, no. 11, Article ID e50094, 2012.

[22] J. J. Hoth, J. D. Wells, E. M. Hiltbold, C. E. McCall, and B. K. Yoza, "Mechanism of neutrophil recruitment to the lung after pulmonary contusion," *Shock*, vol. 35, no. 6, pp. 604–609, 2011.

[23] V. Galani, E. Tatsaki, M. Bai et al., "The role of apoptosis in the pathophysiology of Acute Respiratory Distress Syndrome (ARDS): an up-to-date cell-specific review," *Pathology: Research and Practice*, vol. 206, no. 3, pp. 145–150, 2010.

[24] T. R. Martin, J. C. Mathison, P. S. Tobias et al., "Lipopolysaccharide binding protein enhances the responsiveness of alveolar macrophages to bacterial lipopolysaccharide. Implications for

cytokine production in normal and injured lungs," *The Journal of Clinical Investigation*, vol. 90, no. 6, pp. 2209–2219, 1992.

[25] N. Mariappan, C. M. Elks, B. Fink, and J. Francis, "TNF-induced mitochondrial damage: a link between mitochondrial complex I activity and left ventricular dysfunction," *Free Radical Biology and Medicine*, vol. 46, no. 4, pp. 462–470, 2009.

[26] S. Y. Cheranov and J. H. Jaggar, "TNF-α dilates cerebral arteries via NAD(P)H oxidase-dependent Ca2+ spark activation," *The American Journal of Physiology—Cell Physiology*, vol. 290, no. 4, pp. C964–C971, 2006.

[27] E. K. Wolthuis, A. P. J. Vlaar, G. Choi et al., "Recombinant human soluble tumor necrosis factor-alpha receptor fusion protein partly attenuates ventilator-induced lung injury," *Shock*, vol. 31, no. 3, pp. 262–266, 2009.

[28] M. A. Olman, K. E. White, L. B. Ware, M. T. Cross, S. Zhu, and M. A. Matthay, "Microarray analysis indicates that pulmonary edema fluid from patients with acute lung injury mediates inflammation, mitogen gene expression, and fibroblast proliferation through bioactive interleukin-1," *Chest*, vol. 121, supplement, no. 3, pp. 69S–70S, 2002.

[29] L. J. M. Cross and M. A. Matthay, "Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury," *Critical Care Clinics*, vol. 27, no. 2, pp. 355–377, 2011.

[30] R. Di Paola, E. Mazzon, C. Muià et al., "Protective effect of Hypericum perforatum in zymosan-induced multiple organ dysfunction syndrome: relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity," *Nitric Oxide—Biology and Chemistry*, vol. 16, no. 1, pp. 118–130, 2007.

[31] F. C. R. Souza, N. B. Gobbato, R. G. Maciel et al., "Effects of corticosteroid, montelukast and iNOS inhibition on distal lung with chronic inflammation," *Respiratory Physiology and Neurobiology*, vol. 185, no. 2, pp. 435–445, 2013.

[32] S. Cuzzocrea, E. Mazzon, L. Dugo et al., "Inducible nitric oxide synthase knockout mice exhibit resistance to the multiple organ failure induced by zymosan," *Shock*, vol. 16, no. 1, pp. 51–58, 2001.

[33] Q. Xie, Y. Kashiwabara, and C. Nathan, "Role of transcription factor NF-κB/Rel in induction of nitric oxide synthase," *The Journal of Biological Chemistry*, vol. 269, no. 7, pp. 4705–4708, 1994.

[34] Z. You, D. Feng, H. Xu et al., "Nuclear factor-κ B mediates one-lung ventilation-induced acute lung injury in rabbits," *Journal of Investigative Surgery*, vol. 25, no. 2, pp. 78–85, 2012.

[35] P. J. Yu, J. R. Li, Z. G. Zhu et al., "Praeruptorin D and e attenuate lipopolysaccharide/hydrochloric acid induced acute lung injury in mice," *European Journal of Pharmacology*, vol. 710, no. 1–3, pp. 39–48, 2013.

[36] A. B. Nathens, R. Bitar, C. Davreux et al., "Pyrrolidine dithiocarbamate attenuates endotoxin-induced acute lung injury," *American Journal of Respiratory Cell and Molecular Biology*, vol. 17, no. 5, pp. 608–616, 1997.

[37] A. B. Lentsch, B. J. Czermak, N. M. Bless, N. van Rooijen, and P. A. Ward, "Essential role of alveolar macrophages in intrapulmonary activation of NF-κB," *The American Journal of Respiratory Cell and Molecular Biology*, vol. 20, no. 4, pp. 692–698, 1999.

[38] S. M. Sameer, S. S. Chakraborty, and R. R. Ugale, "Agmatine attenuates acquisition but not the expression of ethanol conditioned place preference in mice: a role for imidazoline receptors," *Behavioural Pharmacology*, vol. 24, no. 2, pp. 87–94, 2013.

[39] S. Bhalla, I. Ali, H. Lee, S. V. Andurkar, and A. Gulati, "Potentiation of oxycodone antinociception in mice by agmatine and BMS182874 via an imidazoline I2 receptor-mediated mechanism," *Pharmacology Biochemistry and Behavior*, vol. 103, no. 3, pp. 550–560, 2013.

[40] G. Olmos, N. deGregorio-Rocasolano, M. P. Regalado et al., "Protection by-imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor," *British Journal of Pharmacology*, vol. 127, no. 6, pp. 1317–1326, 1999.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 803561, 8 pages
http://dx.doi.org/10.1155/2014/803561

Research Article

Early Response Roles for Prolactin Cortisol and Circulating and Cellular Levels of Heat Shock Proteins 72 and 90α in Severe Sepsis and SIRS

K. Vardas,[1] K. Apostolou,[1] E. Briassouli,[2] D. Goukos,[2] K. Psarra,[3] E. Botoula,[4] S. Tsagarakis,[4] E. Magira,[1] C. Routsi,[1] S. Nanas,[1] and G. Briassoulis[5]

[1] *First Critical Care Department, Evangelismos Hospital, National and Kapodistrian University of Athens, Ipsilantou 45, 10676 Athens, Greece*
[2] *1st Department of Propaedeutic Internal Medicine, Laiko University General Hospital, University of Athens, 17 Agiou Thoma, 115 27 Athens, Greece*
[3] *Immunology-Histocompatibility Department, Evangelismos Hospital, Ipsilantou 45, 10676 Athens, Greece*
[4] *Department of Endocrinology and Diabetes, Evangelismos Hospital, Ipsilantou 45, 10676 Athens, Greece*
[5] *Pediatric Intensive Care Unit, School of Health Sciences, University Hospital, University of Crete, 71110 Heraklion, Greece*

Correspondence should be addressed to G. Briassoulis; ggbriass@otenet.gr

Received 7 March 2014; Accepted 24 July 2014; Published 27 August 2014

Academic Editor: Baoli Cheng

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Objective. To evaluate the early heat shock protein (HSP) and hormonal stress response of intensive care unit (ICU) patients with severe sepsis/septic shock (SS) or systemic inflammatory response syndrome (SIRS) compared to healthy subjects (H). *Methods.* Patients with early (first 48 hrs) SS ($n = 29$) or SIRS ($n = 29$) admitted to a university ICU and 16 H were enrolled in the study. Serum prolactin, cortisol, and plasma ACTH were determined using immunoassay analyzers. ELISA was used to evaluate extracellular HSPs (eHSP90α, eHSP72) and interleukins. Mean fluorescence intensity (MFI) values for intracellular HSPs (iHSP72, iHSP90α) were measured using 4-colour flow-cytometry. *Results.* Prolactin, cortisol, and eHSP90α levels were significantly increased in SS patients compared to SIRS and H ($P < 0.003$). ACTH and eHSP72 were significantly higher in SS and SIRS compared to H ($P < 0.005$). SS monocytes expressed lower iHSP72 MFI levels compared to H ($P = 0.03$). Prolactin was related with SAPS III and APACHE II scores and cortisol with eHSP90α, IL-6, and lactate ($P < 0.05$). In SS and SIRS eHSP90α was related with eHSP72, IL-6, and IL-10. *Conclusion.* Prolactin, apart from cortisol, may have a role in the acute stress response in severe sepsis. In this early-onset inflammatory process, cortisol relates to eHSP90α, monocytes suppress iHSP72, and plasma eHSP72 increases.

1. Introduction

Severe sepsis and septic shock are leading causes of death in intensive care units (ICU) worldwide and despite efforts in understanding their pathophysiology and implementing effective treatment, their annual incidence has been projected to increase by 1.5% per year [1]. Sepsis is accompanied by major changes in the hypothalamus-pituitary-adrenal axis through multiple interactions between the autonomous nervous system and immune systems [2]. During early sepsis, initial activation of the pituitary adrenal axis depends on activation of hypothalamus and pituitary by cytokines, while in late sepsis a shift from neuroendocrine to local adrenal regulation of glucocorticoid production has been proposed [3]. The initial release of cytokines from immune cells participates in homeostasis of the body by acting as paracrine, autocrine, and hormonal agents and elevating corticotropin releasing hormone levels (CRH) [3]. Except for CRH, septic patients have increased ACTH, growth hormone, and prolactin levels in the early stage of sepsis [2].

The association of prolactin with modulation of the immune system during sepsis has been studied in septic mice, where administration of prolactin was associated with decreased survival and alterations in immune response [4].

It has been found that prolactin serum levels in critical ill children are usually low early after pediatric intensive care admission [5]. The role of prolactin as an early acute stress in adult ICU sepsis and trauma patients has not been elucidated yet [6]. In an animal model, a possible relationship between heat shock proteins (HSPs) and prolactin receptors has been previously reported [7].

Intracellular HSPs (iHSPs) are high evolutionary conservative proteins that play an important role in regulating host response against infections, thermal injury, oxidative damage, and hypoxia [8]. Particularly, the major heat shock proteins iHSP70 and iHSP90α confer tolerance to sepsis by maintaining the conformational homeostasis, exerting antiapoptotic effects, and mediating LPS-signaling as a part of the LPS receptor cluster [9]. However, although animal studies have demonstrated a protective effect of iHSP72 in sepsis, human studies are inconclusive showing either protection or relation to mortality and infections [10]. To add more questions about the extracellular HSPs (eHSPs) function and their role in sepsis [11, 12], eHSP90α levels were recently shown to decline in controls and remain increased in septic patients, contrasting eHSP72, which increased over time in both groups [13]. Thus, the involvement of extracellular bound HSPs as signals for activation of the immune system and especially macrophages [14] raises interest about the role of these proteins in sepsis. Apart from septic patients, serum levels of eHSP72 measured early after injury in trauma patients correlated with survival, with significantly higher levels in trauma patients who survived compared to nonsurvivors [15].

In this study, we evaluated the early (first 48 hours) serum levels of prolactin, cortisol, and interleukins and plasma levels of ACTH, eHSP90α, and eHSP72 and measured mean fluorescence intensity (MFI) of iHSP72 and iHSP90α in ICU patients with severe sepsis and septic shock (SS) or systemic inflammatory response syndrome (SIRS) compared to healthy control subjects (H). We also correlated their expression with interleukins (ILs), severity scoring systems, clinical and laboratory data, and outcome.

2. Materials and Methods

2.1. Patients. The study was approved by the institutional review board of Evangelismos Hospital and was performed during a 14-month period between October 2012 and December 2013. Informed consent form was obtained from the relatives of patients admitted to the ICU. Consecutively admitted patients >18 years and <75 years with early (<48 h) severe sepsis, septic shock, or SIRS admitted to the ICU were eligible for enrolment and were divided in two groups. The SIRS group included trauma patients ($n = 29$) who met at least two of the four conventional criteria for SIRS. The severe sepsis or septic shock group (SS) included patients ($n = 29$) with an identified source of infection. Sepsis, severe sepsis, septic shock, and SIRS were defined according to the Surviving Sepsis Campaign Guidelines [16]. The third group included healthy volunteers (H) ($n = 16$) matched for age and sex to the ICU patients. Exclusion criteria were (a) malignancy, (b) autoimmune diseases, (c) prior use of corticoids, (d) immunosuppressive illness, and (e) late sepsis or SIRS 48 h after admission. Acute physiology and chronic evaluation (APACHE II) [17], sequential organ failure assessment (SOFA) [18], and simplified acute physiology score III (SAPS III) [19] scores were recorded on admission. Demographics, date of hospital, and ICU admission, ICU and in-hospital mortality, length of stay, and laboratory tests were also recorded for all patients.

2.2. Laboratory Assays

2.2.1. Prolactin, Cortisol, and ACTH. Blood was drawn between 8 and 9 a.m in the first 48 h after ICU admission. For ACTH measurement, blood was collected into ethylenediaminetetraacetic acid (EDTA) containing tube, immediately centrifuged at 4°C, plasma-pooled, and finally stored at −80°C until measurement. For cortisol and prolactin measurement blood was collected in tubes containing clot and gel for serum separation and centrifuged at 4°C and serum was also stored at −80°C until measurement. Serum cortisol and prolactin levels were determined using the ADVIA Centaur Immunoassay Analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) while plasma ACTH was measured using the Immulite 2000 Immunoassay Analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

2.2.2. Cytokines and Extracellular Heat Shock Proteins. Cytokine levels of serum IL-6, IL-10, IL-17, and IFN-γ were measured by ELISA as mentioned by the kit instructions and extracellular plasma levels of HSPs (eHSP72 and eHSP90α) were analyzed by ELISA assay according to the manufacturers' instructions (Invitrogen Carlsbad, CA, USA, and Enzo Life Sciences, Ann Arbor, MI, USA, resp.). The inter- and intra-assay CV for each analyte were as follows: 6.2 and 7.8 for IL-6, 3.25 and 2.75 for IL-10, 3.7 and in process for IL-17, 3.5 and 7.3 for IFN-γ, 7.1 and 15.2 for hsp72, and <10 for hsp90α. The sensitivities of the assays were <2 pg/mL for IL-6, <1 pg/mL for IL-10, 2 pg/mL for IL-17, 0.03 IU/mL for IFN-γ, 90 pg/mL for hsp72, and 50 pg/mL for hsp90α.

2.2.3. Intracellular HSPs. EDTA-anticoagulated blood (100 μL) was used for flow cytometric analysis of fresh peripheral blood mononuclear cells (PBMCs). Monocytes iHSP72 and iHSP90α expressed as mean fluorescence intensity (MFI) were determined after staining with 5 μL surface antigens CD33-PE/Cy5 (BioLegend, San Diego, CA, USA) and 5 μL CD45 PE/Cy7 (BioLegend, San Diego, USA) followed by either 5 μL HSP72-FITC (Enzo Life Sciences, Ann Arbor, MI, USA) or 5 μL HSP90α-PE (Enzo Life Sciences, Ann Arbor, MI, USA) intracellular staining. Assays were performed according to the manufacturer's instructions using 4-colour flow cytometry FC-500 (Beckman Coulter, Miami, FL, USA).

2.2.4. Statistical Analysis. All results are presented as means ± standard deviation. The results were analyzed using SPSS software (version 21.0, SPSS, Chicago, Ill). Group comparisons were performed using the Kruskal-Wallis test. The variables

Table 1: Demographic characteristics and severity scores by group (healthy subjects $n = 16$, SIRS $n = 29$, severe sepsis $n = 29$)**.

	Healthy subjects	SIRS	Severe sepsis	P value*
Age (years)	47.3 ± 9.7	42.5 ± 13.0	54.7 ± 14.5[ab]	0.003
Gender (male/female)	10 M/6 F	24 M/5 F	17 M/12 F	0.118
APACHE II		15.0 ± 5.4	22.0 ± 9.7[b]	0.007
SOFA		8.0 ± 3.0	11.0 ± 4.0[b]	0.006
SAPS III		50.0 ± 10.0	68.0 ± 14.0[b]	0.0001

APACHE: acute physiology and chronic evaluation; SOFA: sequential organ failure assessment; SAPS III: simplified acute physiology score; SIRS: systematic inflammatory response syndrome, [a]$P < 0.05$ between healthy subjects and severe sepsis groups, and [b]$P < 0.05$ between SIRS and severe sepsis group.
*The P value was calculated using the Kruskal-Wallis test and a P value <0.05 was considered statistically significant.
**Data are expressed as means ± SD (standard deviation).

Table 2: Hormonal, cytokine, and heat shock protein measurements by group (healthy subjects $n = 16$, SIRS $n = 29$, severe sepsis $n = 29$)**.

	Healthy subjects	SIRS	Severe sepsis	P value*
Prolactin (ng/mL)	5.97 ± 2.80	18.26 ± 10.64[a]	23.17 ± 18.38[b]	0.003
Cortisol (μg/dL)	12.07 ± 5.03	21.74 ± 16.09[a]	39.64 ± 23.52[bc]	0.000
ACTH (pg/mL)	25.97 ± 19.05	30.71 ± 69.54[a]	31.26 ± 25.18[c]	0.002
eHSP90α (ng/mL)	21.50 ± 10.08	58.70 ± 44.3[a]	81.89 ± 41.81[bc]	0.003
eHSP72 (ng/mL)	0.07 ± 0.06	0.35 ± 0.26[a]	1.43 ± 2.76[b]	0.005
iHSP90α (MFI)	49.73 ± 30.87	41.20 ± 31.62	37.36 ± 28.96	0.490
iHSP72 (MFI)	38.08 ± 24.76	36.65 ± 31.38	24.66 ± 21.15[b]	0.093
IL-6 (pg/mL)	1.70 ± 1.50	188.10 ± 203.92[a]	358.49 ± 377.40[b]	0.001
IL-10 (ng/mL)	0.01 ± 0.001	5.16 ± 7.10	35.29 ± 60.60[b]	0.037
IL-17 (ng/mL)	9.75 ± 16.65	20.86 ± 60.96	25.14 ± 93.56	0.969
IFN-γ (pg/mL)	0.52 ± 1.07	0.19 ± 0.10	0.92 ± 1.89[c]	0.070

eHSP: extracellular heat shock protein; iHSP: intracellular heat shock protein; IL: interleukins; IFN-γ: interferon gamma.
[a]$P < 0.05$ between healthy subjects and SIRS groups.
[b]$P < 0.05$ between healthy subjects and severe sepsis groups.
[c]$P < 0.05$ between SIRS and severe sepsis group.
*The P value was calculated using the Kruskal-Wallis test and a P value <0.05 was considered statistically significant.
**Data are expressed as means ± SD (standard deviation).

that showed differences among groups were compared group by group by the Mann-Whitney test. Paired differences for continuous variables in the same subjects were analyzed using the Wilcoxon signed-rank test. The correlation between variables was analyzed by the Spearman correlation test. The level of significance between groups was set on $P < 0.05$.

3. Results

Anthropometric characteristics and severity scores of the (H), (SIRS), and (SS) groups are summarized in Table 1. Hormonal profile, extracellular heat shock protein and cytokine measurements, and mean fluorescence intensity of heat shock proteins in monocytes are summarized in Table 2.

Prolactin, cortisol, and ACTH levels differed significantly between groups (Figure 1). Prolactin was correlated with SAPS III ($r = 0.42$, $P = 0.004$) and APACHE II ($r = 0.3$, $P = 0.04$) scores; cortisol was correlated with eHSP90α ($r = 0.47$, $P = 0.013$), IL-6 ($r = 0.25$, $P = 0.05$), and maximum admission day lactate ($r = 0.30$, $P = 0.03$) and negatively with HCO_3 ($r = -0.50$, $P = 0.001$).

The eHSP90α levels in the SS group were increased in comparison to the H and SIRS groups. The eHSP72 levels in the SS group were increased compared to H (Figure 2). Both eHSP90a and eHSP72 were significantly increased in SIRS compared to H ($P < 0.02$).

Extracellular HSP72 in the SS and SIRS groups correlated with the severity of illness scores (Figure 3): APACHE II ($r = 0.45$, $P = 0.034$), SOFA ($r = 0.6$, $P = 0.002$), and SAPS III ($r = 0.5$, $P = 0.004$). There was a positive correlation of eHSP72 and eHSP90α levels in all groups (Table 3). The eHSP90α levels showed a positive correlation with IL-6 and IL-10 but not with IL-17 or IF-γ. Also, eHSP72 levels were only correlated with IL-10 (Table 3).

Septic monocytes expressed significantly lower iHSP72 MFI levels compared to the H group (Table 2). Although a positive correlation of iHSP90α with iHSP72 monocyte levels was found in the SS and SIRS groups, only iHSP72 correlated negatively with severity of illness scores: Apache II ($r = -0.32$, $P = 0.004$), SOFA ($r = -0.32$, $P = 0.017$), and SAPS III ($r = -0.34$, $P = 0.012$).

4. Discussion

In this study we evaluated the early inflammatory and hormonal stress response in SS and SIRS patients in an

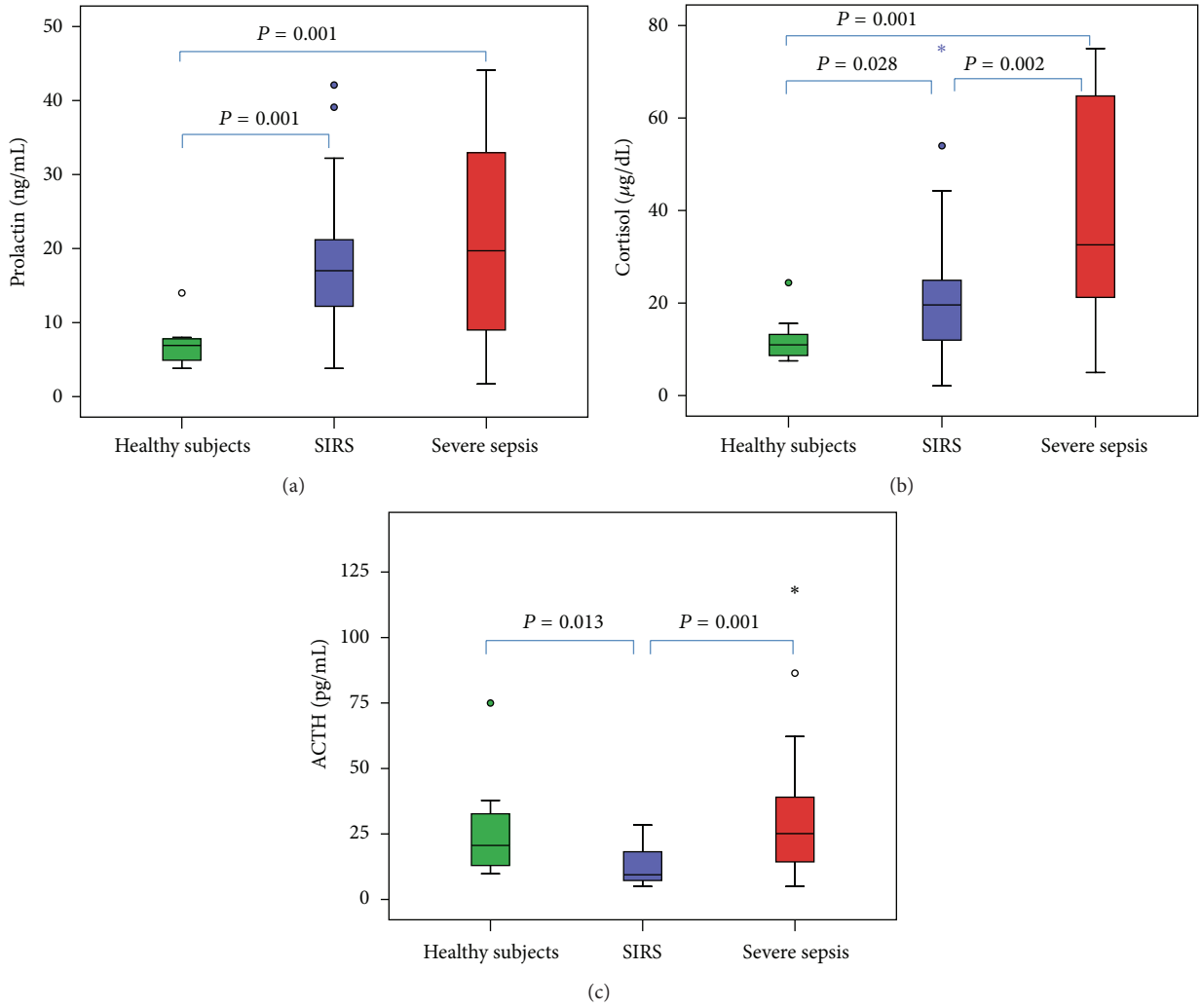

FIGURE 1: Blood concentrations of (a) prolactin; (b) cortisol; and (c) ACTH for the first 48 hours of ICU admission. The P value was calculated using the Mann-Whitney U test and a P value <0.05 (two sided) was considered statistically significant. The box-whisker plots show the median (horizontal line within the box) and the 10th and 90th percentiles (whiskers). The box length is the interquartile range. Solid circles represent outliers and stars extremes.

ICU setting. We showed that in these critically ill patients prolactin levels, along with cortisol, were significantly higher in SS compared to H and SIRS groups. Hormonal increase was related to the severity of illness but only cortisol, not prolactin, was correlated with eHSP90α. Thus, our study demonstrated that stress response of prolactin and its relation to the severity of sepsis might not have been induced through the iHSP72 or iHSP90α "danger signal" pathways.

Patients suffering early sepsis induce elevation of baseline cortisol levels and decrease in blood cortisol to ACTH ratio compared to nonseptic patients admitted to the ICU [20]. Plasma ACTH and prolactin are increased within the few minutes following the insult of a pathogen [2]. Our findings of significantly increased ACTH, cortisol, and prolactin levels in severe sepsis and septic shock also support the hypothesis that prolactin, apart from cortisol, may have a role in the acute stress hormonal response in the early-onset inflammatory process. It has been previously suggested that, with nitric oxide as a key mediator, sepsis elicits a very reproducible pattern of pituitary hormone secretion, with plasma ACTH and prolactin increasing within a few minutes following the insult and with a rapid inhibition of secretion of luteinizing and thyroid-stimulatory hormone [2]. This is further supported by a strongly positive relationship of prolactin with the evaluated in this study's severity scoring systems.

Cortisol and ACTH levels have extensively been studied in a critical ill setting. On the contrary, prolactin's role as an immunomodulator in septic patients needs to be further elucidated. A recent study has suggested an association of increased prolactin mRNA expression in monocytes with better outcome in hemato-oncological septic patients [21]. It has been also found that anterior pituitary cells recognize and respond to fungal cell wall glucans by appropriately stimulating the secretion of prolactin, a hormone that plays an important role in the response to fungal infection [22]. In addition, the increased prolactin levels in our trauma patients might

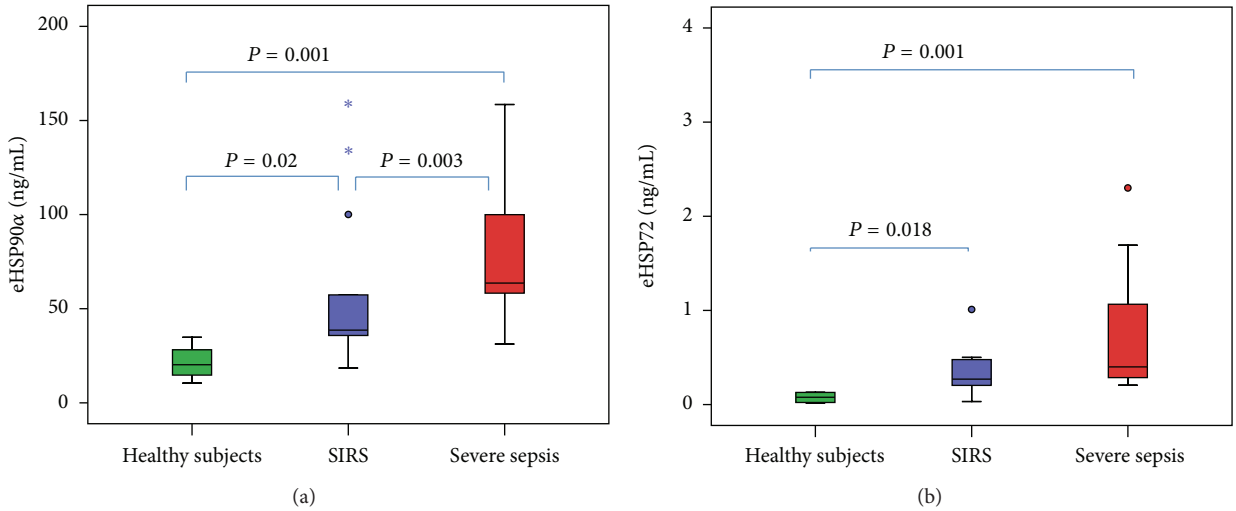

Figure 2: Extracellular heat shock protein (eHSP) levels of (a) eHSP90α and (b) eHSP72 in healthy subjects, SIRS, and severe sepsis patients. The P value was calculated using the Mann-Whitney U test and a P value <0.05 (two sided) was considered statistically significant. The box-whisker plots show the median (horizontal line within the box) and the 10th and 90th percentiles (whiskers). The box length is the interquartile range. Solid circles represent outliers and stars extremes.

Table 3: Pearson's coefficient correlations of hormones, extracellular heat shock proteins, and interleukins.

Prolactin		Prolactin	Cortisol	ACTH	eHSP90α	eHSP72	IL-6	IL-10	IL-17
Cortisol	r	**0.24**							
	p	**0.05**							
ACTH	r	−0.1	0.05						
	p	0.3	0.68						
eHSP90α	r	0.09	**0.47**	0.02					
	p	0.62	**0.013**	0.9					
eHSP72	r	0.35	0.26	−0.02	**0.553**				
	p	0.08	0.23	0.95	**0.002**				
IL-6	r	0.08	0.24	0.02	**0.5**	−0.01			
	p	0.5	0.05	0.8	**0.007**	0.9			
IL-10	r	0.16	0.2	−0.07	**0.6**	**0.7**	**0.27**		
	p	0.3	0.2	0.60	**0.001**	**0.000**	**0.06**		
IL-17	r	−0.11	0.02	−0.080	−0.136	−0.007	−0.4	−0.08	
	p	0.48	0.90	0.596	0.467	0.97	0.3	0.58	
IFN-γ	r	−0.07	0.195	−0.05	0.04	−0.067	0.08	**0.45**	−0.05
	p	0.58	0.125	0.69	0.84	0.733	0.5	**0.02**	0.7

eHSP: extracellular heat shock protein; iHSP: intracellular heat shock protein; IL: interleukins; IFN-γ: interferon gamma.

further suggest that the prolactin's response role is equally important to that of cortisol in homeostasis not only in infectious but also in noninfectious SIRS.

Although none of the eHSPs correlated with prolactin levels, cortisol was correlated with eHSP90α. It has been previously shown that eHSP90α plays a crucial role in antigen presenting in dendritic cells through the major histocompatibility complex (MHC) type 1 [23] and stimulates monocytes [24]. To the best of our knowledge, this is the first time that a significant early increase of plasma eHSP90α is shown in septic patients compared to healthy subjects and severely injured patients. In addition, the strong correlation of eHSP90α with eHSP72 found in our study might further suggest a common pathophysiologic mechanism of their expression. Thus, extracellular levels of both HSPs were significantly elevated in severely septic patients as compared to healthy volunteers; moreover, eHSP90α levels of SS were higher when compared to trauma (SIRS) patients. Importantly, in both SS and SIRS groups, eHSP72 levels correlated with the severity of illness, further supporting the hypothesis that eHSPs may act as danger signals to modulate the immune system [8].

Increased eHSP72 levels have been found in inflammatory myopathy [25], lung injury [26], acute coronary syndrome and stable angina [27], trauma [15], inflammation,

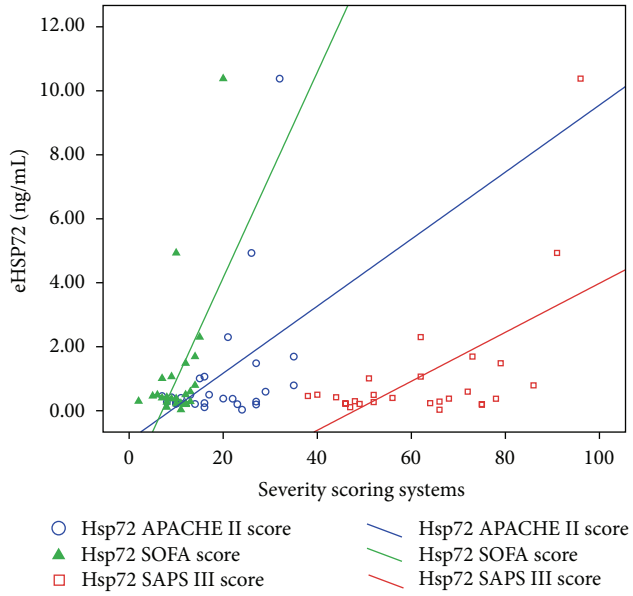

Figure 3: Extracellular heat shock protein (eHSP) levels of eHSP72 in SIRS and sepsis correlated with severity of illness scores APACHE II ($r = 0.45$, $P = 0.034$), SOFA ($r = 0.6$, $P = 0.002$), and SAPS III ($r = 0.5$, $P = 0.004$).

and sepsis [12]. Only two in vivo studies of eHSP72 and none of eHSP90α were identified in a PubMed database research in human severe sepsis (1992–2012) [12, 28]. Wheeler et al. [28] found that serum levels of eHSP72 in children with septic shock were significantly elevated as compared to noncritically ill children undergoing elective surgical procedures and that the increase was related to mortality. Analysis of oxidative parameters in septic patients by Gelain et al. revealed that serum levels of eHSP72 were modulated according to oxidative stress [12]. In our study we further showed a significant early increase of eHSP72 in both septic and traumatic adult patients compared to controls.

It has been suggested that stimulation of toll-like receptors (TLR)-dependent signaling pathways by lipopolysaccharides (LPS) increases iHSP72 expression and its release in the extracellular environment both in in vitro cell cultures and in vivo [29]. The release of eHSPs appears to be a very complex phenomenon encompassing different alternative pathways containing nonconsensus secretory signals [30]. Proposed mechanisms for their release are translocation across the plasma membrane, release associated with lipid vesicles, passive release after cell death by necrosis via extracellular vesicles [11], and through nonclassical exomal nonsecretory pathways [31]. Although in septic patients stimulation of LPS pathways has been proposed to explain the extracellular release of eHSPs, it might not clarify the increased eHSPs levels in our SIRS patients. Instead, it has been hypothesized that eHSPs may be released from damaged tissue and necrotic cells after trauma contributing to trauma induced immunosuppression [32].

In severe sepsis, in contrast to the extracellular monocyte-HSPs, intracellular monocyte-HSPs had a tendency to decrease in comparison to control subjects. These findings are in accordance with those of another prospective observational study in patients with severe sepsis demonstrating a reduced iHSP72 expression in PBMCs and a LPS dose-dependent inhibition of its expression [33]. In patients with inflammation, however, iHSPs were found increased compared to healthy control subjects [34]. Although SIRS was shown to increase iHSP72 and iHSP90α expression in monocytes indicating a protective function of iHSPs during the acute phase of stress, the downregulation of iHSP-positive cells in SS seems to be a result of (mal-)adaptation mechanism to severity of illness [35]. Preliminary data showed that iHSP72 and iHSP90α monocyte expressions follow different longitudinal courses in SS, not related to the metabolic response to stress [36].

The main limitations of our study are the small number of patients and an age difference among groups. Enrollment of septic patients has been proven to be difficult since most of them met exclusion criteria (immunosuppression, cancer). In addition, since that was a prospective study with consecutively admitted patients in ICU, the mean age of trauma patients was, as expected, lower than the mean age of septic patients. The mean age of healthy subjects, however, was matched with the mean age of ICU patients included in our study. Finally, we did not find any correlation between hormones and age, so that the importance of the age difference might not have significantly influenced results.

5. Conclusions

Prolactin, apart from cortisol, may have an early role in the acute stress hormonal response in severe sepsis and septic shock. Extracellular levels of HSP72 and HSP90α are significantly elevated in septic patients as compared to healthy volunteers supporting the hypothesis that eHSPs may act as danger signals to activate the immune system. Simultaneously, however, monocytes suppress iHSP72 expression early in septic patients. To the best of our knowledge, this is the first time that a significant early increase of plasma eHSP90α is shown in septic patients compared to healthy subjects and severely injured patients and that this increase is related to increased cortisol levels.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

This research has been cofinanced by the European Union (European Social Fund (ESF)) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) Research Funding Program: THALES.

References

[1] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, "Epidemiology of severe sepsis in the

United States: Analysis of incidence, outcome, and associated costs of care," *Critical Care Medicine*, vol. 29, no. 7, pp. 1303–1310, 2001.

[2] V. Maxime, S. Siami, and D. Annane, "Metabolism modulators in sepsis: the abnormal pituitary response," *Critical Care Medicine*, vol. 35, no. 9, pp. S596–S601, 2007.

[3] W. Kanczkowski, V. Alexaki, N. Tran et al., "Hypothalamo-pituitary and immune-dependent adrenal regulation during systemic inflammation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 36, pp. 14801–14806, 2013.

[4] R. Oberbeck, D. Schmitz, K. Wilsenack et al., "Prolactin modulates survival and cellular immune functions in septic mice," *Journal of Surgical Research*, vol. 113, no. 2, pp. 248–256, 2003.

[5] S. M. Heidemann, R. Holubkov, K. L. Meert et al., "Baseline serum concentrations of zinc, selenium, and prolactin in critically ill children," *Pediatric Critical Care Medicine*, vol. 14, no. 4, pp. e202–e206, 2013.

[6] L. A. Dossett, B. R. Swenson, H. L. Evans, H. Bonatti, R. G. Sawyer, and A. K. May, "Serum estradiol concentration as a predictor of death in critically ill and injured adults," *Surgical Infections*, vol. 9, no. 1, pp. 41–48, 2008.

[7] B. Saribek, Y. Jin, M. Saigo, K. Eto, and S. Abe, "HSP90β is involved in signaling prolactin-induced apoptosis in newt testis," *Biochemical and Biophysical Research Communications*, vol. 349, no. 4, pp. 1190–1197, 2006.

[8] K. W. McConnell, A. C. Fox, A. T. Clark et al., "The role of heat shock protein 70 in mediating age-dependent mortality in sepsis," *Journal of Immunology*, vol. 186, no. 6, pp. 3718–3725, 2011.

[9] A. Ambade, D. Catalano, A. Lim, and P. Mandrekar, "Inhibition of heat shock protein (molecular weight 90 kDa) attenuates proinflammatory cytokines and prevents lipopolysaccharide-induced liver injury in mice," *Hepatology*, vol. 55, no. 5, pp. 1585–1595, 2012.

[10] G. Briassoulis, E. Briassouli, D. M. Fitrolaki et al., "Heat shock protein 72 expressing stress in sepsis: unbridgeable gap between animal and human studies—A Hypothetical "Comparative" study," *BioMed Research International*, vol. 2014, Article ID 101023, 17 pages, 2014.

[11] A. De Maio and D. Vazquez, "Extracellular heat shock proteins: a new location, a new function," *Shock*, vol. 40, no. 4, pp. 239–246, 2013.

[12] D. P. Gelain, M. A. De Bittencourt Pasquali, C. M. Comim et al., "Serum heat shock protein 70 levels, oxidant status, and mortality in sepsis," *Shock*, vol. 35, no. 5, pp. 466–470, 2011.

[13] E. Briassouli, D. Goukos, G. Daikos et al., "Glutamine suppresses Hsp72 not Hsp90α and is not inducing Th1, Th2, or Th17 cytokine responses in human septic PBMCs," *Nutrition*, 2014.

[14] V. L. Vega, M. Rodríguez-Silva, T. Frey et al., "Hsp70 translocates into the plasma membrane after stress and is released into the extracellular environment in a membrane-associated form that activates macrophages," *Journal of Immunology*, vol. 180, no. 6, pp. 4299–4307, 2008.

[15] J. Pittet, H. Lee, D. Morabito, M. B. Howard, W. J. Welch, and R. C. Mackersie, "Serum levels of Hsp 72 measured early after trauma correlate with survival," *The Journal of Trauma*, vol. 52, no. 4, pp. 611–617, 2002.

[16] R. P. Dellinger, M. M. Levy, A. Rhodes et al., "Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Surviving Sepsis Campaign Guidelines Committee including the Pediatric Subgroup," *Intensive Care Medicine*, vol. 41, no. 2, pp. 580–637, 2013.

[17] W. A. Knaus, E. A. Draper, D. P. Wagner, and J. E. Zimmerman, "APACHE II: a severity of disease classification system," *Critical Care Medicine*, vol. 13, no. 10, pp. 818–829, 1985.

[18] J.-L. Vincent, R. Moreno, J. Takala et al., "The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure," *Intensive Care Medicine*, vol. 22, no. 7, pp. 707–710, 1996.

[19] R. P. Moreno, P. G. H. Metnitz, B. Metnitz, P. Bauer, S. Afonso de Carvalho, and A. Hoechtl, "Modeling in-hospital patient survival during the first 28 days after intensive care unit admission. A prognostic model for clinical trials in general critically ill patients," *The Journal of Critical Care*, vol. 23, no. 3, pp. 339–348, 2008.

[20] O. Lesur, J. Roussy, F. Chagnon et al., "Proven infection-related sepsis induces a differential stress response early after ICU admission," *Critical Care*, vol. 14, no. 4, article R131, 2010.

[21] P. Cejková, V. Chromá, M. Cerná et al., "Monitoring of the course of sepsis in hematooncological patients by extrapituitary prolactin expression in peripheral blood monocytes," *Physiological Research*, vol. 61, no. 5, pp. 481–488, 2012.

[22] K. F. Breuel, P. Kougias, P. J. Rice et al., "Anterior pituitary cells express pattern recognition receptors for fungal glucans: implications for neuroendocrine immune involvement in response to fungal infections," *NeuroImmunoModulation*, vol. 11, no. 1, pp. 1–9, 2004.

[23] J. Oura, Y. Tamura, K. Kamiguchi et al., "Extracellular heat shock protein 90 plays a role in translocating chaperoned antigen from endosome to proteasome for generating antigenic peptide to be cross-presented by dendritic cells," *International Immunology*, vol. 23, no. 4, pp. 223–237, 2011.

[24] P. Cecchini, R. Tavano, P. Polverino de Laureto et al., "The soluble recombinant Neisseria meningitidis adhesin NadA δ351-405 stimulates human monocytes by binding to extracellular Hsp90," *PLoS ONE*, vol. 6, no. 9, Article ID e25089, 2011.

[25] T. Svitalkova, M. Remakova, L. Plestilova et al., "Plasma level of HSP70 protein is increased in czech patients with idiopathic inflammatory myopathy," *Annals of the Rheumatic Diseases*, vol. 1, no. 73, supplement 1, p. A54, 2014.

[26] M. T. Ganter, L. B. Ware, M. Howard et al., "Extracellular heat shock protein 72 is a marker of the stress protein response in acute lung injury," *The American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 291, no. 3, pp. L354–L361, 2006.

[27] X. Zhang, Z. Xu, L. Zhou et al., "Plasma levels of Hsp70 and anti-Hsp70 antibody predict risk of acute coronary syndrome," *Cell Stress and Chaperones*, vol. 15, no. 5, pp. 675–686, 2010.

[28] D. S. Wheeler, L. E. Fisher Jr., J. D. Catravas, B. R. Jacobs, J. A. Carcillo, and H. R. Wong, "Extracellular hsp70 levels in children with septic shock," *Pediatric Critical Care Medicine*, vol. 6, no. 3, pp. 308–311, 2005.

[29] A. Gupta, Z. A. Cooper, M. E. Tulapurkar et al., "Toll-like receptor agonists and febrile range hyperthermia synergize to induce heat shock protein 70 expression and extracellular release," *Journal of Biological Chemistry*, vol. 288, no. 4, pp. 2756–2766, 2013.

[30] A. de Maio, "Extracellular heat shock proteins, cellular export vesicles, and the Stress Observation System: a form of communication during injury, infection, and cell damage: It is never

known how far a controversial finding will go! Dedicated to Ferruccio Ritossa," *Cell Stress and Chaperones*, vol. 16, no. 3, pp. 235–249, 2011.

[31] W. Li, D. Sahu, and F. Tsen, "Secreted heat shock protein-90 (Hsp90) in wound healing and cancer," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1823, no. 3, pp. 730–741, 2012.

[32] S. B. Flohé, J. M. Bangen, S. Flohé, H. Agrawal, K. Bergmann, and F. U. Schade, "Origin of immunomodulation after soft tissue trauma: Potential involvement of extracellular heat-shock proteins," *Shock*, vol. 27, no. 5, pp. 494–502, 2007.

[33] S. Schroeder, J. Bischoff, L. E. Lehmann et al., "Endotoxin inhibits heat shock protein 70 (HSP7O) expression in peripheral blood mononuclear cells of patients with severe sepsis," *Intensive Care Medicine*, vol. 25, no. 1, pp. 52–57, 1999.

[34] R. Njemini, I. Bautmans, M. Lambert, C. Demanet, and T. Mets, "Heat shock proteins and chemokine/cytokine secretion profile in ageing and inflammation," *Mechanisms of Ageing and Development*, vol. 128, no. 7-8, pp. 450–454, 2007.

[35] K. T. Apostolou, K. Psara, E. Briassouli et al., "Heat shock protein 72 and 90 intracellular monocyte expression in patients with sepsis or sirs: preliminary data," *Intensive Care Medicine*, vol. 39, supplement 2, p. S240, 2013.

[36] T. Tavladaki, A. M. Spanaki, H. Dimitriou et al., "Expression of intracellular HSPs in neutrophil and monocyte subjects during acute phase of stress in critical ill patients: preliminary data," *Intensive Care Medicine*, vol. 39, supplement 2, pp. S240–S241, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 254686, 8 pages
http://dx.doi.org/10.1155/2014/254686

Research Article

Epinephrine Enhances the Response of Macrophages under LPS Stimulation

Jianyun Zhou,[1,2] Jun Yan,[1] Huaping Liang,[1] and Jianxin Jiang[1]

[1] *State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Surgery Research, Daping Hospital, Third Military Medical University, Chongqing 400042, China*
[2] *Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China*

Correspondence should be addressed to Jun Yan; 13883092250@163.com and Jianxin Jiang; hellojjx@126.com

Received 16 February 2014; Revised 8 July 2014; Accepted 29 July 2014; Published 26 August 2014

Academic Editor: Baoli Cheng

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Trauma associated with infection may directly trigger a neuroendocrine reaction *in vivo* while the hormone epinephrine is known to mediate immune responses to inflammation after injury. However, the role of epinephrine during the earliest stage of trauma still remains unclear. We therefore explored the role of epinephrine on activated macrophages under LPS stimulation *in vitro* as well as the mechanisms underlying its effect. Dose- and time-dependent effects of epinephrine on macrophage immune function were assessed after LPS activation. We also employed CD14 siRNA interference to investigate whether CD14 played a role in the mechanism underlying the effect of epinephrine on LPS-induced macrophage responses. Our results showed that epinephrine pretreatment (10 ng/mL) significantly promoted immune responses from LPS stimulated macrophages, including phagocytic rate, phagocytic index, TNFα/IL-1β/IL-10 secretion, and CD14 expression ($P < 0.05$). Moreover, TNFα/IL-1β/IL-10 levels attained their peak value 1 hour after incubation with 10 ng/mL epinephrine ($P < 0.05$), and CD14 siRNA transfection dramatically decreased phagocytosis and cytokine secretion by LPS-activated macrophages ($P < 0.05$). We therefore conclude that 10 ng/mL epinephrine enhances immune responses from macrophages under LPS stimulation and that the underlying mechanism may relate to CD14 upregulation on the surface of macrophages.

1. Introduction

A frequent complication arising from severe trauma is infection. Trauma-associated infection may directly trigger a neuroendocrine reaction *in vivo*. This complicated cross-interaction between the immune and neuroendocrine systems mediated by endogenous hormones can influence the homeostasis of host health [1].

Macrophages are the most important immune effector cells during the earliest stage of trauma and are activated by recognizing pathogen-associated molecular patterns (PAMPs) during the immune-defense reaction against infection. Gram-negative bacteria express a variety of PAMPs, including lipopolysaccharide (LPS), the main component of endotoxin that is present in their cell wall. Pattern recognition receptors (PRRs) expressed on macrophages, such as CD14, therefore play a decisive role in recognizing LPS and triggering the subsequent release of inflammatory factors that mediate cell activation.

Epinephrine, a major effector hormone of the sympathetic-adrenal medulla (SAM) axis, has gradually gained attention for its role in the innate immune response during the earliest stage of infection [2–4]. However, its influence on macrophage activation after trauma-associated infection remains unclear, especially its relationship with PRRs expressed on the surface of macrophages.

In this study, the role and mechanism underlying the effect of epinephrine on macrophages in the earliest stage of trauma-associated infection were examined *in vitro* using primary peritoneal macrophages from Sprague-Dawley (SD) rats. Their phagocytic and cytokine-secretion responses were evaluated after pretreatment with epinephrine at various doses and time points which was followed by stimulation with LPS (10 ng/mL) for 1 hour. To test whether CD14 played a

role in this response, the same methodology was also adopted under CD14 siRNA interference conditions.

2. Materials and Methods

2.1. Experimental Animals. Adult Sprague-Dawley (SD) rats were purchased from the experimental animal center at the Surgery Research, Daping Hospital, Third Military Medical University. All animals were bred in the animal facility under specific pathogen-free (SPF) conditions, and experimental procedures were performed in strict accordance with the guidelines set forth by the Research Council and Animal Use and Care Committee of the Third Military Medical University.

2.2. Dose-Dependent Effect of Epinephrine on Macrophage Responses under LPS Stimulation. Peritoneal macrophages were collected from rats and purified [5, 6]. Macrophages (2×10^6 cells/well) were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS) (Hyclone, USA) in a 24-well plate overnight at 37°C and 5% CO_2. After cells were washed with PBS, epinephrine (Sigma, USA) was added at various concentrations (2, 10, 50, and 100 ng/mL) into respective wells and incubated for an additional hour at 37°C and 5% CO_2. Then, macrophages were stimulated with 10 ng/mL LPS (*Escherichia coli* O26:B6) for another hour under the same conditions. Supernatant was collected, and the expressions of TNFα, IL-1β, IL-6, and IL-10 were detected by an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, USA; Boster Biotechnology, China) [5, 6]. Meanwhile, the adherent cell monolayer was washed with PBS, and 2×10^8 colony-forming units/mL of red-fluorescently labeled *E. coli* BI21 was added. After a 30 min incubation, the plate was washed 5x with PBS and fixed with 4% paraformaldehyde. *E. coli* BI21 phagocytosis was observed by confocal microscopy (TCSSP2; Leica Microsystems, Wetzlar, Germany) to acquire the phagocytic rate (% of macrophages containing at least one ingested bacterium) and the phagocytic index (the mean number of phagocytosed bacteria observed in the macrophage cytoplasm). In addition, total macrophage RNA was extracted by TRIzol reagent (Invitrogen, Karlsruhe, Germany). One microgram of total RNA was transcribed into cDNA with Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, California, USA) by oligo dT priming. CD14 expression was measured by reverse transcription polymerase chain reaction (RT-PCR) (Table 1). PCR products were analyzed on a 2% (w/v) agarose-ethidium bromide gel with a computer-linked phosphoimaging system (Gel Doc 2000; Bio-Rad, Hercules, California, USA). Semiquantitative analysis of CD14 expression was conducted with the software of GelDoc 2000 Imaging System.

2.3. Time-Dependent Effect of Epinephrine on Macrophage Responses under LPS Stimulation. The collection, purification, and incubation of primary rat peritoneal macrophages were performed as described above. In a second set of plates, 10 ng/mL epinephrine was added to each well, and the cells were incubated for varying periods of time (0, 0.5, 1, 2, 3, and 5 hours). Thereafter, 10 ng/mL LPS (*E. coli* O26:B6) was added into each well for another hour. Supernatant was collected, and the expressions of TNFα, IL-1β, IL-6, and IL-10 were assayed by ELISA.

2.4. CD14 siRNA Interference Effect on Macrophage Responses. Peritoneal macrophages were acquired as described above. Macrophages (2×10^6 cells/well) were incubated in 24-well plate at 37°C and 5% CO_2 for 24 h before CD14 siRNA transfection. According to the recommended Lipofectamine reagent kit protocol (Santa Cruz, USA), we first determined the optimal time interval by observing cell morphology and CD14 expression level. The macrophages were then divided into the following 3 groups: negative control, siRNA control, and CD14 siRNA. At the optimal time interval, epinephrine (10 ng/mL) was added into the CD14 siRNA group for 1 h, followed by LPS (10 ng/mL) stimulation for another hour. Cell supernatant was collected to assay TNFα expression levels by ELISA. Macrophage phagocytosis was measured as described above. Total RNA was isolated from the macrophages in each group and transcribed into cDNA, as described above. CD14 expression was evaluated by RT-PCR (Table 1), and semiquantitative analysis of CD14 was performed.

2.5. Statistical Analysis. The data were presented as mean ± standard deviation (SD) ($x \pm s$). Origin 7.5 software was used to analyze the role of epinephrine in macrophage activation under LPS stimulation, while one-way analysis of variance (one-way ANOVA) by SPSS11.5 software was used to analyze normality, homogeneity of variance, and interblock contrast. The significance level was set at 95%.

3. Results

3.1. Epinephrine Effects on Phagocytosis Function by LPS-Stimulated Macrophages. Epinephrine at 10 ng/mL significantly increased the phagocytic rate of LPS-treated macrophages ($P < 0.01$). However, whether higher epinephrine concentrations (50–100 ng/mL) influenced the phagocytic rate was not clear. Meanwhile, lower epinephrine concentrations (2–50 ng/mL) enhanced the macrophage phagocytic index to some extent, especially at the 10 ng/mL concentration ($P < 0.01$), although this result was not found at the 100 ng/mL concentration (Figure 1).

3.2. Epinephrine Effects on TNFα/IL-1β/IL-6/IL-10 Secretion by LPS-Stimulated Macrophages. A series of inflammatory cytokines produced by macrophages, namely, TNFα, IL-1β, IL-6, and IL-10, were used to assess the ability of macrophages to produce cytokines. In the dose-dependent effect study, low epinephrine concentrations (2–10 ng/mL) significantly enhanced TNFα/IL-1β/IL-10 production, especially the 10 ng/mL dose ($P < 0.05$). With further increases in epinephrine concentration, however, TNFα secretion level did not continue to rise (Figure 2).

In the time-dependent effect study, the secretion of TNFα, IL-1β, IL-6, and IL-10 significantly increased during the first hour after exposure to 10 ng/mL epinephrine

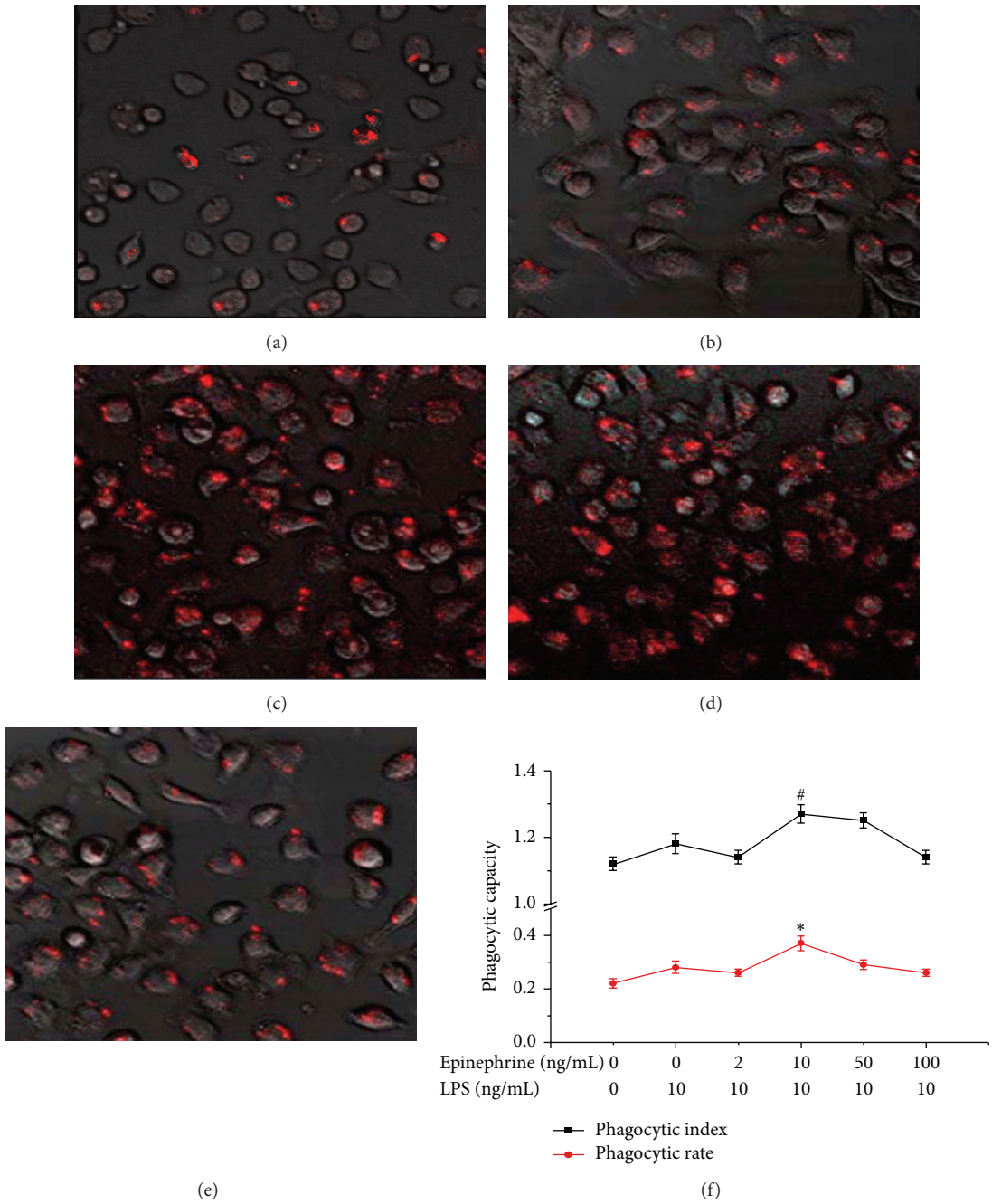

Figure 1: The role of epinephrine in macrophage phagocytosis under LPS stimulation. ((a)–(d)) Confocal images of the following groups are shown (magnification, (a) 200x; ((b)–(d)) 400x). (a) Control macrophages without LPS stimulation; macrophages pretreated with (b) 0 ng/mL, (c) 2 ng/mL, (d) 10 ng/mL, or (e) 100 ng/mL epinephrine under LPS stimulation. (f) Changes in phagocytic index and phagocytic rate at different epinephrine doses compared with the control group. $^{\#}P < 0.01$, $^{*}P < 0.05$.

Table 1: RT-PCR primers.

Primer	Type	Sequence	Product size (bp)
GAPDH	Sense	5′-GCAAGTTCAATGGCACAGTCAAGG-3′	471
	Antisense	5′-TGGATGCAGGGATGATGTTCTGG-3′	
CD14	Sense	5′-CACAAATTCCCGACCCTCCAAGT-3′	425
	Antisense	5′-AAAGTTCCTGACAAGCCCGCTGA-3′	

All primers were synthesized by Sangon (Shanghai, China) with the catalog numbers NM017008 and NM021744, respectively. PCR parameters for rat GAPDH and CD14 proceeded as follows: 94°C for the initial 3 min; 27 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 40 sec; and 72°C for the final 2 min.

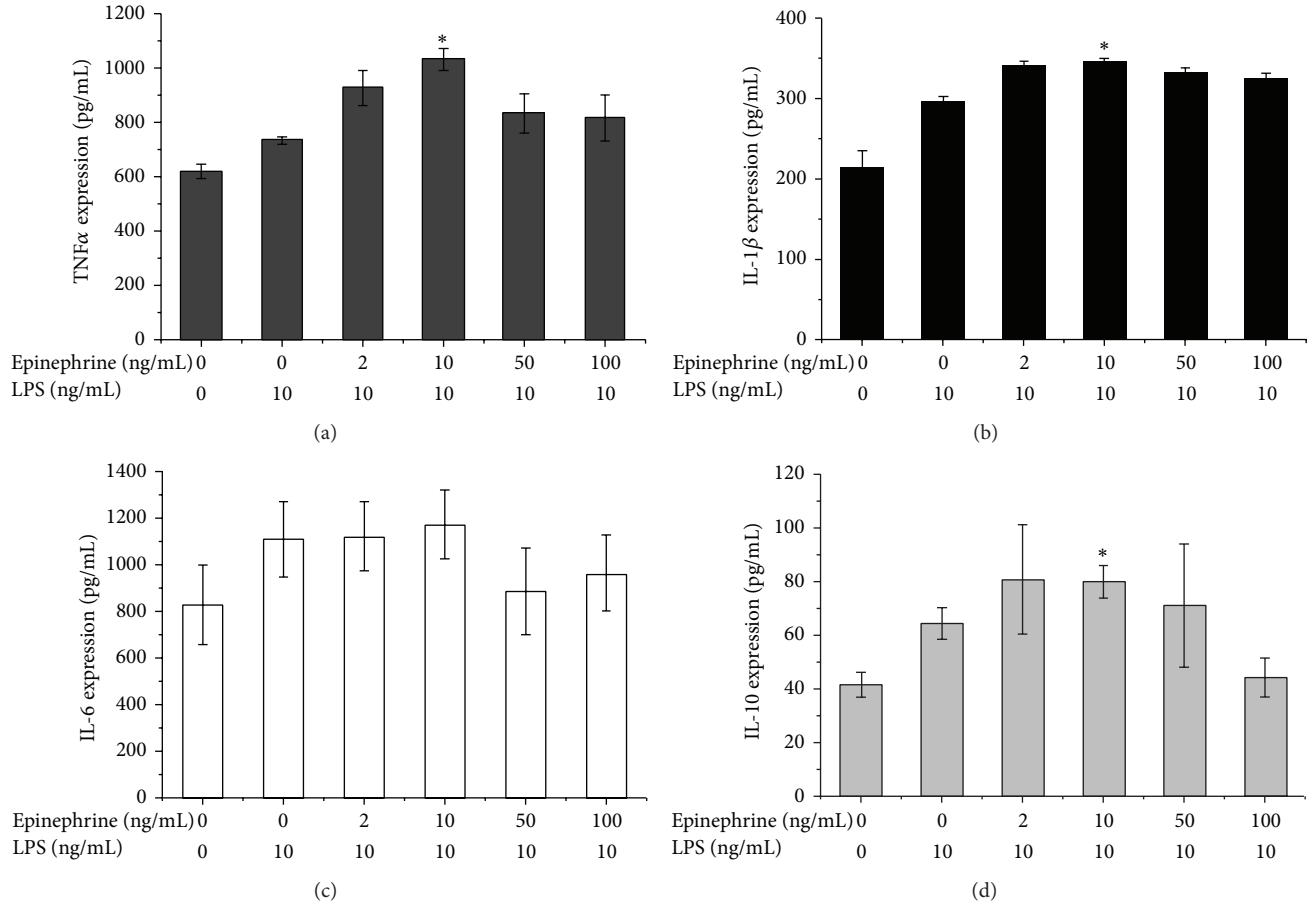

Figure 2: Dose-dependent effect of epinephrine on macrophage secretion under LPS stimulation. The macrophages were pretreated with epinephrine at various concentrations (2 ng/mL, 10 ng/mL, 50 ng/mL, and 100 ng/mL) for 1 hour and then stimulated by 10 ng/mL LPS for another 1 hour. The levels of cytokines including (a) TNFα, (b) IL-1β, (c) IL-6, and (d) IL-10 levels in each group were measured by ELISA. $^{*}P < 0.05$, compared with the 0 ng/mL control group stimulated by LPS only.

($P < 0.05$). However, this same epinephrine dose repressed these cytokines' production when macrophages were exposed for longer time periods (2–5 hours for TNFα, IL-1β, and IL-6; 3–5 hours for IL-10) ($P < 0.05$) (Figure 3).

3.3. Epinephrine Effects on CD14 Expression Levels in LPS-Stimulated Macrophages. CD14 expression level in LPS-stimulated macrophages significantly increased during the first hour of exposure to 10 ng/mL epinephrine ($P < 0.05$). However, higher epinephrine doses (50–100 ng/mL) did not induce the same increase in CD14 expression (Figure 4).

3.4. Effect of CD14 siRNA Interference on Epinephrine-Treated Activated Macrophages. RT-PCR analysis showed that CD14 expression by macrophages significantly decreased 48 hours after transfection with CD14 siRNA, which was maintained for 72 hours. This data demonstrated that the optimal time range for CD14 siRNA-mediated silencing in macrophages was between 48 and 72 hours, during which time the inhibition ratio exceeded 60%. Thus, 52 hours after CD14 siRNA transfection was chosen as the optimal time point to observe macrophage function in the following experiments.

At 52 hours after CD14 siRNA transfection, a 1 h exposure to 10 ng/mL epinephrine inhibited the phagocytic function of macrophages, where the phagocytosis of *E. coli* BI21 significantly decreased ($P < 0.05$) (Figures 5(a)–5(d)). Additionally, compared with the control group ($P < 0.05$) (Figure 5(e)), pretreatment with 10 ng/mL epinephrine led to significantly decreased TNFα secretion by peritoneal macrophages after CD14 siRNA transfection.

4. Discussion

Disease, trauma, inflammation, and infection may elicit a neuroendocrine reaction triggered by exciting the hypothalamic-pituitary-adrenal (HPA) and SAM axes [7, 8]. As major effector hormones secreted by the SAM axis, epinephrine, norepinephrine, and glucocorticoids participate in complicated cross-interaction with innate immunity and influence the homeostasis of host health [1]. Additionally, the movement of Gram-negative bacteria and toxins after severe trauma within hollow organs, such as intestines, is the main source of infection leading to traumatic sepsis. Specifically, bacterial endotoxin plays an important

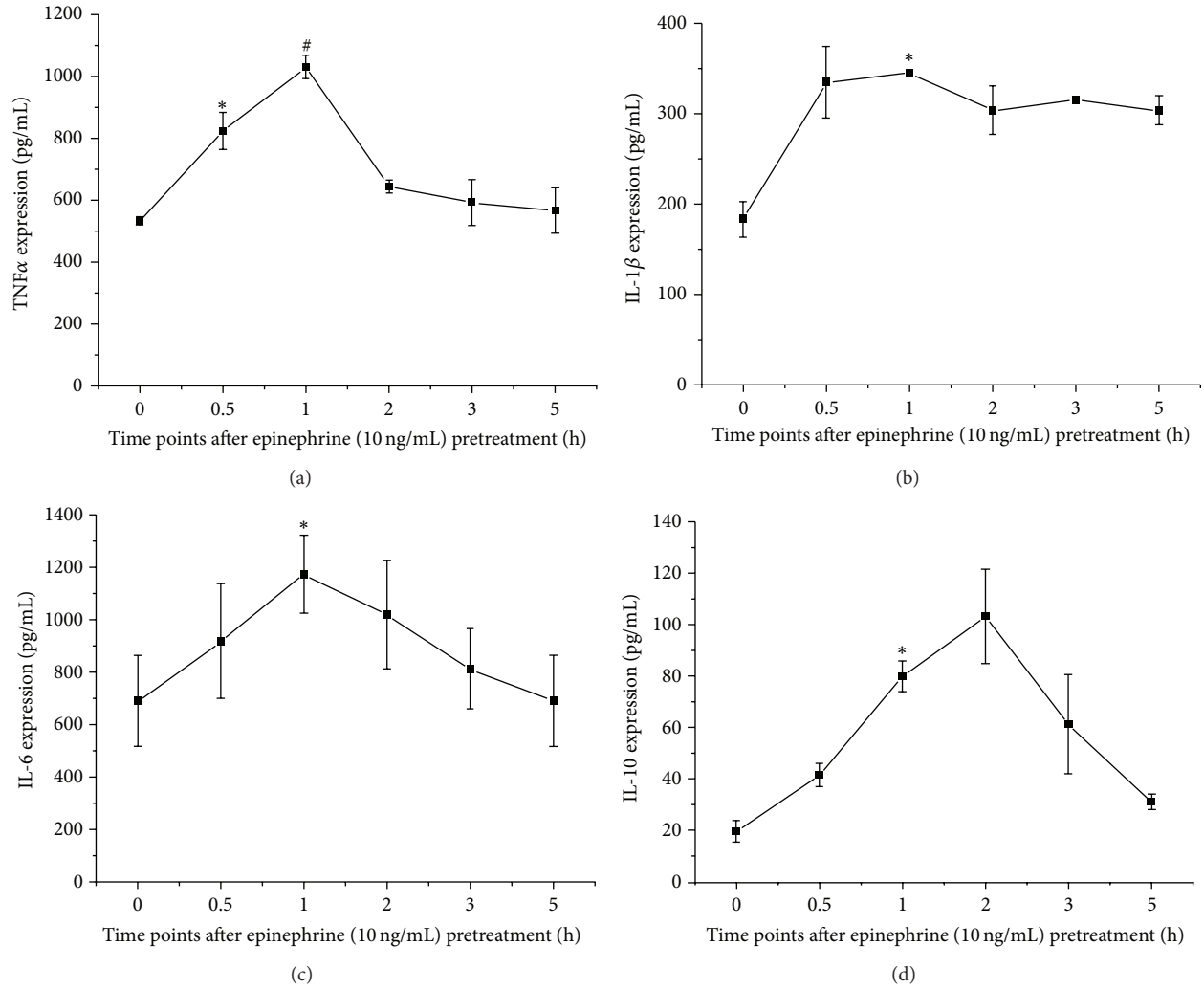

FIGURE 3: Time-dependent effect of epinephrine on macrophage secretion under LPS stimulation. The macrophages were pretreated with 10 ng/mL epinephrine for different intervals (0.5 hours, 1 hour, 2 hours, 3 hours, and 5 hours) and then stimulated by 10 ng/mL LPS for another 1 hour. The levels of cytokines including (a) TNFα, (b) IL-1β, (c) IL-6, and (d) IL-10 levels in each group were measured by ELISA. $^{*}P < 0.05$, $^{\#}P < 0.01$, compared with the 0 h control group.

role in pathogenesis of traumatic sepsis, as PRRs expressed on macrophages recognize LPS, a main component of endotoxin, during the innate immune defense response that leads to cellular activation [9, 10]. Thereafter, macrophages show immunological function, including phagocytosis and cytokine production [11]. However, the role and mechanism by which epinephrine affects macrophage function under LPS stimulation remain unknown.

The *in vitro* study presented here thus explored the relationship among epinephrine, LPS, and macrophages. The results showed that 10 ng/mL epinephrine promoted phagocytosis and TNFα/IL-1β/IL-10 secretion by macrophages, while higher doses of 50 and 100 ng/mL epinephrine inhibited them. Other studies also identified that lower epinephrine doses increased IL-1β secretion, while higher doses did not have the same effect [12–14]. Thus, these results imply that stress hormones at appropriate doses may enhance macrophage activation under LPS stimulation.

The mechanism may have to do with the types and pathways of adrenergic receptors participating in the regulatory course. It has been reported in several studies that the role of lower dose epinephrine is likely to be mediated by α2-adrenergic receptor (AR), which represses the function of adenylate cyclase and causes the amount of cyclic adenosine monophosphate (cAMP) to decrease. As a result, the phosphorylation level of the downstream proteins changes and inflammatory factors are released. On the other hand, it appears that β2-AR mediates the function of higher dose epinephrine and increases the concentration of cAMP. The phagocytosis is inhibited and anti-inflammatory effect is in place in those cases. In addition, the role of epinephrine is related not only to concentrations and pretreatment time but also to the density of AR in different cells. These factors may lead to a completely different immune regulation of epinephrine with various concentrations and pretreatment time. Moreover, the difference among animal strains, organs,

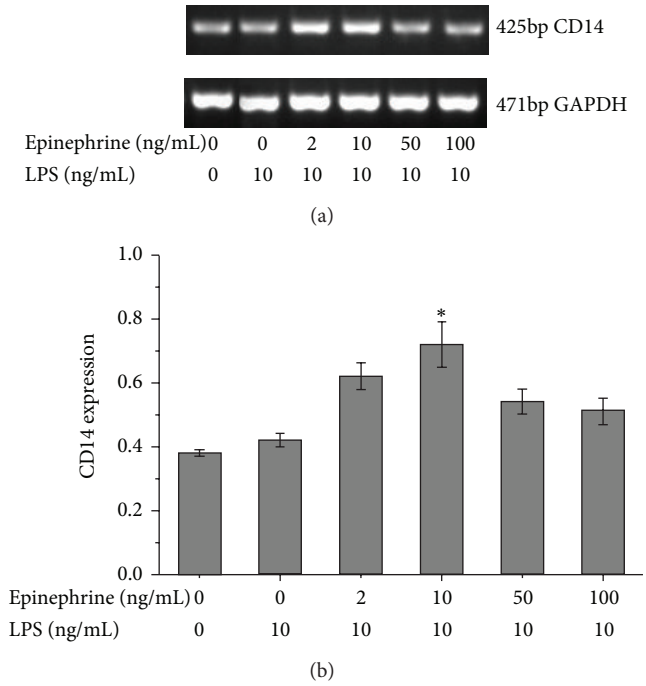

Figure 4: Dose-dependent effect of epinephrine on CD14 expression from macrophages under LPS stimulation. The macrophages were pretreated with epinephrine at various concentrations (2 ng/mL, 10 ng/mL, 50 ng/mL, and 100 ng/mL) for 1 hour and then stimulated by 10 ng/mL LPS for another 1 hour. The expression level of CD14 in each group was measured by RT-PCR. The data were showed in (a) electrophoresis result and (b) semiquantitative analysis. $^{*}P < 0.05$, compared with the 0 ng/mL control group stimulated by LPS only.

and cell types will influence the role of epinephrine too. So far, the regulatory mechanism of epinephrine on immunological cells remains unclear.

LPS is an essential component of the Gram-negative bacterial cell wall and is an important link between the innate immune self-defense response and host signal transduction, since innate immune cells recognize LPS to initiate signal-transduction pathways. PRRs mediate LPS recognition, and transmembrane signal transduction through the PRRs is initiated upstream of the whole signaling pathway. Moreover, this interaction plays a decisive role in cellular activation [15, 16]. LPS is usually first recognized by CD14 in combination with a Toll-like receptor (TLR), which transfers the signal from the cellular membrane to the cytoplasm. The scavenger receptor (SR) participates in regulating endotoxin removal by macrophages and also mediates their host defense function [17–20]. As one type of PRRs, CD14 expression is therefore an important pathway that regulates macrophage activation [21, 22].

In this study, CD14 expression was initially measured after epinephrine treatment, and the results showed that 10 ng/mL epinephrine promoted the expression of CD14 and SR (data not shown) on macrophages. Other studies also confirm that appropriate doses of epinephrine increased SR expression levels in THP-1 cells [23]. In addition, traumatic stress may also promote CD14 expression in macrophages [24]. These above results imply that CD14, as one type of PRRs, participates in macrophage activation triggered by epinephrine under LPS stimulation.

This study further investigated the role of CD14 in macrophage activation under the above conditions by adopting CD14 siRNA technology. The results showed that CD14 siRNA interfered with CD14 expression in macrophages. Moreover, the phagocytosis and cytokine-production functions by macrophages were lower in the transfection group than in the control siRNA group, demonstrating that CD14 siRNA significantly inhibited the sensitivity of LPS-activated macrophages to lower doses of epinephrine. In addition, other studies illustrated that macrophage activation was significantly inhibited after treatment with an antagonistic anti-CD14 antibody [25, 26]. These data further confirm that CD14 plays an important role during epinephrine regulation of macrophage activation under LPS stimulation.

As to the relationship between CD14 and cytokines such as TNFα, it appears that the expression of cytokines (TNFα, IL-1β, IL-6, and IL-10) could be effected by CD14 rather than the reverse. Our results showed that the pattern of CD14 expression was more or less similar to that of cytokines including TNFα, IL-1β, IL-6, and IL-10. With the increasing of the concentration of pretreated epinephrine, the amount of their respective expression changed from low to high and then to low again. Furthermore, their expression levels all attained the peak value when they were pretreated by epinephrine at 10 ng/mL. Meanwhile, in our CD14 siRNA experiment, the expression level of TNFα decreased when the function of CD14 was inhibited. The mechanism may be as follows. As an important pattern recognition receptor, CD14 mediates the recognition and signal transduction of

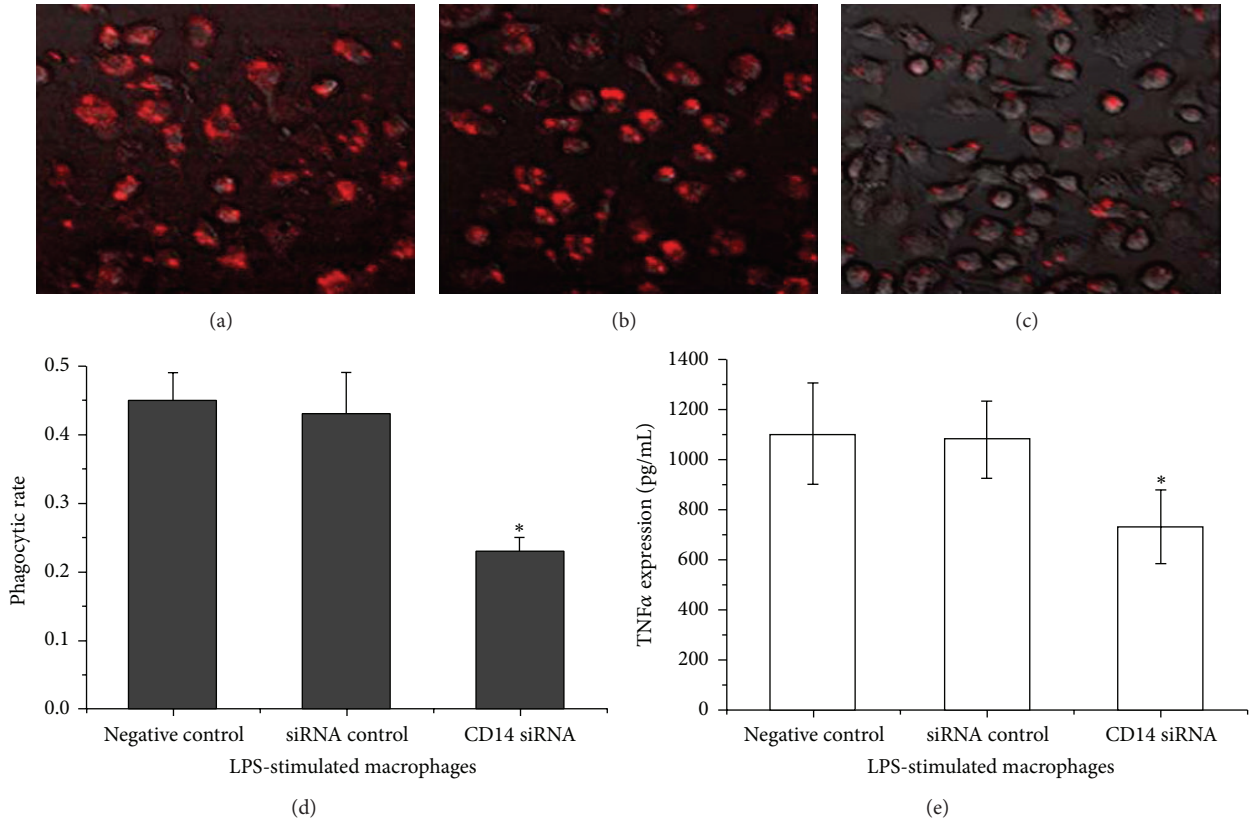

Figure 5: The role of epinephrine on phagocytosis and TNFα secretion by macrophages under LPS stimulation after CD14 siRNA interference. Confocal images of the following groups are shown (magnification, 400x): (a) negative control, (b) siRNA control, and (c) CD14 siRNA. (d) Changes in phagocytic rate from macrophages in the CD14 siRNA group compared to the negative control group and the siRNA control group. (e) Changes in TNFα secretion from macrophages in the CD14 siRNA group compared to the negative control group and the siRNA control group. $^{*}P < 0.05$.

macrophage on LPS. The latter in turn promotes the activation of macrophages and makes them secrete these cytokines. Although the expressions of these cytokines play a feedback role on CD14 to some extent, it appears that the role of CD14 is the most important.

Thus, epinephrine at appropriate concentrations, such as 10 ng/mL, may enhance responses from macrophages stimulated by 10 ng/mL LPS. The underlying mechanism likely relates to upregulated CD14 expression on the cell surface of macrophages. That being said, the precise regulatory mechanism remains unknown in light of the influence that many other stress hormone factors have on innate immunity after LPS infection. We will investigate this matter further in the future research. It must be noted that this investigation discusses a research *in vitro* and the goal is to explore whether the epinephrine pretreatment may influence the immunity function of peritoneal macrophage under LPS stimulation at the early stage (within 6 hours in general). The whole experiment does not involve the role of pretreated epinephrine in mortality and organ injury after LPS stimulated *in vivo*. Nonetheless, our results in this investigation will provide evidential support for the study on the role of epinephrine *in vivo*, especially under the severe trauma combined LPS stimulation.

Conflict of Interests

The authors declare that no conflict of interests exists.

Acknowledgments

The authors thank Dr. Xue Yan, Dr. Guoping Li, Ms. Qing Liu, Ms. Suna Huang, and Ms. Ying Chen for their kind assistance. This work was supported by the National Natural Science Foundation of China (81070624, 81273227), Natural Science Foundation Project of CQ CSTC (2011BB5034), and Open Foundation of State Key Laboratory of Trauma, Burns and Combined Injury (SKLKF201107).

References

[1] J. Jiang, "Posttraumatic stress and immune dissonance," *Chinese Journal of Traumatology*, vol. 11, no. 4, pp. 203–208, 2008.

[2] L. B. Ware, M. D. Eisner, and K. R. Flaherty, "Clinical year in review II: sepsis, mechanical ventilation, occupational and environmental lung disease, and sleep," *Proceedings of the American Thoracic Society*, vol. 6, no. 6, pp. 494–499, 2009.

[3] Y. A. Zausig, D. Geilfus, G. Missler, B. Sinner, B. M. Graf, and W. Zink, "Direct cardiac effects of dobutamine, dopamine,

epinephrine, and levosimendan in isolated septic rat hearts," *Shock*, vol. 34, no. 3, pp. 269–274, 2010.

[4] P. Póvoa and A. H. Carneiro, "Adrenergic support in septic shock: a critical review," *Hospital Practice*, vol. 38, no. 1, pp. 62–73, 2010.

[5] C. Yang, J. Zhou, H. Zhong et al., "Exogenous norepinephrine correlates with macrophage endoplasmic reticulum stress response in association with XBP-1," *The Journal of Surgical Research*, vol. 168, no. 2, pp. 262–271, 2011.

[6] J.-Y. Zhou, H.-J. Zhong, C. Yang, J. Yan, H.-Y. Wang, and J.-X. Jiang, "Corticosterone exerts immunostimulatory effects on macrophages via endoplasmic reticulum stress," *The British Journal of Surgery*, vol. 97, no. 2, pp. 281–293, 2010.

[7] S. G. Correa, M. Maccioni, V. E. Rivero, P. Iribarren, C. E. Sotomayor, and C. M. Riera, "Cytokines and the immune-neuroendocrine network: what did we learn from infection and autoimmunity?" *Cytokine and Growth Factor Reviews*, vol. 18, no. 1-2, pp. 125–134, 2007.

[8] B. A. Kohl and C. S. Deutschman, "The inflammatory response to surgery and trauma," *Current Opinion in Critical Care*, vol. 12, no. 4, pp. 325–332, 2006.

[9] J. Zhou, D. Pavlovic, J. Willecke et al., "Activated protein C improves pial microcirculation in experimental endotoxemia in rats," *Microvascular Research*, vol. 83, no. 3, pp. 276–280, 2012.

[10] W. J. Wiersinga, "Current insights in sepsis: from pathogenesis to new treatment targets," *Current Opinion in Critical Care*, vol. 17, no. 5, pp. 480–486, 2011.

[11] U. Koppe, N. Suttorp, and B. Opitz, "Recognition of Streptococcus pneumoniae by the innate immune system," *Cellular Microbiology*, vol. 14, no. 4, pp. 460–466, 2012.

[12] K. L. Engler, M. L. Rudd, J. J. Ryan, J. K. Stewart, and K. Fischer-Stenger, "Autocrine actions of macrophage-derived catecholamines on interleukin-1β," *Journal of Neuroimmunology*, vol. 160, no. 1-2, pp. 87–91, 2005.

[13] M. Miksa, P. Das, M. Zhou et al., "Pivotal role of the α_{2A}-adrenoceptor in producing inflammation and organ injury in a rat model of sepsis," *PLoS ONE*, vol. 4, no. 5, Article ID e5504, 2009.

[14] G. E. Philippakis, A. C. Lazaris, T. G. Papathomas et al., "Adrenaline attenuates the acute lung injury after intratracheal lipopolysaccharide instillation: an experimental study," *Inhalation Toxicology*, vol. 20, no. 4, pp. 445–453, 2008.

[15] S. K. Biswas and E. Lopez-Collazo, "Endotoxin tolerance: new mechanisms, molecules and clinical significance," *Trends in Immunology*, vol. 30, no. 10, pp. 475–487, 2009.

[16] M. Rossol, H. Heine, U. Meusch et al., "LPS-induced cytokine production in human monocytes and macrophages," *Critical Reviews in Immunology*, vol. 31, no. 5, pp. 379–446, 2011.

[17] S. K. Singh and H. J. Girschick, "Toll-like receptors in *Borrelia burgdorferi*-induced inflammation," *Clinical Microbiology and Infection*, vol. 12, no. 8, pp. 705–717, 2006.

[18] I. A. Yang, K. M. Fong, S. T. Holgate, and J. W. Holloway, "The role of Toll-like receptors and related receptors of the innate immune system in asthma," *Current Opinion in Allergy and Clinical Immunology*, vol. 6, no. 1, pp. 23–28, 2006.

[19] G. Schmalz, S. Krifka, and H. Schweikl, "Toll-like receptors, LPS, and dental monomers," *Advances in Dental Research*, vol. 23, no. 3, pp. 302–306, 2011.

[20] A. Kudrin and D. Ray, "Cunning factor: macrophage migration inhibitory factor as a redox-regulated target," *Immunology and Cell Biology*, vol. 86, no. 3, pp. 232–238, 2008.

[21] J. L. Young, A. Mora, A. Cerny et al., "CD14 deficiency impacts glucose homeostasis in mice through altered adrenal tone," *PLoS ONE*, vol. 7, no. 1, Article ID e29688, 2012.

[22] M. Ueta and S. Kinoshita, "Ocular surface inflammation mediated by innate immunity," *Eye & Contact Lens*, vol. 36, no. 5, pp. 269–281, 2010.

[23] P. Lova, G. F. Guidetti, I. Canobbio, S. Catricalà, C. Balduini, and M. Torti, "Epinephrine-mediated protein kinase C and Rap1b activation requires the co-stimulation of Gz-, Gq-, and Gi-coupled receptors," *Thrombosis and Haemostasis*, vol. 105, no. 3, pp. 479–486, 2011.

[24] J. A. Andrews and K. D. Neises, "Cells, biomarkers, and post-traumatic stress disorder: evidence for peripheral involvement in a central disease," *Journal of Neurochemistry*, vol. 120, no. 1, pp. 26–36, 2012.

[25] K. Reinhart, T. Glück, J. Ligtenberg et al., "CD14 receptor occupancy in severe sepsis: results of a phase I clinical trial with a recombinant chimeric CD14 monoclonal antibody (IC14)," *Critical Care Medicine*, vol. 32, no. 5, pp. 1100–1108, 2004.

[26] S. Genth-Zotz, S. von Haehling, A. P. Bolger et al., "The anti-CD14 antibody IC14 suppresses ex vivo endotoxin stimulated tumor necrosis factor-alpha in patients with chronic heart failure," *European Journal of Heart Failure*, vol. 8, no. 4, pp. 366–372, 2006.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 180109, 5 pages
http://dx.doi.org/10.1155/2014/180109

Review Article

Defensins and Sepsis

Guo-Hao Xie, Qi-Xing Chen, Bao-Li Cheng, and Xiang-Ming Fang

Department of Anesthesiology, the First Affiliated Hospital, Zhejiang University School of Medicine, Qingchun Road 79, Hangzhou 31003, China

Correspondence should be addressed to Bao-Li Cheng; tianwai1979@163.com and Xiang-Ming Fang; xiangming_fang@163.com

Received 1 March 2014; Revised 8 May 2014; Accepted 16 June 2014; Published 19 August 2014

Academic Editor: Qiang Shu

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Sepsis is a leading cause of mortality and morbidity in the critical illness. Multiple immune inflammatory processes take part in the pathogenesis of sepsis. Defensins are endogenous antimicrobial peptides with three disulphide bonds created by six cysteine residues. Besides the intrinsic microbicidal properties, defensins are active players which modulate both innate and adaptive immunity against various infections. Defensins can recruit neutrophils, enhance phagocytosis, chemoattract T cells and dendritic cells, promote complement activation, and induce IL-1β production and pyrotosis. Previous publications have documented that defensins play important roles in a series of immune inflammatory diseases including sepsis. This review aims to briefly summarize in vitro, in vivo, and genetic studies on defensins' effects as well as corresponding mechanisms within sepsis and highlights their promising findings which may be potential targets in future therapies of sepsis.

1. Introduction

Sepsis, severe sepsis, and septic shock represent a continuum of clinical syndromes which are common complications observed in patients with infection, trauma, and major surgeries [1–3]. These syndromes start with infection induced systemic inflammatory response syndrome (SIRS) and evolve to sepsis induced acute organ dysfunction and cardiovascular collapse. Epidemiology studies demonstrated that severe sepsis has a population prevalence of 300/100 000 in the United States and counts for 10–30% of the intensive care unit (ICU) patients [4–6]. And severe sepsis has already been acknowledged as the first cause of death in noncoronary ICUs with a high mortality rate of approximately 25–50% [7]. In the past one or two decades, steady progresses in treatment of sepsis have been made due to the advanced supportive care in ICU and the implementation of bundle therapies [7]. However, searching for specific remedies and reliable predictors within the pathophysiological mechanisms of sepsis is still the emphasis of today's studies [8, 9].

Defensins are classified as a subfamily of cationic antimicrobial peptides, which are major components of the human innate immunity. They are small endogenous peptides with three disulphide bonds created by six cysteine residues. Defensins are categorized into three subtypes, α-, β-, and θ-defensin, based on the spatial structure and the locations of three disulphide bonds within the peptide. In the past decade, cumulative evidences have suggested that defensins play an important role and may be a potential intervention target in sepsis. This review hereby will summarize in vitro, in vivo, and genetic studies on defensins' effects as well as corresponding mechanisms within sepsis and its sequential syndromes.

2. Antimicrobial Activities against Invading Pathogens in Sepsis

Defensins have broad spectrum antimicrobial activities against most pathogens in sepsis. The α-defensins are constitutively expressed in human neutrophils (human neutrophil peptides [HNP] 1–4) or intestinal Paneth cells (human defensin [HD] 5-6) [10–12]. They can inhibit a large variety of Gram-positive bacteria, Gram-negative bacteria, and some species of fungi and viruses [11].

The β-defensins are mainly distributed in the epithelial cells of the respiratory system, digestive system, and genitourinary system [10–12]. They can effectively kill a number of Gram-negative bacteria, such as *E. coli* and *P. aeruginosa*, Gram-positive bacteria, such as *S. aureus* and

Streptococcus pyogenes, and *Candida albicans*. β-Defensin-3 even has bactericidal effect towards multiresistant *S. aureus* and vancomycin-resistant *Enterococcus* [11, 13].

The θ-defensins, which have a unique macrocyclic structure, are isolated from leukocytes from some species of monkey and have not been detected in humans [14]. They are also reported to have antimicrobial activity against a spectrum of pathogens including *E. coli*, *S. aureus,* and *C. albicans* [15]. Also, they are found to have protective effect in a mouse model from a lethal pulmonary infection by a mouse adapted strain of SARS-coronavirus [16].

The classic mechanism of defensins' bactericidal effect is the "pore formation" theory. These positively charged antimicrobial peptides target negatively charged bacterial membrane components, such as lipopolysaccharides, teichoic acids, or phospholipids. Then they form transmembrane pores, disrupt cell integrity, and lead to bacteria lysis [10, 11]. Recently, another mechanism has been reported that defensins kill bacteria by inhibiting the synthesis of bacterial cell wall through interaction with certain precursors such as lipid II [17].

Defensins' bactericidal effect can be limited by high salt concentration of local environment where they encounter with the pathogens [18, 19]. Also, the antimicrobial action appears to be regulated by the redox response, as β-defensin-1 become more potent after reduction of disulfide bridges by thioredoxin or a reducing environment [20, 21].

3. Modulators and Alarmins in Immune Inflammatory Response of Sepsis

Defensins are also reported to have modulating effects on both innate and adaptive immune response. It is well known that HNP1-3 participate in the host immune defense via multiple mechanisms, including enhancing macrophage phagocytosis, facilitating neutrophil recruitment, modulating complement activation, and chemoattracting immature T cells and dendritic cells [12, 22].

In vitro studies showed that β-defensins have potent chemotactic effects, leading to the recruitment and maturation of naive dendritic cells and memory T cells in the inflammatory sites and the triggering of specific immune response in the host [23]. As the endogenous ligand of TLR-4, β-defensins interact with TLR-4 of the immune cells and regulate the expression of inflammatory mediators via the NF-κB pathway [18]. In vivo researches have revealed that the abnormal expression of β-defensins is associated with sepsis and various infectious diseases, as levels of β-defensins in both plasma and bronchoalveolar lavage fluid in patients with pulmonary infections are elevated [24–26], transcription of β-defensin-2 in leukocytes of severe septic patients is suppressed [27], expression of β-defensins in burn wound is reduced [28], and impaired expression of β-defensins is associated with inflammatory bowel diseases [29, 30]. In a mouse model of acute lung injury, Shu et al. expressed recombinant β-defensin-2 in lung tissue via recombinant adenovirus to study its protective effect against *P. aeruginosa* infection. Compared with control mice, they found considerably less *P. aeruginosa* in the transinfected lung tissue, as well as alleviated alveolar impairment, interstitial edema, and neutrophil infiltration [31, 32]. In subsequent studies, mice transinfected by adenovirus with or without β-defensin-2 genes received cecal ligation and puncture (CLP) twice to generate sepsis models. The impact of β-defensin-2 on the inflammatory response (e.g., the level of ICAM-1 expression), the severity of lung injury, and the sepsis outcome (7-day survival rate) were observed and evaluated. It was found that recombinant β-defensin-2 could down-regulate the expression of ICAM-1 in lung tissue 24 h, 36 h, and 72 h after CLP and significantly raised the 7-day survival rate in sepsis mice [31, 33]. In the clinical setting, Olbrich et al. found preterm neonates had lower level of β-defensin-2 in cord blood when compared to term neonates [34]. And among these preterm neonates, lower β-defensin-2 level was associated with late-onset sepsis. These studies indicate that β-defensin-2 may play an important role in the immune inflammatory response in sepsis and might influence the outcome of sepsis.

Among the θ-defensins, rhesus macaque θ-defensin (RTD), which has six subtypes, has been extensively studied. Though not expressed in humans, RTDs were reported to significantly reduce levels of TNF-α, IL-1β, IL-6, IL-8, MIP1, and so on, in human peripheral blood leukocytes that are preincubated with various toll-like receptor agonists [35]. Furthermore, in vivo study showed that subcutaneously administration of 5 mg/kg RTD-1 could improve the survival rate and suppress the levels of a number of inflammatory cytokines and chemokines in two sepsis mouse models (received either intraperitoneal injection of *E. coli* or CLP). Although detailed mechanisms of the protective effect of RTD-1 have not been illuminated, the authors suggested that the interaction between RTD-1 and leukocyte is the critical determinant of TNF-α blockade [35]. The latter is a major proinflammatory cytokine and influences the consequent inflammatory cascades. These results indicate that θ-defensins may be a potential immune adjuvant in the treatment of sepsis, though they are not expressed in human.

In sepsis and other inflammatory disorders, defensins are among a group of rapidly-released host endogenous molecules, which are capable of both recruiting and activating APCs and are also termed the alarmins. Recently, in vitro studies have shown that alarmin HNP1-3 have the ability to boost host inflammatory response by promoting macrophage IL-1β production and pyroptosis via purinergic P2X7 receptor [36]. However, this effect is a double-edged sword in sepsis since it can promote pathogen elimination as well as mediate organ dysfunction such as acute lung injury [22, 37].

4. Genetic Polymorphisms and Sepsis Susceptibility

In molecular genetics and molecular biology, knock-out animal model is one of the most convincing means to determine the role of a specific molecule in the physiopathology of a certain disease. However, as members of the defensin

family have overlapped biological functions, the function of the knock-out gene in animal models may probably be compensated by other defensins. Since the gene cluster coding for the entire defensin family cannot be fully knocked out using the present techniques of molecular biology and genetics as well as human defensins lack of absolute animal analogues, genetic association analysis is a good alternative that can effectively explore the relationship between genetic polymorphism and sepsis.

In normal peripheral blood cells, mRNA levels of both β-defensin-1 and β-defensin-2 raise remarkably when stimulated by LPS or *P. aeruginosa* [23]. However, the upregulation of β-defensin-1 and β-defensin-2 varies among individuals, resulting in interindividual differences in host defense capacity and hence influencing the clinical progression of sepsis. Previous studies showed that single nucleotide polymorphism (SNP) of β-defensin-1 gene (DEFB1) correlates with chronic obstructive pulmonary disease, asthma, genetic allergy, HIV infection, and pseudomonas species infection in oral mucosa [38–42]. Since sepsis is a multifactorial disease caused by both environmental factors (pathogenic microbes) and host factors (comorbidities and genetic background), its occurrence and outcome are influenced with individual genetic background [43]. Chen et al. selected 5 SNPs in the promote region of DEFB-1 (–1816A/G, –390A/T, –52A/G, –44C/G, and –20A/G) and one in its extron (1654G/A) as candidate loci and studied 211 patients with severe sepsis and 157 healthy controls [44]. Distribution of alleles, gene types, and haplotypes associating with these loci were studied and compared between septic patients and controls, as well as between survivals and victims of severe sepsis. Association analysis, logistic regression, and linkage disequilibrium study showed that –44G allele was closely related with susceptibility to severe sepsis and poor outcome. And severe septic patients with haplotype –20G/–44G/–52G had even poorer outcome, while individuals with haplotype –20A/–44C/–52G were less susceptible to severe sepsis. The reason why –44C/G is correlated with the occurrence and outcome of severe sepsis may attribute to the following points. It located in the 5′ untranslated region of DEFB1 and its polymorphism may result in changes in the space conformation of mRNA, which would alter the stability of mRNA and the efficiency of translation. And its impact on the protein function is more significant than nonsynonymous SNP in coding region [45], as the quantity of protein would change dramatically. However, as any other genetic association analysis, DEFB1 −44C/G may be only a surface marker of some unknown real genetic marker of sepsis in linkage disequilibrium. Although these hypothesis need to be proved by further researches, the above-mentioned study indicated that β-defensin-1 might be an influential factor in the process of immune defense and inflammation regulation in sepsis, and the locus of −44C/G may be an important genetic warning indicator of susceptibility to severe sepsis and its outcomes.

Copy number variation (CNV) is a kind of genetic polymorphism that accounts for approximately 12% of human genomic DNA. It refers to a large-scale duplication or deletion of certain DNA sections, which causes a variation in the number of copies of one or more genes. Previous publications reported that CNV is present in β-defensin-2 gene (DEFB4), β-defensin-3 gene (DEFB103), β-defensin-4 gene (DEFB104), α-defensin-1 gene (DEFA1), and α-defensin-3 gene (DEFA3) [18, 46–48]. And copy number of DEFB4 has a positive correlation with its mRNA level [35, 45]. Recently, Chen et al. screened 179 severe sepsis and 233 healthy controls for DEFA1 and DEFA3 [49]. An average DEFA1/DEFA3 copy number of 7 per genome was observed in the studied population, with a range of 2 to 15. The authors found that patients with high copy number of DEFA1/DEFA3 were predisposed to severe sepsis and tended to have lower level of plasma HNP1-3 as well as cytokines such as TNF-α, IL-6, and IL-10. They further validated their findings in an independent cohort. These results indicated that CNVs in the defensin gene may be potential genetic markers for identifying high risk patients or providing individual treatment in sepsis.

5. Perspective

Defensins are emerging therapeutic molecules against pathogens in sepsis because of their broad spectrum antimicrobial properties. In the past decade or two, a number of potent and salt insensitive defensins and their analogs have been screened, structurally modified, and synthesized. However, most of these studies are performed in vitro and not much is known about the in vivo roles of these molecules. In fact, chemoattracting and immunomodulating effects make defensins a double-edged sword in the pathogenesis of sepsis, which leads to facilitation of pathogen clearance as well as exacerbation of inflammation and injury of self-tissues. Recently, several investigations showed that the chemoattractant and antimicrobial activities of defensins could be separated, which shed light on the design of defensin-derived pharmaceuticals [50]. In addition, genetic studies help identify high risk patients with susceptibility to sepsis or its adverse outcome, which provides foundation for future individualized sepsis treatments that are targeting defensins.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 81201495) and the research project of the Department of Education of Zhejiang Province (no. Y200909678).

References

[1] R. S. Hotchkiss and I. E. Karl, "The pathophysiology and treatment of sepsis," *The New England Journal of Medicine*, vol. 348, no. 2, pp. 138–150, 2003.

[2] J. A. Russell, "Management of sepsis," *The New England Journal of Medicine*, vol. 355, no. 16, pp. 1699–1713, 2006.

[3] J. Cohen, "The immunopathogenesis of sepsis," *Nature*, vol. 420, no. 6917, pp. 885–891, 2002.

[4] G. S. Martin, D. M. Mannino, S. Eaton, and M. Moss, "The epidemiology of sepsis in the United States from 1979 through 2000," *New England Journal of Medicine*, vol. 348, no. 16, pp. 1546–1554, 2003.

[5] B. Cheng, G. Xie, S. Yao et al., "Epidemiology of severe sepsis in critically ill surgical patients in ten university hospitals in China," *Critical Care Medicine*, vol. 35, no. 11, pp. 2538–2546, 2007.

[6] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, "Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care," *Critical Care Medicine*, vol. 29, no. 7, pp. 1303–1310, 2001.

[7] F. B. Mayr, S. Yende, and D. C. Angus, "Epidemiology of severe sepsis," *Virulence*, vol. 5, no. 1, pp. 4–11, 2014.

[8] R. P. Wenzel, "Treating sepsis," *The New England Journal of Medicine*, vol. 347, no. 13, pp. 966–967, 2002.

[9] R. S. Hotchkiss, G. Monneret, and D. Payen, "Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach," *The Lancet Infectious Diseases*, vol. 13, no. 3, pp. 260–268, 2013.

[10] Y. Yamaguchi and Y. Ouchi, "Antimicrobial peptide defensin identification of novel isoforms and the characterization of their physiological roles and their significance in the pathogenesis of diseases," *Proceedings of the Japan Academy B, Physical and Biological Sciences*, vol. 88, no. 4, pp. 152–166, 2012.

[11] G. Wang, "Human antimicrobial peptides and proteins," *Pharmaceuticals*, vol. 7, no. 5, pp. 545–594, 2014.

[12] M. E. Selsted and A. J. Ouellette, "Mammalian defensins in the antimicrobial immune response," *Nature Immunology*, vol. 6, no. 6, pp. 551–557, 2005.

[13] J. J. Schneider, A. Unholzer, M. Schaller et al., "Human defensins," *Journal of Molecular Medicine*, vol. 83, no. 8, pp. 587–595, 2005.

[14] Y. Tang, J. Yuan, G. Ösapay et al., "A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated α-defensins," *Science*, vol. 286, no. 5439, pp. 498–502, 1999.

[15] P. Tongaonkar, P. Tran, K. Roberts et al., "Rhesus macaque θ-defensin isoforms: expression, antimicrobial activities, and demonstration of a prominent role in neutrophil granule microbicidal activities," *Journal of Leukocyte Biology*, vol. 89, no. 2, pp. 283–290, 2011.

[16] C. L. Wohlford-Lenane, D. K. Meyerholz, S. Perlman et al., "Rhesus theta-defensin prevents death in a mouse model of severe acute respiratory syndrome coronavirus pulmonary disease," *Journal of Virology*, vol. 83, no. 21, pp. 11385–11390, 2009.

[17] K. M. Varney, A. M. Bonvin, and M. Pazgier, "Turning defensin mimetics novel antibiotics targeting lipid II," *PLOS Pathogens*, vol. 9, no. 11, Article ID e1003732, 2013.

[18] T. Ganz, "Defensins: antimicrobial peptides of innate immunity," *Nature Reviews Immunology*, vol. 3, no. 9, pp. 710–720, 2003.

[19] J. Harder, J. Bartels, E. Christophers, and J. Schröder, "Isolation and characterization of human β-defensin-3, a novel human inducible peptide antibiotic," *Journal of Biological Chemistry*, vol. 276, no. 8, pp. 5707–5713, 2001.

[20] B. O. Schroeder, Z. Wu, S. Nuding et al., "Reduction of disulphide bonds unmasks potent antimicrobial activity of human β 2-defensin 1," *Nature*, vol. 469, no. 7330, pp. 419–423, 2011.

[21] S. U. Jaeger, B. O. Schroeder, U. Meyer-Hoffert et al., "Cell-mediated reduction of human β-defensin 1: major role for mucosal thioredoxin," *Mucosal Immunology*, vol. 6, no. 6, pp. 1179–1190, 2013.

[22] Y. Lai and R. L. Gallo, "AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense," *Trends in Immunology*, vol. 30, no. 3, pp. 131–141, 2009.

[23] D. Yang, A. Biragyn, D. M. Hoover, J. Lubkowski, and J. J. Oppenheim, "Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense," *Annual Review of Immunology*, vol. 22, pp. 181–215, 2004.

[24] S. Schaller-Bals, A. Schulze, and R. Bals, "Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection," *The American Journal of Respiratory and Critical Care Medicine*, vol. 165, no. 7, pp. 992–995, 2002.

[25] H. Mukae, H. Ishimoto, S. Yanagi et al., "Elevated BALF concentrations of α- and β-defensins in patients with pulmonary alveolar proteinosis," *Respiratory Medicine*, vol. 101, no. 4, pp. 715–721, 2007.

[26] T. D. Starner, B. Agerberth, G. H. Gudmundsson, and P. B. McCray Jr., "Expression and activity of β-defensins and LL-37 in the developing human lung," *Journal of Immunology*, vol. 174, no. 3, pp. 1608–1615, 2005.

[27] M. Book, Q. Chen, L. E. Lehmann et al., "Inducibility of the endogenous antibiotic peptide β-defensin 2 is impaired in patients with severe sepsis," *Critical Care*, vol. 11, article R19, 2007.

[28] S. M. Milner and M. R. Ortega, "Reduced antimicrobial peptide expression in human burn wounds," *Burns*, vol. 25, no. 5, pp. 411–413, 1999.

[29] K. Fellermann, D. E. Stange, E. Schaeffeler et al., "A chromosome 8 gene-cluster polymorphism with low human geta-defensin 2 gene copy number predisposes to Crohn disease of the colon," *The American Journal of Human Genetics*, vol. 79, no. 3, pp. 439–448, 2006.

[30] A. Fahlgren, S. Hammarström, Å. Danielsson, and M.-L. Hammarström, "β-defensin-3 and -4 in intestinal epithelial cells display increased mRNA expression in ulcerative colitis," *Clinical and Experimental Immunology*, vol. 137, no. 2, pp. 379–385, 2004.

[31] Q. Shu, Z. Shi, Z. Zhao et al., "Protection against *Pseudomonas aeruginosa* pneumonia and sepsis-induced lung injury by overexpression of β-defensin-2 in rats," *Shock*, vol. 26, no. 4, pp. 365–371, 2006.

[32] H. H. Wang, Q. Shu, and Z. Shi, "The protective effect of beta-defensin 2 in acute lung injury induced by respiratory *Pseudomonas aeruginosa* infection in rats," *Chinese Journal of Anesthesiology*, vol. 25, pp. 762–764, 2005.

[33] J. M. Bao, H. P. Yao, Z. Chen et al., "The effect of pretreatment of recombinant beta-defensin 2 on the ICAM-1 expression in lung of rats with acute lung injury," *Chinese Journal of Anesthesiology*, vol. 25, pp. 702–703, 2005.

[34] P. Olbrich, A. Pavón, M. L. Rosso et al., "Association of human beta-defensin-2 serum levels and sepsis in preterm neonates," *Pediatric Critical Care Medicine*, vol. 14, no. 8, pp. 796–800, 2013.

[35] J. B. Schaal, D. Tran, P. Tran et al., "Rhesus macaque theta defensins suppress inflammatory cytokines and enhance survival in mouse models of bacteremic sepsis," *PLoS ONE*, vol. 7, no. 12, Article ID e51337, 2012.

[36] Q. Chen, Y. Jin, K. Zhang et al., "Alarmin HNP-1 promotes pyroptosis and IL-1β release through different roles of NLRP3

inflammasome via P2X7 in LPS-primed macrophages," *Innate Immunity*, vol. 20, no. 3, pp. 290–300, 2014.

[37] K. Bdeir, A. A. Higazi, I. Kulikovskaya et al., "Neutrophil alpha-defensins cause lung injury by disrupting the capillary-epithelial barrier," *American Journal of Respiratory and Critical Care Medicine*, vol. 181, no. 9, pp. 935–946, 2010.

[38] I. Matsushita, K. Hasegawa, K. Nakata, K. Yasuda, K. Tokunaga, and N. Keicho, "Genetic variants of human β-defensin-1 and chronic obstructive pulmonary disease," *Biochemical and Biophysical Research Communications*, vol. 291, no. 1, pp. 17–22, 2002.

[39] H. Levy, B. A. Raby, S. Lake et al., "Association of defensin beta-1 gene polymorphism with asthma," *Journal of Allergy and Clinical Immunology*, vol. 115, no. 2, pp. 252–258, 2005.

[40] L. Braida, M. Boniotto, A. Pontillo, P. A. Tovo, A. Amoroso, and S. Crovella, "A single-nucleotide polymorphism in the human beta-defensin 1 gene as associated with HIV-1 infection in Italian children," *AIDS*, vol. 18, no. 11, pp. 1598–1600, 2004.

[41] R. J. Jurevic, M. Bai, R. B. Chadwick, T. C. White, and B. A. Dale, "Single-nucleotide polymorphisms (SNPs) in human β-defensin 1: high-throughput SNP assays and association with Candida carriage in type I diabetics and nondiabetic controls," *Journal of Clinical Microbiology*, vol. 41, no. 1, pp. 90–96, 2003.

[42] A. M. Wallace, J. He, K. M. Burkett et al., "Contribution of alpha- and beta-defensins to lung function decline and infection in smokers: an association study," *Respiratory Research*, vol. 7, article 76, 2006.

[43] M. T. Lin and T. E. Albertson, "Genomic polymorphisms in sepsis," *Critical Care Medicine*, vol. 32, no. 2, pp. 569–579, 2004.

[44] Q. X. Chen, C. Lv, L. X. Huang et al., "Genomic variations within DEFB1 are associated with the susceptibility to and the fatal outcome of severe sepsis in Chinese Han population," *Genes and Immunity*, vol. 8, no. 5, pp. 439–443, 2007.

[45] A. G. Nackley, S. A. Shabalina, I. E. Tchivileva et al., "Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure," *Science*, vol. 314, no. 5807, pp. 1930–1933, 2006.

[46] E. J. Hollox, J. A. L. Armour, and J. C. K. Barber, "Extensive normal copy number variation of a β-defensin antimicrobial-gene cluster," *The American Journal of Human Genetics*, vol. 73, no. 3, pp. 591–600, 2003.

[47] P. M. R. Aldred, E. J. Hollox, and J. A. L. Armour, "Copy number polymorphism and expression level variation of the human α-defensin genes DEFA1 and DEFA3," *Human Molecular Genetics*, vol. 14, no. 14, pp. 2045–2052, 2005.

[48] Q. Chen, M. Book, X. Fang, A. Hoeft, and F. Stuber, "Screening of copy number polymorphisms in human β-defensin genes using modified real-time quantitative PCR," *Journal of Immunological Methods*, vol. 308, no. 1-2, pp. 231–240, 2006.

[49] Q. Chen, M. Hakimi, S. Wu et al., "Increased genomic copy number of DEFA1/DEFA3 is associated with susceptibility to severe sepsis in Chinese han population," *Anesthesiology*, vol. 112, no. 6, pp. 1428–1434, 2010.

[50] K. Taylor, D. J. Clarke, B. McCullough et al., "Analysis and separation of residues important for the chemoattractant and antimicrobial activities of beta-defensin 3," *The Journal of Biological Chemistry*, vol. 283, no. 11, pp. 6631–6639, 2008.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 984082, 9 pages
http://dx.doi.org/10.1155/2014/984082

Review Article

Fluid Resuscitation in Sepsis: Reexamining the Paradigm

Poorna Madhusudan,[1,2] Bharath Kumar Tirupakuzhi Vijayaraghavan,[2,3] and Matthew Edward Cove[1,2]

[1] *Cardiothoracic Intensive Care Unit, NUHS, Singapore 119074*
[2] *National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074*
[3] *Intensive Care Unit, NUHS, Singapore 119074*

Correspondence should be addressed to Poorna Madhusudan; poorna.madhusudan@gmail.com

Received 25 February 2014; Revised 8 July 2014; Accepted 20 July 2014; Published 11 August 2014

Academic Editor: Baoli Cheng

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Sepsis results in widespread inflammatory responses altering homeostasis. Associated circulatory abnormalities (peripheral vasodilation, intravascular volume depletion, increased cellular metabolism, and myocardial depression) lead to an imbalance between oxygen delivery and demand, triggering end organ injury and failure. Fluid resuscitation is a key part of treatment, but there is little agreement on choice, amount, and end points for fluid resuscitation. Over the past few years, the safety of some fluid preparations has been questioned. Our paper highlights current concerns, reviews the science behind current practices, and aims to clarify some of the controversies surrounding fluid resuscitation in sepsis.

1. Introduction

The incidence of severe sepsis varies between 20 and 30% in most intensive care units and is a leading cause of mortality [1]. Fluid resuscitation is one of the cornerstones of management. Though there is a consensus on the need for adequate fluid therapy, the timing, type, and quantity of fluid resuscitation remain controversial. Furthermore, the optimal monitoring technique to guide fluid therapy continues to be debated; with mounting and sometimes contradicting evidence, the ideal fluid strategy is increasingly elusive.

Contemporary understanding of the pathophysiology of sepsis supports intensive fluid resuscitation in the initial phase. SIRS and sepsis incite widespread inflammatory responses at tissue and cellular levels altering homeostasis. Resultant circulatory abnormalities (peripheral vasodilation, intravascular volume depletion, increased cellular metabolism, and myocardial depression) lead to an imbalance between oxygen delivery and demand, worsening end organ injury and failure.

In a landmark paper, Rivers and colleagues demonstrated early goal directed therapy, targeting a specific central venous pressure (CVP) and mixed central venous oxygen saturation ($ScVO_2$), improved mortality by 16% [2]. In response, the surviving sepsis guidelines recommend early aggressive fluid resuscitation during the "golden" hours [3]. Although adequate fluid resuscitation makes eminent physiological sense, the optimal amount, and type of fluid remain unclear. Our paper aims to clarify these issues by reviewing the latest evidence guiding these practices.

2. Monitoring Fluid Resuscitation

2.1. Static Monitors. In sepsis, it is important to identify which patients will respond to volume resuscitation. In the critically ill, this means identifying the patient whose cardiac output will improve with fluid administration, called preload responsiveness. Traditionally, static indicators such as CVP have guided therapy. However, historic and recent evidence suggest CVP is a poor predictor of fluid responsiveness. In a systematic review on the usefulness of the CVP, Marik et al. concluded that it is neither a good indicator of volume status nor a predictor of responsiveness to fluid therapy [4]. It has been suggested that CVP no longer be used to guide fluid therapy [5]; although it remains in the surviving sepsis guidelines, some authors suggest that these recommendations should be revisited [5]. In fact, recent

evidence suggests CVP guided fluid resuscitation leads to venous congestion increasing the incidence of pulmonary complications in septic shock [6]. However, removal of CVP parameters from the guidelines may result in inadequate volume resuscitation and many centres continue to use static CVP measurement, despite evidence that it is an unhelpful guide for fluid administration. Furthermore, respiratory variation in the CVP is useful for predicting fluid responsiveness in spontaneously breathing patients [7].

Similarly, the pulmonary artery catheter (PAC) is unable to predict fluid responsiveness. Perhaps this is partly why the PAC is not associated with improved outcomes and its use has declined over the past two decades [8]. Although hemodynamic variables available from the PAC, such as the pulmonary capillary wedge pressure (PCWP), cardiac output (CO), and derived variables, are helpful for determining the type of circulatory shock and assessing response to therapy, none of these parameters predict preload responsiveness [9]. Furthermore, recent evidence casts doubt on the accuracy of hemodynamic data obtained from PACs [10].

2.2. Dynamic Monitoring. The most useful indicators of preload responsiveness are phasic changes in stroke volume and systolic blood pressure during positive pressure mechanical ventilation [11]. Stroke volume variation (SVV) is the ratio of maximal stroke volume difference during several respiratory cycles and the mean stroke volume over the same period [12]. Since the arterial pulse pressure depends on the amount of blood ejected during each systole (stroke volume), the pulse pressure variation covaries with SVV [13]. During positive pressure ventilation, inspiration increases the intrathoracic pressure reducing the right ventricular (RV) filling and right ventricular output if the RV is volume responsive. This causes the left ventricular filling and left ventricular (LV) output to decrease over successive beats if the LV is also volume responsive [12]. A SVV of >15% in patients receiving a tidal volume of >8 mL/kg or an SVV of >10% in patients receiving a tidal volume of 6 mL/kg accurately predicts preload responsiveness in patients with a closed chest [14–16].

Commercially available monitors such as the PiCCO, LiDCOplus, Volume View/EV1000, and the FloTrac use pulse contour analysis to indirectly determine the cardiac output and stroke volume variation. Pulse contour analysis is based on the relationship of the stroke volume, aortic compliance, and systemic vascular resistance [17]. Complex algorithms that account for reflection waves and aortic impedance are used to analyse the arterial wave and derive the stroke volume. The LiDCO uses pulse power analysis to convert the arterial waveform into a volume-time waveform which makes it less dependent on the shape of the pulse wave [18]. Although these devices are dependent on accurate calibration to measure CO, SVV and PPV are not dependent on calibration and, therefore, less affected by reliability concerns associated with these devices.

2.3. Indicators of Tissue Perfusion. The ultimate goal of fluid resuscitation is adequate tissue perfusion. However, dynamic monitoring does not measure tissue perfusion. Indicators of adequate perfusion include SVO_2, $ScVO_2$, and lactate. The surviving sepsis group recommends targeting $ScVO_2$ of 70% within the first 6 hours of recognition of sepsis [19]. However, $ScVO_2$ may be normal or even elevated in sepsis, for example, in patients with chronic liver disease. In contrast, hyperlactatemia is a more consistent finding in severe sepsis [20]. Normalization of lactate can be a useful target, alongside other hemodynamic parameters. Jansen et al. demonstrated reduced hospital mortality when targeting normalisation of lactate in a multicentre RCT [21].

A potentially useful measure of tissue perfusion is gastric mucosal pH. Since splanchnic circulation is compromised early during hypoperfusion, gastric blood flow is reduced. Changes in gastric mucosal pH (pHi), measured using a tonometer, reflect the adequacy of splanchnic perfusion [22]. The pHi is determined using a fluid or air filled balloon tipped nasogastric tube. The balloon contents equilibrate with the gases in the gastric lumen; therefore, changes in carbon dioxide (CO_2) in the balloon reflect gastric luminal CO_2. The pHi is calculated from the gastric lumen CO_2 and blood bicarbonate; lower values indicate greater hypoperfusion. Although it is useful in prognosticating multiorgan failure and death in several conditions such as acute pancreatitis [23], trauma [24], and other critically ill patients [25], technical difficulties and potential sources of error in manual tonometer monitoring have prevented its widespread use [22]. Other tissue perfusion monitors such as Sidestream Dark Field imaging technique (SDF) [26], sublingual capnometry [27–29], and near infrared spectroscopy (NIRS) [30–33] have also been studied in critically ill patients. Although some studies have shown benefit, these monitors are not widely available and their clinical utility for delivery of bedside critical care remains to be established [33, 34].

3. Which Fluid?

Intravenous fluid therapy originated during the great cholera outbreak of the nineteenth century [35–38]. Fluids of various compositions were used, and studies tracing their composition indicate they resembled balanced crystalloids [39]. Balanced solutions are those with an electrolyte composition similar to that of plasma. However, the most commonly used crystalloid is 0.9% saline, which is not balanced. About 10 million litres of saline are used each year in the UK and 200 million litres are sold every year in the United States [40].

3.1. Crystalloids (Saline and Balanced Solutions). 0.9% saline is frequently referred to as "normal" saline. However, Awad and colleagues elegantly showed that this term entered medical practice based on colloquialism rather than sound physiological or scientific data [39]. There is certainly nothing normal about "normal" saline. The first documented use of "normal saline" was in the Lancet in 1888 [41]; however the solution described bore no resemblance to 0.9% saline. The widespread adoption of 0.9% saline was likely based on its

isotonicity, as described in a single in vitro experiment on red cell lysis as well as the convenience and low cost of production [42].

Although there is no consensus on the superiority of balanced solutions over 0.9% saline, contemporary understanding of acid-base balance and recent observational evidence favours balanced solutions. The Stewart physicochemical approach [43] to acid-base dictates that infusion of large quantities of 0.9% saline will result in hyperchloremic acidosis. The strong ion difference (SID-sum of all the strong cations minus sum of all the strong anions) of plasma is maintained by the greater concentration of sodium relative to chloride in the plasma. Electroneutrality is maintained by anions such as bicarbonate (HCO_3^-), weak acids (HA), and hydroxyl ion (OH^-). Decreases in the SID decrease the available "space" for these anions, ultimately reducing $[OH^-]$. However, the dissociation of water (kw) must remain constant. Since kw is directly proportional to the product of $[OH^-]$ and hydrogen ion concentration $[H^+]$, decreases in $[OH^-]$ lead to increases in $[H^+]$ causing acidosis. Infusing 0.9% saline provides relatively more chloride, compared to sodium, resulting in a reduction in the strong ion difference which in turn lowers the pH, causing hyperchloremic acidosis [44].

Few studies have compared balanced crystalloids and 0.9% saline in patients with sepsis. However, there is substantial animal evidence that hyperchloremia causes harmful effects. In dogs, hyperchloremia causes progressive renal vasoconstriction and a fall in GFR in denervated kidneys [45]. In animal sepsis models, infusions of 0.9% saline increase inflammatory cytokines, worsen hypotension and hyperlactatemia, are more likely to cause renal failure, and ultimately increase mortality [46–48]. In patients without sepsis undergoing aortic aneurysm, repair use of 0.9% saline (NS), when compared to lactated ringer's (LR), was associated with increased platelet use (223 mL LR group versus 392 mL in NS group, median difference −169, 95% confidence interval (CI) −814 to −13) and increased interventions for acidosis (volume of bicarbonate used for acidosis; 40.2 ± 64.0 mL NS group versus 3.8 ± 15.5 mL LR group) [49]. In a randomized double-blind comparison of lactated Ringer's solution and 0.9% saline during renal transplantation, lactated Ringer's was associated with less hyperkalaemia and acidosis [50]. In a recent large observation study, Shaw et al. compared adult patients undergoing major open abdominal surgery receiving either 0.9% saline (30,994 patients) or a balanced crystalloid solution (PlasmaLyte) (926 patients). Unadjusted hospital mortality was higher in the saline group (5.6% versus 2.9%; $P < 0.001$). After propensity score matching, patients receiving 0.9% saline received more fluid (1976 mL [± 1560] versus 1658 mL [± 1288], $P < 0.001$), more buffer orders (6.3% [95% CI 5.5–7.3] versus 4.2% [95% CI 3.1–5.7], $P = 0.02$), and more transfusions (11.5% [95% CI 10.3–12.7] versus 1.8% [95% CI 1.2–2.9], $P < 0.001$) and the use of dialysis was nearly fivefold greater (1.0% [95% CI 0.05–1.8] versus 4.8% [95% CI 4.1–5.7], $P < 0.001$) when compared to PlasmaLyte [51].

In Sub-Saharan African children with severe sepsis, fluid boluses with saline or 5% Albumin in saline result in increased mortality when compared to no fluid boluses [52]. Although the predominance of malarial sepsis could lead to severe anaemia following fluid boluses, theoretically contributing to this observation, it is intriguing to postulate that, in the absence of modern ICU interventions, the negative effects of 0.9% saline may be magnified. Furthermore, post hoc analysis suggests cardiovascular collapse accounted for the excess mortality in the fluid bolus groups [53]. There is clearly mounting evidence suggesting that hyperchloremia associated with use of 0.9% saline does have significant clinical implications, cannot be ignored and should at least give pause to continued saline only resuscitation in sepsis. Table 1 summarizes the studies examining crystalloids.

3.2. Colloids. There are fundamental differences between crystalloids and colloids. Crystalloids are predominantly based on sterile water to which electrolytes have been added. Colloids have an additional "colloidal" component that does not freely diffuse across semipermeable membranes, in theory making them more effective volume expanders. Colloids are the preferred resuscitation fluids in Europe and Australasia [54]. Albumin, hydroxyethyl starch (HES), and gelatins are the three classes of colloid commonly used.

However, the safety profile of certain colloids in patients with sepsis has recently been challenged. In fact, safety concerns have existed since their introduction. Scheirhout and colleagues, in a meta-analysis of 37 RCTs in critically ill patients, found that resuscitation with colloids (albumins, gelatins, dextrans, and starches) increased risk of mortality by 4% (95% CI 0–8%) [55]. A separate French multicentre study found gelatins [odds ratio (OR) 4.81 (95% CI 2.01–11.51 $P = 0.0005$)] and dextrans [OR 3.83 (95% CI 1.17–12.60 $P = 0.02$)] were independent risk factors for anaphylactoid reactions [56]. Furthermore, Dextran 70 has been shown to decrease Factor VIII procoagulant activity, factor VIII related antigen, and ristocetin cofactor activity [57] resulting in coagulopathies.

Based on adverse outcome reports, such as renal dysfunction and coagulopathy, high molecular weight starches have already been phased out in favour of HES (130/0.42). The adverse effects of HES were considered benign, transitory, dose dependent, and related to only high molecular weight starches. However, HES (130/0.42) is not readily excreted and there is evidence it accumulates in the skin, liver, kidney, and reticuloendothelial system [58]. It is suggested that the lower degree of substitution and lower molecular weight of HES (130/0.42) facilitate greater uptake in the tubular epithelium leading to osmotic nephrosis and requirement of renal replacement therapy and, therefore, could be more harmful than its predecessors [58–60].

In the recent Crystalloid versus Hydroxyethyl starch Trial (CHEST), the effect of fluid resuscitation with HES (130/0.4) was compared with 0.9% saline among 7000 patients admitted to an intensive care unit [61]. The study found no difference in 90 day mortality between the groups; however, patients receiving HES required renal replacement therapy

Table 1: Summary of studies evaluating crystalloids.

Author	Year	Study design	Sample size	Study fluid	Primary endpoint	Comments
Wilcox [45]	1983	Animal experiment	48	Chloride rich solutions	Regulation of renal blood flow	Increased renal vasoconstriction and ↓GFR[a] with chloride rich solutions
Waters et al. [49]	2001	Prospective randomized study	66	0.9% Saline versus lactated Ringer	Multiple outcomes studied	Increased use of blood products and acidosis with 0.9% Saline
O'Malley et al. [50]	2005	Randomised clinical trial	51	0.9% Saline versus lactated Ringer	Creatinine concentration on POD3[b]	No difference; but Ringer's was associated with less hyperkalemia and acidosis
Shaw et al. [51]	2012	Observational	31,920	0.9% Saline versus balanced crystalloid	Major morbidity	Higher mortality, increased transfusion requirements, dialysis requirements, and increased buffer requirements in saline group
Maitland et al. [52]	2011	Multicentric randomized trial	3141	Albumin bolus and saline bolus	Mortality	Boluses resulted in increased mortality

Table summarizing studies evaluating crystalloids. [a]Glomerular Filtration Rate, [b]Postoperative Day 3.

more frequently (RR 1.21, 95% CI 1.00–1.45, $P = 0.04$). The study also demonstrated more adverse events with the use of HES.

A second trial recently randomized 804 patients with severe sepsis to receive either HES (130/0.42) or Ringer's acetate, the 6S trial [62]. The primary outcome of death or dialysis dependence at 90 days occurred in 51% of the HES group compared to 43% in the Ringer's group ($P = 0.03$) with more patients in the HES group receiving renal replacement therapy (22% versus 16%, $P = 0.04$). Since Ringer's acetate was the carrier of HES in the intervention arm, it enabled the investigators to examine only the effects of HES between the two groups. It is conceivable that CHEST study did not identify a mortality difference due to harmful effects exerted by 0.9% saline in the control arm. In addition, a recent meta-analysis by Zarychanski et al. concluded that HES was associated with significant increase in mortality and acute kidney injury in patients with sepsis [63].

However, some studies contradict these findings, suggesting benefit to HES use. The CRYSTMAS trial was a prospective multicentre, double blind randomized study comparing the hemodynamic efficacy and safety of HES (130/0.4) with 0.9% saline in severe sepsis. The authors found significantly less volume of HES was required to achieve hemodynamic stability (1379 ± 886 mL in HES versus 1709 ± 1164 mL in saline group, $P = 0.0185$) and found no difference in the rate of AKI or RRT [64]. Unfortunately, it lacked power to address renal safety, and based on current evidence, the FDA has issued a boxed warning for HES. It would appear the risks associated with HES use in sepsis outweigh any volume expansion benefits and its use in sepsis cannot currently be recommended. Given the concerns with synthetic colloids, albumin has reemerged as a good alternative. The recent surviving sepsis campaign guidelines advocate the use of albumin for volume expansion after the use of crystalloids [19].

Apart from the hemodynamic efficacy that albumin confers, it is reported to have antioxidant and anti-inflammatory activity [65]. The postulated mechanisms include an increase in plasma thiol levels, modulation of cytokine activity, binding of endotoxin, and protection of glycocalyx. It also alters drug binding and reduces nitric oxide, attenuating vasodilatation [66].

The SAFE study compared albumin and saline resuscitation in 6997 patients [67]. Twenty-eight-day mortality was no different between both groups (726 albumin group versus 729 0.9% saline group, $P = 0.87$). The study concluded that albumin and 0.9% saline are clinically equivalent for fluid resuscitation in the ICU. However, post hoc analysis of the sepsis subgroup indicates that resuscitation with albumin may reduce the mortality in patients with severe sepsis, confirming possible additional protective mechanisms conferred by albumin. Furthermore, a large meta-analysis showing resuscitation with albumin solutions in sepsis was associated with lower mortality [68]. Although many of the studies included had not used proper methodology, the results suggest that albumin does not have specific adverse effects in sepsis. Table 2 summarizes studies examining colloids.

A separate multicentre study in Italy, the ALBIOS trial, recruited 1800 patients with sepsis or septic shock and compared resuscitation with 20% albumin or a crystalloid the results of which are not yet published. Similarly, another large trial involving 800 patients with sepsis in France resuscitated either with 20% albumin or normal saline, conducted by the EARSS study group, is yet to publish its results. Together, the results of these two large trials will help confirm whether albumin has additional protective effects.

The recent CRISTAL randomized trial examined the effects of fluid resuscitation with colloids versus crystalloids on mortality in critically ill patients with hypovolemic shock [69]. It was a multicentre, open label randomized clinical trial stratified by case mix (sepsis, trauma, or hypovolemic

Table 2: Summary of studies evaluating colloids.

Author	Year	Study design	Sample size	Study fluid	Primary endpoint	Comments
Schierhout and Roberts [55]	1998	Meta-analysis	1315	All colloids	Mortality	Increased mortality
Laxenaire et al. [56]	1994	Multicentre prospective	19593	All colloids	Adverse effects	Gelatins and dextrans-independent risk for anaphylactoid reactions
Myburgh et al. [61]	2012	RCT[a]	7000	HES[b] versus 0.9% saline	90-day mortality	HES[b] associated with increased incidence of RRT
Perner et al. [62]	2012	RCT[a]	804	HES[b] versus Ringer's acetate	Death/dialysis dependence at 90 days	Death and dialysis dependence more in HES
Zarychanski et al. [63]	2013	Meta-analysis	10,290	HES[b]	Mortality and AKI[c]	Significant increase in risk of mortality and AKI[c]
Guidet et al. [64]	2012	RCT[a]	196	HES[b] versus 0.9% saline	Hemodynamic efficacy and safety	HES[b] better hemodynamic efficacy and no difference in AKI[c]
Finfer et al. [67]	2004	RCT[a]	6997	Albumin versus 0.9% saline	28-day mortality	No difference
Myburgh et al. [72]	2007	Post hoc analysis of SAFE trial	460	Albumin versus 0.9% saline	Safety in TBI[d]	Albumin unsafe for TBI[d]
Delaney et al. [68]	2011	Meta-analysis	1977	Albumin	Safety for resuscitation	Albumin associated with lower mortality
Annane et al. [69]	2013	RCT[a]	2857	Colloids versus crystalloids	28-day mortality	No difference

Table summarizing studies evaluating colloids. [a]Randomized Controlled Trial, [b]Hydroxyethyl starch, [c]Acute Kidney Injury, [d]Traumatic Brain Injury.

shock without sepsis or trauma). They used colloids (n = 1414; gelatins, dextrans, hydroxyethyl starches, 4% or 20% of albumin) or crystalloids (n = 1443; isotonic saline, hypertonic saline, or Ringers lactate) for fluid interventions, other than fluid maintenance throughout the ICU stay. There was no difference in 28-day mortality between the two groups (359 in colloid group versus 390 in crystalloid group, P = 0.26). However, a secondary outcome, 90-day mortality, was lower in patients receiving colloids, but it is difficult to draw strong conclusions from this study due to the heterogeneity of fluid composition in the two groups.

From the many trials that have been carried out so far, it is clear that some synthetic colloids should be avoided in sepsis and that 0.9% saline may have disadvantages over balanced crystalloids. However, whether to select albumin over a crystalloid remains uncertain. Based on the SAFE study, one potential advantage of albumin is that less fluid is ultimately required to achieve hemodynamic end goals. This will only prove beneficial, if a more positive balance is associated with worse outcomes.

4. How Much Fluid?

Boyd and colleagues retrospectively reviewed the association of positive fluid balance at 12 hours and at 4 days in 778 patients of the Vasopressin in Septic Shock (VASST) study [70]. They found that the quartile that had the least positive balance at 12 hours [0.569 (0.405–0.799) for Quartile 1 and 0.581 (0.414–0.816) for Quartile 2] and at 4 days [0.466 (0.299–0.724) for Quartile 1 and 0.512 (0.339–0.775) for Quartile 2] had a lower hazard ratio relative to the quartile with the maximum positive balance. Furthermore we know that a fluid restrictive strategy is beneficial in patients with concomitant ARDS [71]. Although it is expected that 3 to 4 times the volume of crystalloids may be required to achieve the hemodynamic efficacy of colloids, the SAFE study found that the volume of saline used was only 40% more than albumin, perhaps because the clearance of crystalloids is decreased during the stress response of critical illness. Furthermore, there are settings where one fluid is clearly advantageous, such as sepsis patients with traumatic brain injury where albumin and hypotonic resuscitation fluids should be avoided [72]. Similarly, patients requiring fluid restrictive strategy, such as those with ARDS or concomitant abdominal compartment syndrome, might benefit from an albumin based strategy (Figure 1).

5. Conclusion

In conclusion, a perfect one-size-fits-all fluid strategy does not exist. In sepsis, clinicians should understand the limitations and potential benefits of each strategy. Each fluid should be considered a drug, with specific pharmacokinetic, pharmacodynamic, and adverse effect profiles, which can be carefully matched to the patient. Whichever fluid is chosen, resuscitation should be titrated to evidence based

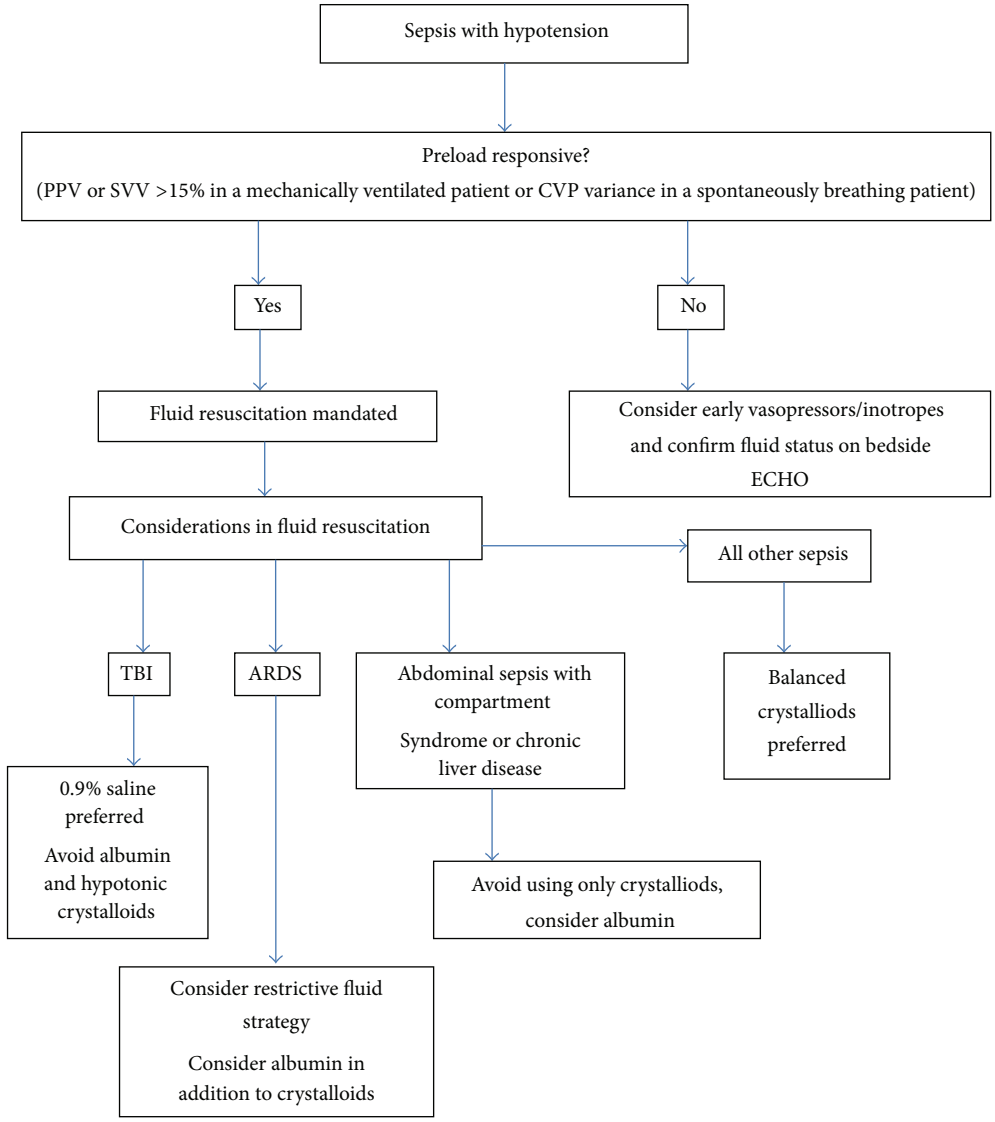

Figure 1: Algorithm to guide fluid therapy in the septic patient.

targets, combining clinical assessment, such as signs of tissue perfusion with dynamic hemodynamic monitoring. Balanced crystalloids may be preferred first choice, followed by albumin, based on their comparative safety profiles. 0.9% saline should only be used after consideration of its potential to cause harm and current evidence would suggest starches (HES) should be avoided in sepsis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

Work was in part funded by New Clinician Scientist Program, National University Health System Singapore (MEC).

References

[1] C. Alberti, C. Brun-Buisson, H. Burchardi et al., "Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study," *Intensive Care Medicine*, vol. 28, no. 2, pp. 108–121, 2002.

[2] E. Rivers, B. Nguyen, S. Havstad et al., "Early goal-directed therapy in the treatment of severe sepsis and septic shock," *The New England Journal of Medicine*, vol. 345, no. 19, pp. 1368–1377, 2001.

[3] R. P. Dellinger, M. M. Levy, J. M. Carlet et al., "Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock," *Critical Care Medicine*, vol. 36, pp. 296–327, 2008.

[4] P. E. Marik, M. Baram, and B. Vahid, "Does the central venous pressure predict fluid responsiveness? A systematic review of the literature and the tale of seven mares," *Chest*, vol. 134, no. 1, pp. 172–178, 2008.

[5] P. E. Marik, X. Monnet, and J. Teboul, "Hemodynamic parameters to guide fluid therapy," *Annals of Intensive Care*, vol. 1, no. 1, pp. 2–9, 2011.

[6] R. Rajendram and J. R. Prowle, "Venous congestion: are we adding insult to kidney injury in sepsis?" *Critical Care*, vol. 18, p. 104, 2014.

[7] S. Magder, G. Georgiadis, and T. Cheong, "Respiratory variations in right atrial pressure predict the response to fluid challenge," *Journal of Critical Care*, vol. 7, no. 2, pp. 76–85, 1992.

[8] S. Harvey, D. A. Harrison, M. Singer et al., "Assessment of the clinical effectiveness of pulmonary artery catheters in management of patients in intensive care (PAC-Man): a randomised controlled trial," *The Lancet*, vol. 366, no. 9484, pp. 472–477, 2005.

[9] A. Kumar, R. Anel, E. Bunnell et al., "Pulmonary artery occlusion pressure and central venous pressure fail to predict ventricular filling volume, cardiac performance, or the response to volume infusion in normal subjects," *Critical Care Medicine*, vol. 32, no. 3, pp. 691–699, 2004.

[10] P. E. Marik, "Obituary: pulmonary artery catheter 1970 to 2013," *Annals of Intensive Care*, vol. 3, article 38, 2013.

[11] P. E. Marik, R. Cavallazzi, T. Vasu, and A. Hirani, "Dynamic changes in arterial waveform derived variables and fluid responsiveness in mechanically ventilated patients: a systematic review of the literature," *Critical Care Medicine*, vol. 37, no. 9, pp. 2642–2647, 2009.

[12] M. E. Cove and M. R. Pinsky, "Perioperative hemodynamic monitoring," *Best Practice and Research: Clinical Anaesthesiology*, vol. 26, no. 4, pp. 453–462, 2012.

[13] J. Erlanger and D. R. Hooker, " An experimental study of blood pressure and of pulse pressure in man," *John Hopkins Hospital Records*, vol. 12, pp. 145–378, 1904.

[14] M. Kobayashi, M. Koh, T. Irinoda, E. Meguro, Y. Hayakawa, and A. Takagane, "Stroke volume variation as a predictor of intravascular volume depression and possible hypotension during the early postoperative period after esophagectomy," *Annals of Surgical Oncology*, vol. 16, no. 5, pp. 1371–1377, 2009.

[15] H. Berkenstadt, N. Margalit, M. Hadani et al., "Stroke volume variation as a predictor of fluid responsiveness in patients undergoing brain surgery," *Anesthesia and Analgesia*, vol. 92, no. 4, pp. 984–989, 2001.

[16] D. A. Reuter, T. W. Felbinger, C. Schmidt et al., "Stroke volume variations for assessment of cardiac responsiveness to volume loading in mechanically ventilated patients after cardiac surgery," *Intensive Care Medicine*, vol. 28, no. 4, pp. 392–398, 2002.

[17] L. J. Montenij, E. E. C. De Waal, and W. F. Buhre, "Arterial waveform analysis in anesthesia and critical care," *Current Opinion in Anaesthesiology*, vol. 24, no. 6, pp. 651–656, 2011.

[18] M. Jonas, D. Hett, and J. Morgan, "Real time, continuous monitoring of cardiac output and oxygen delivery," *International Journal of Intensive Care*, vol. 9, no. 1, pp. 33–42, 2002.

[19] R. P. Dellinger, M. M. Levy, A. Rhodes et al., "Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012," *Critical Care Medicine*, vol. 41, no. 2, pp. 580–637, 2013.

[20] J. Bakker, M. W. N. Nijsten, and T. C. Jansen, "Clinical use of lactate monitoring in critically ill patients," *Annals of Intensive Care*, vol. 3, article 12, 2013.

[21] T. C. Jansen, J. van Bommel, F. J. Schoonderbeek et al., "Early lactate-guided therapy in intensive care unit patients: a multicenter, open-label, randomized controlled trial," *American Journal of Respiratory and Critical Care Medicine*, vol. 182, no. 6, pp. 752–761, 2010.

[22] J. J. Kolkman, J. A. Otte, and A. B. J. Groeneveld, "Gastrointestinal luminal P_{CO_2} tonometry: an update on physiology, methodology and clinical applications," *British Journal of Anaesthesia*, vol. 84, no. 1, pp. 74–86, 2000.

[23] M. J. D. Bonham, F. M. Abu-Zidan, M. O. Simovic, and J. A. Windsor, "Gastric intramucosal pH predicts death in severe acute pancreatitis," *British Journal of Surgery*, vol. 84, no. 12, pp. 1670–1674, 1997.

[24] O. C. Kirton, J. Windsor, R. Wedderburn et al., "Failure of splanchnic resuscitation in the acutely injured trauma patient correlates with multiple organ system failure and length of stay in the ICU," *Chest*, vol. 113, no. 4, pp. 1064–1069, 1998.

[25] N. Maynard, D. Bihari, R. Beale et al., "Assessment of splanchnic oxygenation by gastric tonometry in patients with acute circulatory failure," *Journal of the American Medical Association*, vol. 270, no. 10, pp. 1203–1210, 1993.

[26] P. T. Goedhart, M. Khalilzada, R. Bezemer, J. Merza, and C. Ince, "Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation," *Optics Express*, vol. 15, no. 23, pp. 15101–15114, 2007.

[27] E. C. Rackow, P. O'Neil, M. E. Astiz, and C. M. Carpati, "Sublingual capnometry and indexes of tissue perfusion in patients with circulatory failure," *Chest*, vol. 120, no. 5, pp. 1633–1638, 2001.

[28] P. E. Marik, "Sublingual capnography: a clinical validation study," *Chest*, vol. 120, no. 3, pp. 923–927, 2001.

[29] P. E. Marik and A. Bankov, "Sublingual capnometry versus traditional markers of tissue oxygenation in critically ill patients," *Critical Care Medicine*, vol. 31, no. 3, pp. 818–822, 2003.

[30] H. Gómez, A. Torres, P. Polanco et al., "Use of non-invasive NIRS during a vascular occlusion test to assess dynamic tissue O_2 saturation response," *Intensive Care Medicine*, vol. 34, no. 9, pp. 1600–1607, 2008.

[31] D. E. Skarda, K. E. Mulier, D. E. Myers, J. H. Taylor, and G. J. Beilman, "Dynamic near-infrared spectroscopy measurements in patients with severe sepsis," *Shock*, vol. 27, no. 4, pp. 348–353, 2007.

[32] A. Lima, J. van Bommel, T. C. Jansen, C. Ince, and J. Bakker, "Low tissue oxygen saturation at the end of early goal-directed therapy is associated with worse outcome in critically ill patients," *Critical Care*, vol. 13, supplement 5, article S13, 2009.

[33] A. Lima, J. Van Bommel, K. Sikorska et al., "The relation of near-infrared spectroscopy with changes in peripheral circulation in critically ill patients," *Critical Care Medicine*, vol. 39, no. 7, pp. 1649–1654, 2011.

[34] R. Bezemer, S. A. Bartels, J. Bakker, and C. Ince, "Clinical review: clinical imaging of the sublingual microcirculation in the critically ill—where do we stand?" *Critical Care*, vol. 16, no. 3, article 224, 2012.

[35] N. Howard-jones, "Cholera therapy in the nineteenth century," *Journal of the History of Medicine and Allied Sciences*, vol. 27, no. 4, pp. 373–395, 1972.

[36] J. E. Cosnett, "The origins of intravenous fluid therapy," *The Lancet*, vol. 1, no. 8641, pp. 768–771, 1989.

[37] T. F. Baskett, "The resuscitation greats: William O'Shaughnessy, Thomas Latta and the origins of intravenous saline," *Resuscitation*, vol. 55, no. 3, pp. 231–234, 2002.

[38] B. A. Foëx, "How the cholera epidemic of 1831 resulted in a new technique for fluid resuscitation," *Emergency Medicine Journal*, vol. 20, no. 4, pp. 316–318, 2003.

[39] S. Awad, S. P. Allison, and D. N. Lobo, "The history of 0.9% saline," *Clinical Nutrition*, vol. 27, no. 2, pp. 179–188, 2008.

[40] Baxter Healthcare Deerfield IL, "Derived from IMS Health Hospital supply index, GHX Market Intelligence data and Baxter internal sales data".

[41] Dr. Churton, "A case of scirrhus of the pylorus, with excessive vomiting; repeated intravenous injections of saline solution; remarks," *The Lancet*, vol. 132, no. 3396, pp. 620–621, 1888.

[42] F. A. Coller, V. S. Dick, and W. G. Maddock, "Maintenance of normal water exchange with intravenous fluids," *Journal of the American Medical Association*, vol. 107, pp. 1522–1527, 1936.

[43] P. A. Stewart, "Modern quantitative acid-base chemistry," *Canadian Journal of Physiology and Pharmacology*, vol. 61, no. 12, pp. 1444–1461, 1983.

[44] N. M. Yunos, R. Bellomo, D. Story, and J. Kellum, "Bench-to-bedside review: chloride in critical illness," *Critical Care*, vol. 14, no. 4, article 226, 2010.

[45] C. S. Wilcox, "Regulation of renal blood flow by plasma chloride," *The Journal of Clinical Investigation*, vol. 71, no. 3, pp. 726–735, 1983.

[46] J. A. Kellum, M. Song, and R. Venkataraman, "Effects of hyperchloremic acidosis on arterial pressure and circulating inflammatory molecules in experimental sepsis," *Chest*, vol. 125, no. 1, pp. 243–248, 2004.

[47] J. A. Kellum, M. Song, and E. Almasri, "Hyperchloremic acidosis increases circulating inflammatory molecules in experimental sepsis," *Chest*, vol. 130, no. 4, pp. 962–967, 2006.

[48] F. Zhou, Z. Y. Peng, J. V. Bishop et al., "Effects of Fluid Resuscitation With 0.9% Saline Versus a Balanced Electrolyte Solution on Acute Kidney Injury in a Rat Model of Sepsis," *Critical Care Medicine*, vol. 42, 2014.

[49] J. H. Waters, A. Gottlieb, P. Schoenwald, M. J. Popovich, J. Sprung, and D. R. Nelson, "Normal saline versus lactated Ringer's solution for intraoperative fluid management in patients undergoing abdominal aortic aneurysm repair: an outcome study," *Anesthesia and Analgesia*, vol. 93, no. 4, pp. 817–822, 2001.

[50] C. M. N. O'Malley, R. J. Frumento, M. A. Hardy et al., "A randomized, double-blind comparison of lactated ringer's solution and 0.9% NaCl during renal transplantation," *Anesthesia and Analgesia*, vol. 100, no. 5, pp. 1518–1524, 2005.

[51] A. D. Shaw, S. M. Bagshaw, S. L. Goldstein et al., "Major complications, mortality, and resource utilization after open abdominal surgery: 0.9% saline compared to plasma-lyte," *Annals of Surgery*, vol. 255, no. 5, pp. 821–829, 2012.

[52] K. Maitland, S. Kiguli, R. O. Opoka et al., "Mortality after fluid bolus in African children with severe infection," *The New England Journal of Medicine*, vol. 364, no. 26, pp. 2483–2495, 2011.

[53] K. Maitland, E. C. George, J. A. Evans et al., "Exploring mechanisms of excess mortality with early fluid resuscitation: Insights from the FEAST trial," *BMC Medicine*, vol. 11, no. 1, article no. 68, 2013.

[54] S. Finfer, B. Liu, C. Taylor et al., "Resuscitation fluid use in critically ill adults: an international cross-sectional study in 391 intensive care units," *Critical Care*, vol. 14, article R185, 2010.

[55] G. Schierhout and I. Roberts, "Fluid resuscitation with colloid or crystalloid solutions in critically ill patients: a systematic review of randomised trials," *British Medical Journal*, vol. 316, no. 7136, pp. 961–964, 1998.

[56] M. C. Laxenaire, C. Charpentier, and L. Feldman, "Anaphylactoid reactions to colloid plasma substitutes: incidence, risk factors, mechanisms. A French multicenter prospective study," *Annales Francaises d'Anesthesie et de Reanimation*, vol. 13, no. 3, pp. 301–310, 1994.

[57] J. Batlle, F. Del Rio, and L. Fernandez, "Effect of dextran on factor VIII/von Willebrand factor structure and function," *Thrombosis and Haemostasis*, vol. 54, no. 3, pp. 697–699, 1985.

[58] R. Bellmann, C. Feistritzer, and C. J. Wiedermann, "Effect of molecular weight and substitution on tissue uptake of hydroxyethyl starch: a meta-analysis of clinical studies," *Clinical Pharmacokinetics*, vol. 51, no. 4, pp. 225–236, 2012.

[59] H. P. Ferber, E. Nitsch, and H. Förster, "Studies on hydroxyethyl starch. Part II: changes of the molecular weight distribution for hydroxyethyl starch types 450/0.7, 450/0.5, 450/0.3, 300/0.4, 200/0.7, 200/0.5, 200/0.3 and 200/0.1 after infusion in serum and urine of volunteers," *Arzneimittelforschung*, vol. 35, no. 3, pp. 615–622, 1985.

[60] M. L. Cittanova, I. Leblanc, C. Legendre, C. Mouquet, B. Riou, and P. Coriat, "Effect of hydroxyethylstarch in brain-dead kidney donors on renal function in kidney-transplant recipients," *The Lancet*, vol. 348, no. 9042, pp. 1620–1622, 1996.

[61] J. A. Myburgh, S. Finfer, R. Bellomo et al., "Hydroxyethyl starch or saline for fluid resuscitation in intensive care," *The New England Journal of Medicine*, vol. 367, no. 20, pp. 1901–1911, 2012.

[62] A. Perner, N. Haase, A. B. Guttormsen et al., "Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis," *The New England Journal of Medicine*, vol. 367, no. 2, pp. 124–134, 2012.

[63] R. Zarychanski, A. M. Abou-Setta, A. F. Turgeon et al., "Association of hydroxyethyl starch administration with mortality and acute kidney injury in critically ill patients requiring volume resuscitation," *Journal of the American Medical Association*, vol. 309, no. 7, pp. 678–688, 2013.

[64] B. Guidet, O. Martinet, T. Boulain et al., "Assessment of hemodynamic efficacy and safety of 6% hydroxyethylstarch 130/0.4 versus 0.9% NaCl fluid replacement in patients with severe sepsis: the CRYSTMAS study," *Critical Care*, vol. 16, article R94, 2012.

[65] M. Roche, P. Rondeau, N. R. Singh, E. Tarnus, and E. Bourdon, "The antioxidant properties of serum albumin," *FEBS Letters*, vol. 582, no. 13, pp. 1783–1787, 2008.

[66] J. S. Stamler, O. Jaraki, J. Osborne et al., "Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 16, pp. 7674–7677, 1992.

[67] S. Finfer, R. Bellomo, N. Boyce, J. French, J. Myburgh, and R. Norton, "A comparison of albumin and saline for fluid resuscitation in the intensive care unit," *The New England Journal of Medicine*, vol. 350, no. 22, pp. 2247–2256, 2004.

[68] A. P. Delaney, A. Dan, J. McCaffrey, and S. Finfer, "The role of albumin as a resuscitation fluid for patients with sepsis: a systematic review and meta-analysis," *Critical Care Medicine*, vol. 39, no. 2, pp. 386–391, 2011.

[69] D. Annane, S. Siami, S. Jaber et al., "Effects of fluid resuscitation with colloids versus crystalloids on mortality in critically ill patients presenting with hypovolemic shock: the CRISTAL randomized trial," *The Journal of the American Medical Association*, vol. 310, no. 17, pp. 1809–1817, 2013.

[70] J. H. Boyd, J. Forbes, T. Nakada, K. R. Walley, and J. A. Russell, "Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality," *Critical Care Medicine*, vol. 39, no. 2, pp. 259–265, 2011.

[71] A. L. Rosenberg, R. E. Dechert, P. K. Park, and R. H. Bartlett, "Review of a large clinical series: association of cumulative fluid balance on outcome in acute lung injury: a retrospective review of the ARDSnet tidal volume study cohort," *Journal of Intensive Care Medicine*, vol. 24, no. 1, pp. 35–46, 2009.

[72] J. Myburgh, D. J. Cooper, S. Finfer et al., "Saline or albumin for fluid resuscitation in patients with traumatic brain injury," *The New England Journal of Medicine*, vol. 357, no. 9, pp. 874–884, 2007.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 390760, 8 pages
http://dx.doi.org/10.1155/2014/390760

Research Article

Role of AMPKα in Skeletal Muscle Glycometabolism Regulation and Adaptation in relation to Sepsis

Xia Zheng, Mi Xu, and Qiang Fang

Intensive Care Unit, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310003, China

Correspondence should be addressed to Qiang Fang; guyan7769@163.com

Received 30 January 2014; Accepted 16 June 2014; Published 29 June 2014

Academic Editor: Baoli Cheng

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Background. AMP-activated protein kinase (AMPK) and the translocation of glucose transporter 4 (GLUT4) protein always involve disturbance of carbohydrate metabolism. *Objective.* To determine whether the change of blood glucose in the early stage of septic rat is associated with the alteration of AMPKα protein expression and GLUT4 protein translocation expression. *Methods.* Animal models of sepsis were induced by tail vein injection of LPS in Wistar rats. The dynamic values of blood glucose within 2 hours after injection of LPS were observed. AMPKα protein and GLUT4 protein translocation in different tissues (such as soleus muscle and extensor digitorum longus) were assessed by western blot. *Results.* Blood glucose levels appeared to rise at 0.5 h after injection of LPS, arrived the peak value at 1 h, then fell at 1.5 h and 2 h Animals in LPS group experienced the increase of phos-AMPKα protein and GLUT4 protein translocation expression in soleus muscle and extensor digitorum longus. *Conclusion.* The dynamic change of blood glucose, represented in a form of initiative increase and subsequent decrease in the early stage of sepsis, may be related to glycometabolism disorder in the skeletal muscle, coming down to enhancement of GLUT4 translocation expression promoted by activation of AMPKα.

1. Introduction

Sepsis is a serious medical condition that is characterized by a whole-body inflammatory state, resulting from the systemic response to bacterial infection. If the bacteria die, the endotoxin will be released into the bloodstream. Sepsis remains one of the leading causes of morbidity and mortality in critically ill intensive care unit patients [1]. The systemic administration of lipopolysaccharide (LPS), an outer component of the gram-negative bacterial wall, has been applied as an experimental model to mimic some of the clinical findings of human septic shock [2]. The kind of vicious stimulus, at the same time, leads to severe metabolic disorder. Baseline hyperglycemia, including stress-induced hyperglycemia, is common in patients with severe sepsis. Similarly, stress-induced hyperglycemia is associated with adverse outcomes in septic patients [3–7]. In a 2001 study of critically ill intensive care unit (ICU) patients, van den Berghe and associates demonstrated that aggressive insulin therapy to maintain blood glucose between 4.4 and 6.1 mmol/L reduced mortality from 8.0% with conventional treatment to 4.6%, a relative reduction of 42% [3]. Mortality reduction in the intensive insulin treatment group was attributed to lower rates of organ failure and bacteremia. Mackenzie and colleagues recently reported that when intensive glycemic control was managed by the bedside nurse, average morning glucose concentration was 7.0 ± 2.4 mmol/L, but 42% of patients suffered hypoglycemic episodes, defined as a serum glucose <2.2 mmol/L [8]; thus studies recommend a cautious approach to the control of glucose levels in acutely ill emergency department patients, with a target glucose of below 8 to 9 mmol/L [9]. In present, we only try to control blood glucose with insulin therapy. In fact these metabolic effects induced by 5′-adenosine monophosphate-activated protein kinase (AMPK) are associated with lowering blood glucose levels in hyperglycemic individuals [10]. AMPK is widely present in eukaryotic cells, sensing the changes of cellular energy metabolism, known as the "cellular energy regulator." Impaired glucose metabolism regulated by activated AMPK is the response to cellular stress, such as exercise, hypoxic

stress, and ischemic stimulus [11–14]. It is well known that sepsis is a sophisticated morbid process and this unique model of sepsis induced by LPS always relates to the change of AMP/ATP ratio, ischemia, hypoxia, nutrition, and metabolic disorders. Therefore, as a new target for antidiabetic drugs, AMPK expression in sepsis should be noted.

Recent study showed that patients with type 2 diabetes were more prone to develop dysregulated glucose disposal, which was associated with altered AMPK phosphorylation in skeletal muscle [15]. GLUT4 is a glucose transport protein found in fat and striated muscle cells [16]. When carbohydrates are ingested, the major cellular mechanism that diminishes blood glucose is insulin-stimulated glucose transport into skeletal muscle. Skeletal muscle both stores glucose as glycogen and oxidizes it to produce energy following the transport step. The principal glucose transporter protein that mediates this uptake is GLUT4, which plays a key role in regulating whole body glucose homeostasis [17]. When insulin receptor is activated, it induces the GLUT4 protein to move from reserves held inside cells. GLUT4 can also be recruited to the cell surface through muscle contraction. In the absence of insulin or muscle contraction, GLUT4 is stored in vesicles within the cell. In addition to insulin, skeletal muscle glucose transport is possible stimulated by other media or by other pathways. AMPK is really another known regulator of glucose metabolism in skeletal muscle [18]. Activation of AMPK in muscle leads to an increase in glucose transport, accompanied by increased translocation of GLUT4 to the plasma membrane [19]. Therefore, as the important targets which always involve disturbance of carbohydrate metabolism, whether AMPK and the translocation of GLUT4 protein expression appear to change to adapt the stress hyperglycemia in early stage of sepsis still needs to be paid attention to. Thus the present study is designed to explore whether the acute blood glucose dynamic changes are partly based on translocation of GLUT4 regulated by AMPK signal pathway in the early stage of sepsis.

2. Materials and Methods

2.1. Main Materials. Anti-Phos-AMPKα-Thr172 antibody and anti-AMPKα antibody were purchased from the U.S. Cell Signaling, Inc.; anti-GLUT4 antibody was obtained from Santa Cruz Biotechnology; anti-α-tubulin antibody was obtained from Merck Millipore, Billerica, MA; lipopolysaccharide (LPS, Escherichia coli 0111: B4) was purchased from USA Sigma Company; insulin kit was purchased from the U.S. Adlitteram Diagnostic Laboratories Inc.; Membrane Protein Extraction Kit was purchased from the Fermentas International Inc.

2.2. Animal Model. 12 healthy male Wistar rats (8 weeks old, 200 to 250 g) were purchased from Experiment Animal Center of Chinese Academy of Sciences in Shanghai (SCXK (Shanghai) 2007-0005). The rats were divided into two groups: LPS group (received LPS 5 mg/kg (concentration of 2 mg/mL) by tail vein injection, to establish the septic rat model) and control group (given normal saline (NS) 2.5 mL/kg by tail vein injection) [20]. Body temperature of the rat was measured using the rectal probe. The procedures in our experiments were approved by the Animal Care and Use Committee of Zhejiang University, China.

2.3. The Determination of Blood Glucose and Insulin Levels. Blood glucose levels were determined at 0 h, 0.5 h, 1 h, 1.5 h, and 2 h after injection of LPS or NS with an Accu-chek glucometer (Roche, Mannheim, Germany) from tail-bled samples (made with a needle stick). At 2 hours, anesthesia was executed by 3% pentobarbital sodium (0.15 mL/100 g) intraperitoneal injection. 4–6 mL blood was taken from carotid artery; serum was segregated and stored at −20°C for measurement of insulin level. Insulin levels were determined using an Ultrasensitive Insulin ELISA kit according to the manufacturer's instructions.

2.4. Western Blot. The samples of heart, liver, soleus muscle, and extensor digitorum longus were frozen into liquid nitrogen and stored. 100 mg of each tissue was homogenized in 1 mL modified lysis buffer (0.3 mol/L sucrose, 10 mmol/L imidazole, 10 mmol/L sodium metabisulfite, 1 mmol/L DTT, 0.3 mmol/L PMSF) [21]. The protein concentration was determined by the Bradford method.

Western blot analysis of AMPKα and Pho-AMPKα protein and α-tubulin were performed in heart, liver, soleus muscle, and extensor digitorum longus, while western blot analysis of GLUT4 was performed only in soleus muscle and extensor digitorum longus. Aliquots containing the protein for Phos-AMPKα-Thr172, AMPKα, GLUT4, and α-tubulin were loaded on the SDS-polyacrylamide gel with 10% acrylamide separating gel, respectively, and separated by electrophoresis for 30 min. The separated Phos-AMPKα-Thr172, AMPKα, GLUT4, and α-tubulin proteins were electrophoretically transferred onto nitrocellulose membranes (Amersham Life Science). All of the membranes were incubated at 4°C overnight with anti-Phos-AMPKα-Thr172 antibody (1 : 1000), or anti-AMPKα antibody (1 : 1000) or anti-GLUT4 (1 : 3000), or anti-α-tubulin antibody (1 : 1000) in 5% Carnation instant milk/TBS. After incubating with a secondary antibody (1 : 500) (Beijing Zhongshan Biotechnology, China) in 5% Carnation instant milk-TBS-Tween 20, the blots were developed using enhanced chemiluminescence according to the manual (Biological Industries, Beit Haemerk LTD, Israel) and exposed to X-ray film [22]. Normalization of protein expression was carried out using α-tubulin as control.

2.5. GLUT4 Translocation Analysis. Preparation of plasma membrane fraction from the skeletal muscles was performed as described by Dombrowski et al. [23]. Briefly, three grams of the SOL or EDL muscles were homogenized in 10 mM sodium bicarbonate, 0.25 M sucrose, 5 mM sodium azide, and 100 μM PMSF. The homogenate was subjected to specific centrifugations for subcellular fractionation. The crude membrane was separated from homogenized tissue by use of triple centrifugation at 1200, 9000, and 19 000 ×g, respectively. The plasma membrane fractions were further separated by sucrose density-gradient centrifugation (25%, 32%, and 35%)

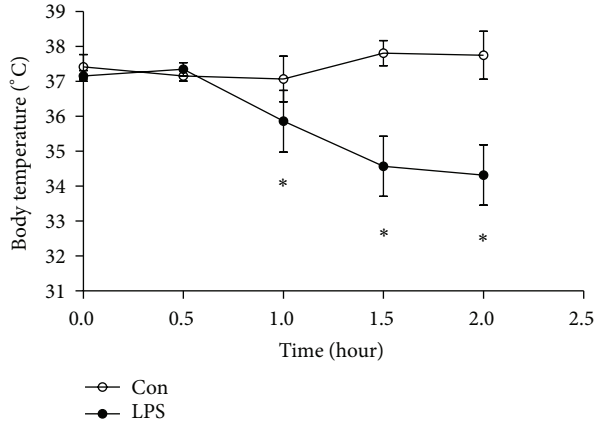


Figure 1: Change of body temperature induced by LPS. The change in body temperature of the rat was dynamically measured at 0 h, 0.5 h, 1 h, 1.5 h, and 2 h after injection of LPS or NS. Data are expressed as mean ± S.D. ($n = 6$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$ LPS group (LPS) versus control group (Con).

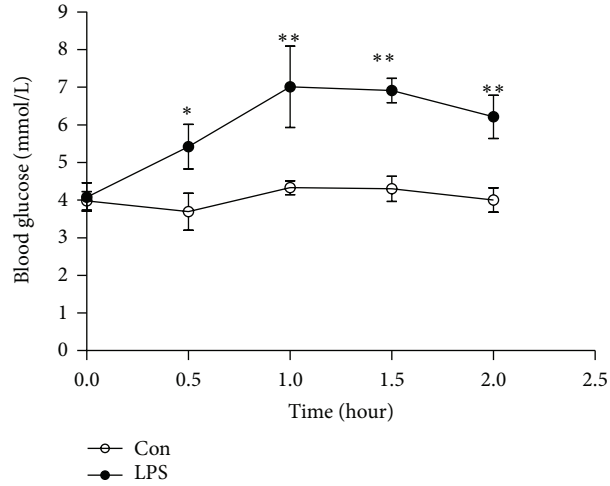


Figure 2: Change of blood glucose induced by LPS. Blood glucose levels were determined at 0 h, 0.5 h, 1 h, 1.5 h, and 2 h after injection of LPS or NS. Data are expressed as mean ± S.D. ($n = 6$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$ LPS group (LPS) versus control group (Con).

at 150 000 ×g for 16 h. The plasma membrane GLUT4 (m-GLUT4) protein was collected from the fraction of 25% sucrose solution, subjected to 190 000 ×g for 60 min, and analyzed by Western blot. Immunoblotting of the tissue protein extracts was performed using anti-GLUT4 antiserum (1 : 3000). The blotted protein was quantified using quantity one software system [24–26].

2.6. Statistical Analysis. Data were reported as means plus or minus Standard Deviation (SD). The various kinds of indexes between control group and LPS-treated groups were compared using analysis of one-way ANOVA with SPSS 16 software. Values were considered significantly different when $P < 0.05$.

3. Results

3.1. General State of the Rats. Rats in control group were still active as usual, with good state, while those in LPS group showed mental weaknesses, physical inactivity dull coat, breathing frequently, greedy overdrink, and abnormal body temperature. Body temperature represented in a form with a rapid decline after 0.5 h and then kept lower within 2 h. In an hour after treatment, there was statistically significant effect on half-hourly body temperature between LPS group and control group (35.86 ± 0.88 versus 37.07 ± 0.65 at 1 h, $P < 0.05$; 34.57 ± 0.86 versus 37.81 ± 0.36 at 1.5 h, $P < 0.05$; 34.32 ± 0.86 versus 37.75 ± 0.69 at 2 h, $P < 0.05$, separately) (see Figure 1).

3.2. Dynamic Change of Blood Glucose. Blood glucose levels appeared to rise at 0.5 h after injection of LPS, arrived the peak value at 1 h, then fell at 1.5 h and 2 h in LPS group. In half an hour after treatment, there was statistically significant effect on half-hourly blood glucose between LPS group and control group (3.69 ± 1.21 versus 5.42 ± 1.45 at 0.5 h, $P < 0.05$; 4.33 ± 0.45 versus 7.01 ± 2.65 at 1 h, $P < 0.01$; 4.30 ± 0.82 versus 6.91 ± 0.79 at 1.5 h, $P < 0.01$; 4.00 ± 0.79 versus 6.21 ± 1.40 at 2 h, $P < 0.01$, separately) (see Figure 2).

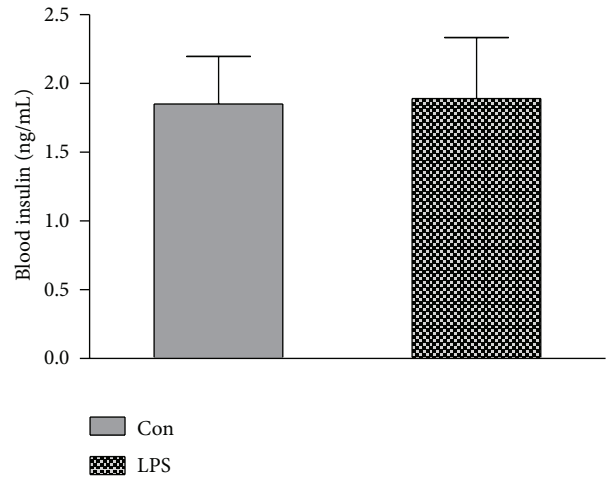


Figure 3: Change of blood insulin induced by LPS. At 2 hours after injection of LPS or NS, 4–6 mL blood was taken from carotid artery; serum was segregated for measurement of insulin level. Data are expressed as mean ± S.D. ($n = 6$ per group). $^{*}P < 0.05$, $^{**}P < 0.01$ LPS group (LPS) versus control group (Con).

3.3. Changes of Plasma Insulin. At 2 hour, there was no significant different of serum insulin level between LPS and control group (1.85 ± 0.85 versus 1.89 ± 1.09, $P > 0.05$) (see Figure 3).

3.4. Effects of LPS on Protein Expression of Phos-AMPKα and AMPKα. LPS failed to alter the protein expression of AMPKα in different tissues (soleus muscle 0.78 ± 0.55 versus 1.03 ± 0.52, $P > 0.05$; extensor digitorum longus 1.05 ± 0.26

versus 1.28 ± 0.32, $P > 0.05$; liver 1.28 ± 0.24 versus 1.43 ± 0.22, $P > 0.05$; and myocardium 2.52 ± 1.26 versus 3.00 ± 0.82, $P > 0.05$). No impact of LPS on abundance of Phos-AMPKα proteins of cardiac (2.77 ± 0.80 versus 2.80 ± 0.53, $P > 0.05$) and liver (1.03±0.70 versus 1.22±0.68, $P > 0.05$) was exhibited in this study. However, LPS induced significant increase of Phos-AMPKα proteins in soleus muscle (1.03 ± 0.29 versus 0.52 ± 0.29, $P < 0.01$) and extensor digitorum longus (1.20 ± 0.21 versus 0.73 ± 0.33, $P < 0.01$) (see Figure 4)

3.5. Effects of LPS on Expression of GLUT4 Protein Translocation in Skeletal Muscle. GLUT4 and m-GLUT4 expression levels in skeletal muscle by Western blot. As shown in Figures 5(a) and 5(b), no significant differences in total GLUT4 protein in soleus muscle (1.15 ± 0.08 versus 1.10 ± 0.12, $P > 0.05$) and extensor digitorum longus (1.17 ± 0.23 versus 1.21 ± 0.17, $P > 0.05$) were observed between LPS and control group. However, LPS induced the increase in the expression of GLUT4 protein translocation of soleus muscle (0.84 ± 0.06 versus 0.67 ± 0.08, $P < 0.01$) and extensor digitorum longus (0.74 ± 0.12 versus 0.57 ± 0.13, $P < 0.05$).

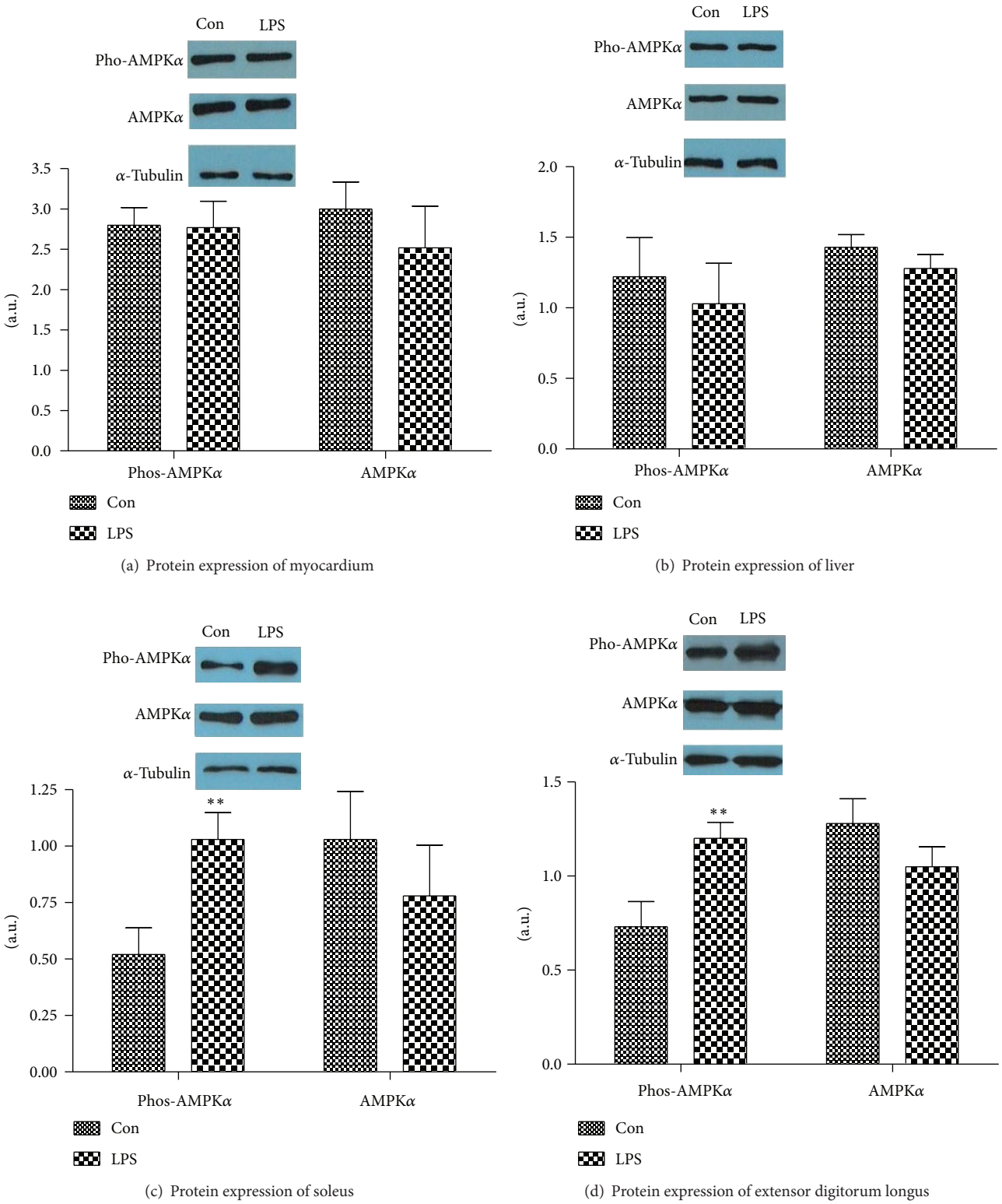
4. Discussion

Sepsis is a kind of severe illness, caused by infection in the body. When inflammation overwhelms the host, simple infections will develop into sepsis. Sepsis is associated with various metabolic and endocrine disorders that can be confusing [27]. On the one hand, metabolic disorders in sepsis express high catabolic state with increased energy consumption. These patients often exhibit a well-defined endocrine and metabolic adaptive response to stressor agents, partly characterized by incremented resting energy expenditure (hypermetabolism, which is believed to signify increased energy requirements) [28]. That is to say, a cardinal manifestation is hyperglycemia [27]. On the other hand, some metabolic pathways were demolished in sepsis. For example, prolonged sepsis and exposure to an inflammatory milieu decreases muscle protein synthesis and reduces muscle mass [29]. Hyperglycemia is frequently easily observed during bacterial infection and it is a marker of a poor clinical outcome in critically ill patients. Lipopolysaccharides (LPS) of the cell wall of Gram (−) bacteria trigger inflammation, which is associated with marked changes in glucose metabolism; thus recently more and more attention has been paid to LPS-induced glucose metabolism disorder, which is a prominent pathological problem [30, 31].

Our experiment showed that, blood glucose levels were elevated in 0.5 h after injection of LPS, and there was statistically significant effect on half-hourly blood glucose between LPS group and control group from 0.5 h to 2 h. In fact, physical trauma, surgical-site infection, and many forms of severe stress can temporarily increase glucose levels [32–34]. Even only hypothermia can have the "perverse result." For example, adverse events may develop when a patient is treated with hypothermia [35]. One of the adverse events associated with hypothermic therapy is a decrease in insulin sensitivity and insulin secretion, which can lead to hyperglycemia [35]. In our experiment, body temperature represented in a form with rapidly decline after 0.5 h induced by LPS, then kept lower within 2 hours. In fact, sepsis is a complex pathological process, and multiple factors are involved in abnormally high blood sugar. So far the mechanism of stress hyperglycemia in early stage of sepsis still leaves a puzzle. Stress in early stage of sepsis can increase sympathetic nerve activity, then the autonomic nervous system regulated adrenocortical function, and catecholamines facilitated the action of glucocorticoids. In addition to pituitary adrenocorticotropin, there are other extrapituitary factors regulating adrenal steroidogenesis in septic shock [36]. Similarly, glucagon was triggered to increase the level of glucose [36, 37]. In present, even plasma glucose >120 mg/dL in the absence of diabetes is a clinical sign of sepsis. Of course, hyperglycemia may be associated with increased mortality, while strict regulation of glucose levels has been found to decrease mortality and length of stay in the ICU. Now we only try to control blood glucose with insulin therapy. However, in our experiment, no significant changes in insulin levels were observed after 2 h of LPS injection, similar with the results of D. T. Yates et al. [38]. It is speculated that only 2 hours after LPS injection were too little time to finish the desired changes of insulin levels. However, in 2 hours after LPS injection, blood glucose levels significantly fell a long way from their peak simultaneously. Thus, rather than insulin action, another way, such as 5′-adenosine monophosphate-activated protein kinase (AMPK), maybe become the pathway to affect the self-regulation of blood glucose after IV bolus of LPS.

It is well known that as a highly conserved serine/threonine protein kinase, AMPK can become the important metabolic stress protein kinases, constituted by α, β, and γ 3 subunit. Once activated, AMPK phosphorylates several downstream substrates, the overall effect of which is to switch off ATP-consuming pathways (e.g., fatty acid synthesis and cholesterol synthesis) and to switch on ATP-generating pathways (e.g., fatty acid oxidation and glycolysis) [39]; thus the phosphorylation of AMPK become a central link of cellular energy regulation. And AMPK on the regulation of carbohydrate metabolism is mainly reflected the promotion of glucose uptake, glycolysis, inhibit gluconeogenesis, and glycogen synthesis. Even in recent years AMPK has become an attractive pharmacological target for the treatment of insulin resistance and type 2 diabetes-associated dyslipidaemia [40].

Patients with sepsis have a hypermetabolic and hypercatabolic state, which can be represented by increased oxygen demand on the body tissue and reduced oxygen consumption because of microcirculatory disturbance, thus ATP generation is decreased, the AMP to ATP ratio is increased, and AMPK is activated at last. It is well known that AMPK activation favors carbohydrate metabolism under some certain conditions. For example, activation of AMPK is thought to mediate, at least partially, the increases in skeletal muscle fatty acid oxidation and glucose transport that occur during acute exercise [41]. AMPK activation is a complex and elaborates the regulating process. As the action sites, α-subunit 172 threonine can provide the key role on AMPK activation. Therefore, activation of AMPK requires its phosphorylation



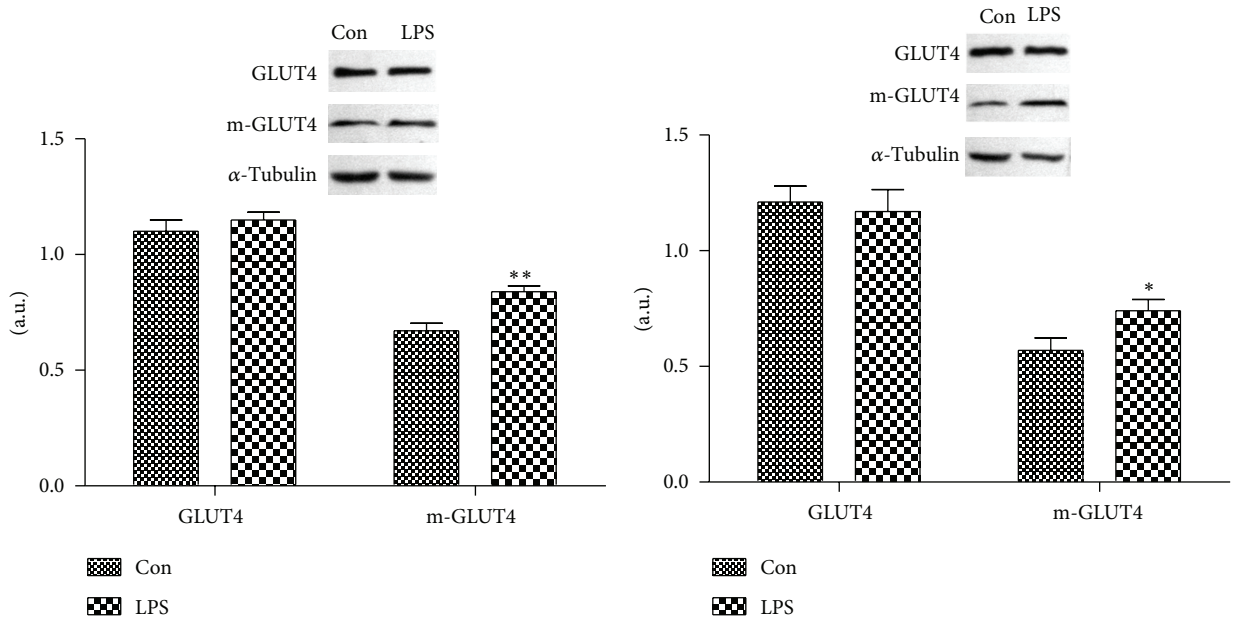
(a) Protein expression of myocardium

(b) Protein expression of liver

(c) Protein expression of soleus

(d) Protein expression of extensor digitorum longus

Figure 4: The effects of LPS on the protein expression of phos-AMPKα and AMPKα in different tissues: heart (a), liver (b), soleus muscle (c), and extensor digitorum longus (d). Equal amounts of protein were subjected to electrophoresis and immunoblotted, as described. Data were represented as mean ± S.D. ($n = 6$, per group) $^{*}P < 0.05$, $^{**}P < 0.01$ LPS group (LPS) versus control group (Con).



(a) Total GLUT4 and m-GLUT4 translocation in soleus muscles

(b) Total GLUT4 and m-GLUT4 translocation in extensor digitorum longus

Figure 5: The effect of LPS on total GLUT4 and m-GLUT4 translocation in skeletal muscle (soleus muscle or extensor digitorum longus). Preparation of plasma membrane fraction from the skeletal muscles was performed. The proteins were analyzed by western blot. Results were normalized by α-tubulin, and the m-GLUT4 was normalized by the total protein. Data were represented as mean ± S.D. ($n = 6$, per group) $^{*}P < 0.05$, $^{**}P < 0.01$ LPS group (LPS) versus control group (Con).

at Thr 172 site [42]. Our experiment showed that AMPKα and Phos-AMPKα in myocardium and liver tissue of septic rats had no significant difference, compared with those in control group, after 2 h of LPS injection. However, the levels of Phos-AMPKα in the soleus muscle and extensor digitorum longus were significantly increased, although the expression of AMPKα was not impaired. In association with the alteration of blood glucose, it was speculated AMPK activation in exercising muscles could take part in the glycometabolism process in early stage of sepsis, while the metabolic capacity of blood glucose was not relate to AMPK activation in myocardial and liver tissue.

The signaling mechanism, downstream of AMPK, which regulates muscle glucose transport, is unclear in septic rat. Previous studies showed that, in skeletal muscle, AMPK was activated by exercise/contraction, metformin, and thiazolidinediones resulting in an increase in glucose uptake [43]. The skeletal muscle is the main peripheral tissue of glucose metabolism. The rate-limiting step of glucose metabolism is the pathway of glucose into skeletal muscle cells, which requires direct involvement of GLUT4 on the cell membrane. In cell culture, Edward O. Ojuka et al. [44] found AICAR (5-amino-4-ammonia ribonucleotide formyl imidazole), as AMPK activator, could activate AMPK to divert GLUT4 within the cell toward cytomembrane. And Bergeron et al. [45] showed that, in the quiet state, AICAR could activate AMPK, promoting GLUT4 protein translocation in cell membrane, which would increase glucose transport and uptake in skeletal muscle.

The adjustment mechanism of AMPK has been confirmed in state of exercise. On the one hand, islet β-cell insulin receptor, insulin-like growth factor receptor and peripheral insulin receptors mRNA expression, and protein expression can be adjusted by activation of AMPK [46]. On the other hand, AMPK can be activated by noninsulin signals in skeletal cells, so that GLUT4 within cytoplasm will shift to Cytolemma and various plasma membrane, enhancing the capacity of glucose transport [47]. In the experiment, LPS induced the increase in the expression of GLUT4 protein translocation of soleus muscle and extensor digitorum longus. Prompt decline in blood glucose at this time may be related to activation of AMPK regulation of skeletal muscle glucose metabolism [44, 48]. Because the result in this study showed that the level of insulin in LPS group did not alter; thus, in the early stage of sepsis, GLUT4 protein translocation by noninsulin dependent pathway can be actually a mechanism for glucose metabolism in skeletal muscle.

Generally skeletal muscle fibers are a mixture of 3 types of muscle fibers: type I (red fibers, slow-twitch, and slow oxidative), type II a (red fibers, fast-twitch, and fast oxidative), and type II b (white fibers, fast-twitch, fast glycolytic). Soleus muscle fibers mainly belong to type I, while extensor digitorum longus muscle fiber belongs to type II. To the different muscle fiber types, AMPK response is various. AMPK may be involved in the signal transduction pathway induced by fast muscle movement, while AMPK is not related to the slow-twitch fibers [49–51]. But in this experiment,

Phos-AMPKα expression and GLUT4 protein translocation expression of the soleus muscle and extensor digitorum longus all increased in 2 h after LPS injection. Therefore, it is deduced that, in early stage of acute sepsis, the effect of AMPK on glucose metabolism in skeletal muscle may not be related to muscle fiber type.

In conclusion, the dynamic changes of blood glucose appeared to be an increase at first and then a drop in early stage of acute sepsis. The changes of blood glucose have no bearing on glucose metabolism in cardiac muscle and liver tissue. Non-insulin-dependent AMPK signaling pathway can increase the expression of GLUT4 protein translocation to promote skeletal muscle glucose metabolism. Activation of AMPK on the regulation of glucose metabolism in skeletal muscle has no relation to muscle fiber type.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This project is supported by National Science Foundation of China (Grant no. 81101445) and by Medical and Health Projects of Zhejiang Province, China (Grant no. 2013KYA063).

References

[1] G. M. L. Bearman and R. P. Wenzel, "Bacteremias: a leading cause of death," *Archives of Medical Research*, vol. 36, no. 6, pp. 646–659, 2005.

[2] R. S. Saia, G. Bertozi, F. L. Mestriner, J. Antunes-Rodrigues, F. Queiróz Cunha, and E. C. Cárnio, "Cardiovascular and inflammatory response to cholecystokinin during endotoxemic shock," *Shock*, vol. 39, no. 1, pp. 104–113, 2013.

[3] G. van den Berghe, P. Wouters, F. Weekers et al., "Intensive insulin therapy in critically ill patients," *The New England Journal of Medicine*, vol. 345, no. 19, pp. 1359–1367, 2001.

[4] S. J. Finney, C. Zekveld, A. Elia, and T. W. Evans, "Glucose Control and Mortality in Critically Ill Patients," *Journal of the American Medical Association*, vol. 290, no. 15, pp. 2041–2047, 2003.

[5] J. S. Krinsley, "Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients," *Mayo Clinic Proceedings*, vol. 78, no. 12, pp. 1471–1478, 2003.

[6] G. van den Berghe, A. Wilmer, G. Hermans et al., "Intensive insulin therapy in the medical ICU," *The New England Journal of Medicine*, vol. 354, no. 5, pp. 449–461, 2006.

[7] J. S. Krinsley, "Effect of an intensive glucose management protocol on the mortality of critically Ill adult patients," *Mayo Clinic Proceedings*, vol. 79, no. 8, pp. 992–1000, 2004.

[8] I. Mackenzie, S. Ingle, S. Zaidi, and S. Buczaski, "Tight glycaemic control: a survey of intensive care practice in large English hospitals," *Intensive Care Medicine*, vol. 31, no. 8, p. 1136, 2005.

[9] W. R. Henderson, D. R. Chittock, V. K. Dhingra, and J. J. Ronco, "Hyperglycemia in acutely ill emergency patients—cause or effect? State of the art," *Canadian Journal of Emergency Medicine*, vol. 8, no. 5, pp. 339–343, 2006.

[10] A. Gruzman, G. Babai, and S. Sasson, "Adenosine monophosphate-activated protein kinase (AMPK) as a new target for antidiabetic drugs: a review on metabolic, pharmacological and chemical considerations," *Review of Diabetic Studies*, vol. 6, no. 1, pp. 13–36, 2009.

[11] Y. Xing, N. Musi, N. Fujii et al., "Glucose metabolism and energy homeostasis in mouse hearts overexpressing dominant negative α2 subunit of AMP-activated protein kinase," *The Journal of Biological Chemistry*, vol. 278, no. 31, pp. 28372–28377, 2003.

[12] S. C. Stein, A. Woods, N. A. Jones, M. D. Davison, and D. Cabling, "The regulation of AMP-activated protein kinase by phosphorylation," *Biochemical Journal*, vol. 345, no. 3, pp. 437–443, 2000.

[13] A. S. Marsin, L. Bertrand, M. H. Rider et al., "Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia," *Current Biology*, vol. 10, no. 20, pp. 1247–1255, 2000.

[14] L. G. D. Fryer and D. Carling, "AMP-activated protein kinase and the metabolic syndrome," *Biochemical Society Transactions*, vol. 33, part 2, pp. 362–366, 2005.

[15] A. S. Andreasen, M. Kelly, R. M. Berg, K. Møller, and B. K. Pedersen, "Type 2 diabetes is associated with altered NF-κB DNA binding activity, JNK phosphorylation, and AMPK phosphorylation in skeletal muscle after LPS," *PLoS ONE*, vol. 6, no. 9, Article ID e23999, 2011.

[16] G. D. Holman and I. V. Sandoval, "Moving the insulin-regulated glucose transporter GLUT4 into and out of storage," *Trends in Cell Biology*, vol. 11, no. 4, pp. 173–179, 2001.

[17] S. Huang and M. P. Czech, "The GLUT4 Glucose Transporter," *Cell Metabolism*, vol. 5, no. 4, pp. 237–252, 2007.

[18] J. F. P. Wojtaszewski, J. N. Nielsen, S. B. Jørgensen, C. Frøsig, J. B. Birk, and E. A. Richter, "Transgenic models—a scientific tool to understand exercise-induced metabolism: the regulatory role of AMPK (5′-AMP-activated protein kinase) in glucose transport and glycogen synthase activity in skeletal muscle," *Biochemical Society Transactions*, vol. 31, part 6, pp. 1290–1294, 2003.

[19] A. Fritah, J. H. Steel, N. Parker et al., "Absence of RIP140 reveals a pathway regulating glut4-dependent glucose uptake in oxidative skeletal muscle through UCP1-mediated activation of AMPK," *PLoS ONE*, vol. 7, no. 2, Article ID e32520, 2012.

[20] S. Li, H. Bao, L. Han, and L. Liu, "Effects of propofol on early and late cytokines in lipopolysaccharide-induced septic shock in rats," *Journal of Biomedical Research*, vol. 24, no. 5, pp. 389–394, 2010.

[21] W. Luo, B. M. Wolska, I. L. Grupp et al., "Phospholamban gene dosage effects in the mammalian heart," *Circulation Research*, vol. 78, no. 5, pp. 839–847, 1996.

[22] A. Tominaga, N. Ishizaki, Y. Naruse, H. Kitakoji, and Y. Yamamura, "Repeated application of low-frequency electroacupuncture improves high-fructose diet-induced insulin resistance in rats," *Acupuncture in Medicine*, vol. 29, no. 4, pp. 276–283, 2011.

[23] L. Dombrowski, D. Roy, B. Marcotte, and A. Marette, "A new procedure for the isolation of plasma membranes, T tubules, and internal membranes from skeletal muscle," *The American Journal of Physiology*, vol. 270, no. 4, part 1, pp. E667–E676, 1996.

[24] C. Sunil, V. Duraipandiyan, P. Agastian, and S. Ignacimuthu, "Antidiabetic effect of plumbagin isolated from *Plumbago*

zeylanica L. root and its effect on GLUT4 translocation in streptozotocin-induced diabetic rats," *Food and Chemical Toxicology*, vol. 50, no. 12, pp. 4356–4363, 2012.

[25] E. L. Brunetto, S. S. Teixeira, G. Giannocco, U. F. MacHado, and M. T. Nunes, "T3 rapidly increases SLC2A4 gene expression and GLUT4 trafficking to the plasma membrane in skeletal muscle of rat and improves glucose homeostasis," *Thyroid*, vol. 22, no. 1, pp. 70–79, 2012.

[26] S. Hu, Y. Chang, J. Wang et al., "Fucosylated chondroitin sulfate from Acaudina molpadioides improves hyperglycemia via activation of PKB/GLUT4 signaling in skeletal muscle of insulin resistant mice," *Food & Function*, vol. 4, pp. 1639–1646, 2013.

[27] R. Khardori and D. Castillo, "Endocrine and metabolic changes during sepsis: an update," *Medical Clinics of North America*, vol. 96, no. 6, pp. 1095–1105, 2012.

[28] J. F. Patiño, S. Echeverri de Pimiento, A. Vergara, P. Savino, M. Rodríguez, and J. Escallón, "Hypocaloric support in the critically ill," *World Journal of Surgery*, vol. 23, no. 6, pp. 553–559, 1999.

[29] C. H. Lang, R. A. Frost, and T. C. Vary, "Regulation of muscle protein synthesis during sepsis and inflammation," *The American Journal of Physiology: Endocrinology and Metabolism*, vol. 293, no. 2, pp. E453–E459, 2007.

[30] S. E. Hussey, H. Liang, S. R. Costford et al., "TAK-242, a small-molecule inhibitor of Toll-like receptor 4 signalling, unveils similarities and differences in lipopolysaccharide- and lipidinduced inflammation and insulin resistance in muscle cells," *Bioscience Reports*, vol. 33, no. 1, pp. 37–47, 2013.

[31] Q.-M. Ge, S.-C. Du, F. Bian, N. Lin, and Q. Su, "Effects of lipopolysaccharides on TLR4 expression in INS-1 rat insulinoma cells," *Cellular and Molecular Biology*, vol. 57, pp. OL1513–OL1519, 2011.

[32] P. L. Bosarge and J. D. Kerby, "Stress-induced hyperglycemia. Is it harmful following trauma?" *Advances in Surgery*, vol. 47, no. 1, pp. 287–297, 2013.

[33] J. E. Richards, R. M. Kauffmann, W. T. Obremskey, and A. K. May, "Stress-induced hyperglycemia as a risk factor for surgical-site infection in nondiabetic orthopedic trauma patients admitted to the intensive care unit," *Journal of Orthopaedic Trauma*, vol. 27, no. 1, pp. 16–21, 2013.

[34] V. Srinivasan, "Stress hyperglycemia in pediatric critical illness: the intensive care unit adds to the stress!," *Journal of Diabetes Science and Technology*, vol. 6, no. 1, pp. 37–47, 2012.

[35] T. Melhuish, "Linking hypothermia and hyperglycemia," *Nursing Management*, vol. 40, no. 12, pp. 42–45, 2009.

[36] S. R. Bornstein and J. Briegel, "A new role for glucocorticoids in septic shock: balancing the immune response.," *The American Journal of Respiratory and Critical Care Medicine*, vol. 167, no. 4, pp. 485–486, 2003.

[37] S. R. Maitra, M. L. Gestring, M. R. El-Maghrabi, C. H. Lang, and M. C. Henry, "Endotoxin-induced alterations in hepatic glucose-6-phosphatase activity and gene expression," *Molecular and Cellular Biochemistry*, vol. 196, no. 1-2, pp. 79–83, 1999.

[38] D. T. Yates, C. A. Löest, T. T. Ross, D. M. Hallford, B. H. Carter, and S. W. Limesand, "Effects of bacterial lipopolysaccharide injection on white blood cell counts, hematological variables, and serum glucose, insulin, and cortisol concentrations in ewes fed low- or high-protein diets," *Journal of Animal Science*, vol. 89, no. 12, pp. 4286–4293, 2011.

[39] D. Carling, "The AMP-activated protein kinase cascade—a unifying system for energy control," *Trends in Biochemical Sciences*, vol. 29, no. 1, pp. 18–24, 2004.

[40] A. Ben Djoudi Ouadda, E. Levy, E. Ziv et al., "Increased hepatic lipogenesis in insulin resistance and type 2 diabetes is associated with AMPK signalling pathway up-regulation in *Psammomys obesus*," *Bioscience Reports*, vol. 29, no. 5, pp. 283–292, 2009.

[41] A. Sriwijitkamol, D. K. Coletta, E. Wajcberg et al., "Effect of acute exercise on AMPK signaling in skeletal muscle of subjects with type 2 diabetes: a time-course and dose-response study," *Diabetes*, vol. 56, no. 3, pp. 836–848, 2007.

[42] S. A. Hawley, M. Davison, A. Woods et al., "Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase," *Journal of Biological Chemistry*, vol. 271, no. 44, pp. 27879–27887, 1996.

[43] F. Mauvais-Jarvis, F. Andreelli, H. Hanaire-Broutin, B. Charbonnel, and J. Girard, "Therapeutic perspectives for type 2 diabetes mellitus: Molecular and clinical insights," *Diabetes and Metabolism I*, vol. 27, no. 4, pp. 415–423, 2001.

[44] E. O. Ojuka, L. A. Nolte, and J. O. Holloszy, "Increased expression of GLUT-4 and hexokinase in rat epitrochlearis muscles exposed to AICAR in vitro," *Journal of Applied Physiology*, vol. 88, no. 3, pp. 1072–1075, 2000.

[45] R. Bergeron, R. R. Russell III, L. H. Young et al., "Effect of AMPK activation on muscle glucose metabolism in conscious rats," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 276, no. 5, part 1, pp. E938–E944, 1999.

[46] K. Raile, J. Klammt, S. Laue et al., "Glucose concentration and AMP-dependent kinase activation regulate expression of insulin receptor family members in rat islets and INS-1E beta cells," *Diabetologia*, vol. 48, no. 9, pp. 1798–1809, 2005.

[47] F. Golami, J. Mohiti Ardakani, A. Moradi, and F. Daneshpouya, "Anti diabetic effect of curcuma longa extract via non-insulin dependent cellular pathway AMPK," *The Journal of Sonoma State University*, vol. 21, no. 3, pp. 311–318, 2013.

[48] I. Webster, S. O. Friedrich, A. Lochner, and B. Huisamen, "AMP kinase activation and glut4 translocation in isolated cardiomyocytes," *Cardiovascular Journal of Africa*, vol. 21, no. 2, pp. 72–78, 2010.

[49] W. W. Winder and B. F. Holmes, "Insulin stimulation of glucose uptake fails to decrease palmitate oxidation in muscle if AMPK is activated," *Journal of Applied Physiology*, vol. 89, no. 6, pp. 2430–2437, 2000.

[50] N. Fujii, M. F. Hirshman, E. M. Kane et al., "AMP-activated protein kinase $\alpha 2$ activity is not essential for contraction-and hyperosmolarity-induced glucose transport in skeletal muscle," *The Journal of Biological Chemistry*, vol. 280, no. 47, pp. 39033–39041, 2005.

[51] D. C. Wright, P. C. Geiger, J. O. Holloszy, and D. H. Han, "Contraction- and hypoxia-stimulated glucose transport is mediated by a Ca^{2+}-dependent mechanism in slow-twitch rat soleus muscle," *The American Journal of Physiology: Endocrinology and Metabolism*, vol. 288, no. 6, pp. E1062–E1066, 2005.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 698265, 10 pages
http://dx.doi.org/10.1155/2014/698265

Review Article

Intensive Insulin Therapy for Septic Patients: A Meta-Analysis of Randomized Controlled Trials

Fang Song,[1] Liu-Jun Zhong,[2] Liang Han,[2] Guo-Hao Xie,[2] Cheng Xiao,[3] Bing Zhao,[2] Yao-Qin Hu,[4] Shu-Yan Wang,[2] Chao-Jin Qin,[4] Yan Zhang,[2] Deng-Ming Lai,[4] Ping Cui,[2] and Xiang-Ming Fang[2]

[1] *School of Medicine, Zhejiang University, Yuhangtang Road 866, Hangzhou 310058, China*
[2] *Department of Anesthesiology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Qingchun Road 79, Hangzhou 310003, China*
[3] *Department of Surgical Oncology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Qingchun Road 79, Hangzhou 310003, China*
[4] *Department of Surgery, Children's Hospital, School of Medicine, Zhejiang University, Zhuganxiang 57, Hangzhou 310003, China*

Correspondence should be addressed to Xiang-Ming Fang; xiangming_fang@163.com

Received 7 January 2014; Accepted 18 March 2014; Published 18 June 2014

Academic Editor: Baoli Cheng

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Background. Studies on the effect of intensive insulin therapy (IIT) in septic patients with hyperglycemia have given inconsistent results. The primary purpose of this meta-analysis was to evaluate whether it is effective in reducing mortality. *Methods*. We searched PubMed, Embase, the Cochrane Library, clinicaltrials.gov, and relevant reference lists up to September 2013 and including randomized controlled trials that compared IIT with conventional glucose management in septic patients. Study quality was assessed using the Cochrane Risk of Bias Tool. And our primary outcome measure was pooled in the random effects model. *Results*. We identified twelve randomized controlled trials involving 4100 patients. Meta-analysis showed that IIT did not reduce any of the outcomes: overall mortality (risk ratio [RR] = 0.98, 95% CI [0.85, 1.15], $P = 0.84$), 28-day mortality (RR = 0.66, 95% CI [0.40, 1.10], $P = 0.11$), 90-day mortality (RR = 1.10, 95% CI [0.97, 1.26], $P = 0.13$), ICU mortality (RR = 0.94, 95% CI [0.77, 1.14], $P = 0.52$), hospital mortality (RR = 0.98, 95% CI [0.86, 1.11], $P = 0.71$), severity of illness, and length of ICU stay. Conversely, the incidence of hypoglycemia was markedly higher in the IIT (RR = 2.93, 95% CI [1.69, 5.06], $P = 0.0001$). *Conclusions*. For patients with sepsis, IIT and conservative glucose management show similar efficacy, but ITT is associated with a higher incidence of hypoglycemia.

1. Introduction

Sepsis has been a long withstanding issue in modern medicine that in too many instances leads to mortality. Every year in the United States there are approximately 750,000 documented cases, of which at least 225,000 are fatal [1]. Though there have been advances in intensive care, the mortality rate of patients with sepsis has remained between 20% and 30% over the past three decades [1, 2]. One pathophysiological component in septic patients is hypermetabolism, including perturbations of glucose metabolism resulting in hyperglycemia [3].

Hyperglycemia is prevalent in ICU patients, especially those with sepsis [4–6]. Hyperglycemia is associated with many adverse outcomes, including immune disorder, oxidative stress, susceptibility to infection, and endothelial dysfunction [7, 8]. Its impact is believed by research that has found hyperglycemia to be independently associated with increased mortality in patients with sepsis because it enhances the inflammatory response [6, 9]. Some randomized controlled clinical trials have attempted to determine whether intensive insulin therapy targeted on establishing normoglycemia could benefit septic patients [10–14].

In 2001, a randomized controlled trial showed that intensive treatment with insulin (80–110 mg/dL) resulted in a lower hospital mortality in the surgical ICU, which was attributed to a reduction of mortality in patients with sepsis [15]. Conversely, the VISEP study, the first to specifically

investigate intensive insulin therapy for septic patients, found no significant reduction in mortality [10]. Then, further randomized controlled trials failed to replicate the mortality benefit in septic patients [16, 17]. Despite this continuing debate, the Surviving Sepsis Campaign included an upper limit for blood glucose of 180 mg/dL in their guidelines based upon systematic reviews of studies involving critically ill patients [18]. Although sepsis is the chief cause of death in ICUs, whether the impact and safety of intensive insulin therapy in septic patients are the same as those in critically ill patients is uncertain.

In order to clarify this matter, we conducted a meta-analysis to assess the use of intensive insulin therapy in managing glycemic control for septic patients. The primary purpose was to evaluate the effects of tight glycemic control on mortality stratified into four subgroups (90-day and 28-day mortality and hospital and ICU mortality).

2. Methods

2.1. Search Strategy. We searched for randomized controlled trials of intensive insulin therapy targeting euglycemia among septic patients in PubMed, Embase, the Cochrane Library, and clinicaltrials.gov dating up to September 2013 without language restriction. We used the exploded Medical Subject Heading (MeSH) terms "insulin," "blood glucose," and "hypoglycemic agents," with the text words "hyperglycemia," "insulin," "blood glucose," and "glycemic control," for the intensive insulin filter. The MeSH term "sepsis" with the text words "sepsis," "severe sepsis," "septic shock," and "septicemia" for the sepsis filter. Additionally, a highly sensitive search strategy described in the Cochrane Handbook was utilized for the randomized controlled trials filter [19]. The MeSH terms and text words were combined with the Boolean operator OR, and then the three filters were combined with the Boolean AND operator. We also checked the reference lists of retrieved reviews and clinical trials to identify additional studies.

2.2. Study Selection. Two investigators independently reviewed all of the titles and abstracts. Included articles met the following criteria: (1) trials had a randomized controlled clinical design, with or without blinding; (2) patients were adults with sepsis; (3) the intensive insulin therapy group had a targeted glucose concentration of ≤150 mg/dL, and the control group had a higher glucose level; and (4) the outcome measures included at least one of the following: mortality, severity of illness, length of ICU stay, and hypoglycemia (≤40 mg/dL). We also included data from trials in critically ill patients if the data concerning sepsis could be extracted.

2.3. Data Abstraction and Quality Assessment. Independently, the same two investigators abstracted the data and assessed the methodological quality of eligible trials. If there was any disagreement, a third investigator participated in a group discussion and made the final decision. The abstracted data was as follows: first author, year of publication, region or country, number of study site, sample size, population,

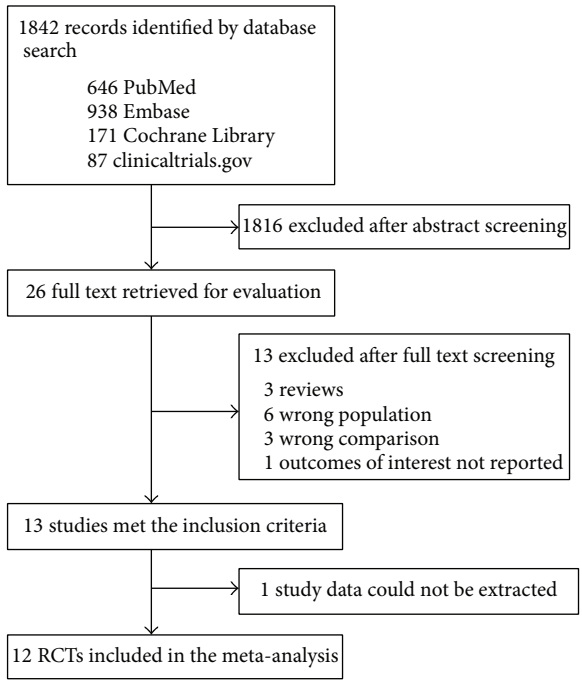

Figure 1: Flow diagram of study selection.

patient age, history of diabetes mellitus, initial glucose level, targeted glucose level, and achieved mean glucose value. The primary outcome was mortality with a preference for 90-day mortality. If this was not reported in the outcome, we used hospital mortality, 28-day mortality, or ICU mortality, in that order. The secondary outcomes were severity of illness, length of ICU stay, and hypoglycemia. We also stratified mortality by 90-day and 28-day mortality, as well as hospital and ICU mortality in subgroup analyses.

The methodological quality was formally evaluated using the Cochrane risk of bias assessment tool, which incorporates random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other potential sources of bias [19]. Each item was stratified into one of three categories: (1) high risk, which represented low quality, (2) low risk, which represented high quality, or (3) unclear, in which there was insufficient information to judge or the study did not involve this outcome.

2.4. Statistical Analysis. We used the Review Manager software to conduct the statistical analyses [20]. For each outcome measure, we used the relative risk (RR) for dichotomous data and the standardized mean difference (SMD) for continuous data. We used a random-effect model for all analyses which provides a more conservative pooled estimate than a fixed-effect model, considering the anticipated clinical heterogeneity among eligible articles [21]. Some data were presented with means and 95% confidence intervals, necessitating that we calculate standard deviations from the data provided. We assessed the heterogeneity among studies using

Table 1: Characteristics of included randomized controlled trials.

First author, year (country)	Number of study sites	Sample size	Population, %	Mean age, (years)	Diabetic, %	Initial glucose, mg/dL	Glucose goal, mg/dL	Glucose achieved, mean (SD), mg/dL
Cappi 2012 (Brazil) [14]	1	63	Severe sepsis Septic shock	53	24	IIT 144 [97–182]* Control 141 [101–160]*	IIT 80–110 Control 140–180	IIT 99 (18) Control 155 (39.6)
COIITSS 2010 (France) [16]	11	509	Septic shock 100	64	NA	IIT 216 (NA) Control 204 (NA)	IIT 80–110 Control 180–200	IIT 120–140 Control 140–160
Savioli 2009 (Italy) [12]	3	90	Severe sepsis 65.6 Septic shock 34.4	61	13.3	IIT 175 (101) Control 160 (74)	IIT 80–110 Control 180–200	IIT 112 (23) Control 159 (31)
Iapichino 2008 (Italy) [11]	3	72	Severe sepsis 19.4 Septic shock 80.6	62.3	17	IIT 137 (45) Control 151.7 (36.6)	IIT 80–110 Control 180–200	IIT 110 (17) Control 163 (29)
Brunkhorst 2008 (Germany) [10]	18	537	Septic shock 100	64.6	30	IIT 130 [108–167]* Control 138 [111–184]*	IIT 80–110 Control 180–200	IIT 112 (NA) Control 151 (NA)
Ellger 2008 (Belgium) [25]	1	950	Severe sepsis 51.4 Septic shock 48.6	62	14	IIT 163 (73) Control 161 (70)	IIT 80–110 Control 180–200	IIT 106 (26) Control 150 (30)
Yu 2005 (China) [13]	1	55	Sepsis	46	NA	IIT 153 (61) Control 151 (65)	IIT 80–110 Control 180–200	IIT 103 (22) Control 198 (29)
Dong 2009 (China) [27]	1	27	Septic shock 100	44	0	IIT157 (45) Control 159 (39.6)	IIT 74–110 Control 112–150	IIT 108 (27) Control 148 (34)
NICE-SUGAR 2009 (Australia and New Zealand—Canada) [17]	42	1299	Severe sepsis Septic shock	60.2	20.1	IIT 146 (52.3) Control 144 (49.1)	IIT 81–108 Control 180 or less	IIT 115 (18) Control 144 (23)
Arabi 2008 (Saudi Arabia) [24]	1	122	Severe sepsis Septic shock	52.4	40.0	IIT 195 (75) Control 211 (81)	IIT 80–110 Control 180–200	IIT 115 (18) Control 171 (34)
Zhang 2008 (China) [26]	1	22	Sepsis	66.3	27.5	IIT 165 (55) Control 200 (100)	IIT 80–110 Control 130–150	IIT 119 (7.6) Control 141 (7.9)
Jin 2009# (China) [23]	14	356	Severe sepsis Septic shock	65.7	NA	IIT NA Control NA	IIT 80–110; 120–150 Control 180–200	IIT 80–110: 99 (31); 120–150: 133 (34) Control 189 (40)

IIT: intensive insulin therapy; NA: not available from article or author.
*Median (interquartile range).
#Abstract only.

Cochran's Q-test ($P < 0.10$ for statistical significance) and the I^2 statistic (I^2 value >50% for substantial heterogeneity). To eliminate the heterogeneity, we conducted either a sensitivity analysis or subgroup analysis.

3. Results

3.1. Literature Search. Our predefined search strategy yielded a total of 1,842 abstracts (Figure 1). After reviewing the titles and abstracts, we excluded 1,816 studies because they

Table 2: Risk of bias in included randomized controlled trials.

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Cappi 2012 [14]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
COIITSS 2010 (France) [16]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	High risk[a]
Savioli 2009 [12]	Low risk	Unclear	High risk	Low risk	Low risk	Low risk	Low risk
Iapichino 2008 [11]	Low risk	Unclear	High risk	High risk	Low risk	Unclear	Low risk
Brunkhorst 2008 [10]	Low risk	Low risk	High risk	High risk	Low risk	Low risk	High risk[a,b]
Ellger 2008 [25]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Yu 2005 [13]	Unclear	Unclear	High risk	Low risk	Low risk	High risk	Low risk
Dong 2009 [27]	Unclear	Unclear	High risk	High risk	Low risk	Unclear	Low risk
NICE-SUGAR 2009 (Australia and New Zealand—Canada) [17]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	High risk[c]
Arabi 2008 [24]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	High risk[d]
Zhang 2008 [26]	Unclear	Unclear	High risk	Low risk	Low risk	Unclear	Low risk
Jin 2009* [23]	NA	NA	NA	NA	NA	NA	NA

*Abstract only.
NA: not available from article or author.
[a]The two treatment groups differ in the use of medications other than insulin.
[b]Intensive insulin therapy was terminated early because of increasing hypoglycemic events.
[c]Inclusion used subjective criteria.
[d]It had a different baseline.

were nonrandomized controlled trials, not specific to septic patients or pertained to interventions other than intensive insulin therapy. We checked the full text of the remaining 26 articles. One trial that met our inclusion criteria was excluded because the data were presented in diagrams from which we were unable to abstract values [22]. No additional studies were found in the screened reference lists. Finally, 12 randomized controlled trials were included in our meta-analysis [10–14, 16, 17, 23–27]. One trial was only an abstract, despite contacting the authors to request a copy of the full article [23].

3.2. Study Characteristics. The 12 randomized controlled trials included 4,100 patients in all, of whom 2,094 were assigned to the intensive insulin group and 2,006 to the control group. The details of the included studies are listed in Table 1. For the intervention, all the trials used tight glycemic control (80–110 mg/dL) except for one trial used that used two glycemic subcategories: 80–110 mg/dL or 120–150 mg/dL [23]. Because our inclusion criterion was an intensive insulin therapy group targeting a glucose concentration of ≤150 mg/dL, we combined the data of the two glycemic subcategories for mortality analysis. The mean glucose concentration of included patients varied significantly among eligible trials, from 130 mg/dL to 216 mg/dL. The proportion of patients with septic shock ranged widely (32.7–100%). In 3 trials, the baseline parameters of the septic patients were undocumented in the intensive and the control groups [17, 23, 24]. Most of the included trials used a specific method of random sequence generation (Table 2). None of the trials met the "blinding of participants and personnel" bias criterion and thus were rated as high-risk. On the contrary, all studies were identified as low risk because they did not have "incomplete outcome data."

3.3. Primary Outcome: Mortality. The all-cause mortality was reported in the 12 randomized controlled trials [10–14, 16, 17,

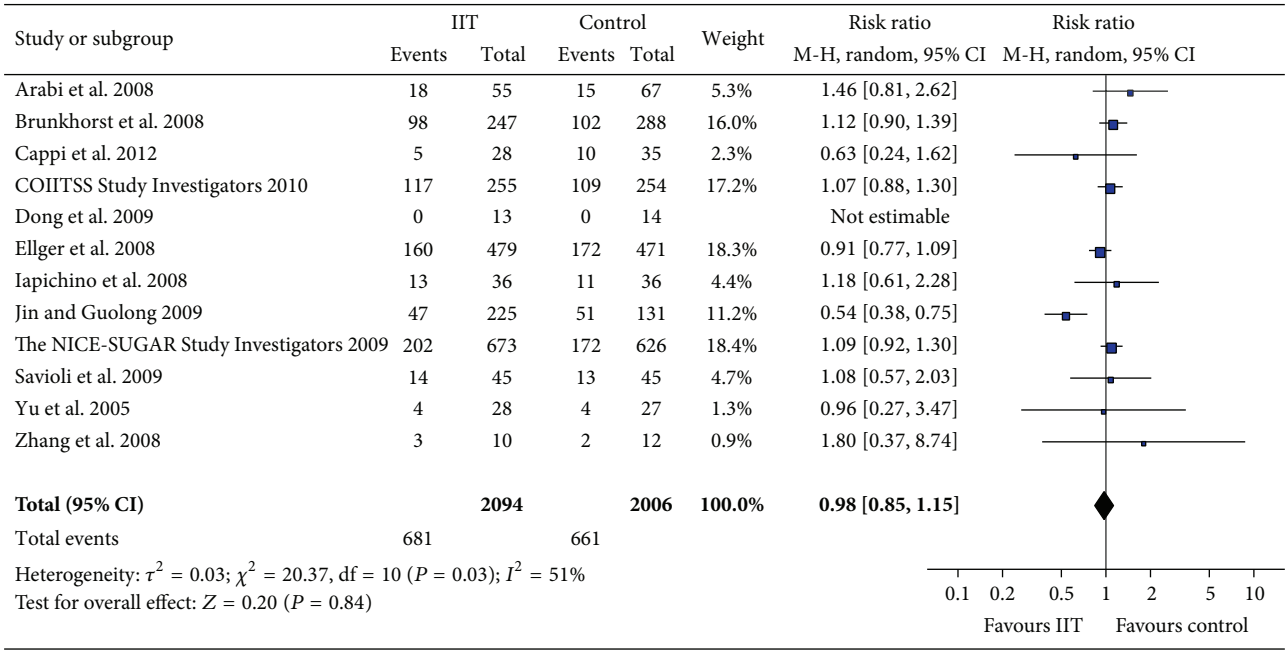

Figure 2: Forest plot with all-cause mortality showing no significant difference between IIT and control group (RR = 0.98, 95% CI [0.85, 1.15]). IIT: intensive insulin therapy. CI: confidence interval. M-H: Mantel-Haenszel.

23–27]. There were 681/2,094 (32.5%) deaths in the intensive insulin intervention group and 661/2,006 (33%) in the control group. Meta-analysis showed that the rate of death did not differ significantly between the two groups (RR = 0.98, 95% CI [0.85, 1.15], $P = 0.84$) (Figure 2).

In the subgroup analysis, there was no significant difference in 28-day mortality (RR = 0.66, 95% CI [0.40, 1.10], $P = 0.11$), 90-day mortality (RR = 1.10, 95% CI [0.97, 1.26], $P = 0.13$), ICU mortality (RR = 0.94, 95% CI [0.77, 1.14], $P = 0.52$), or hospital mortality (RR = 0.98, 95% CI [0.86, 1.11], $P = 0.71$) (Figure 3).

The statistical heterogeneity was substantial for all-cause mortality ($I^2 = 51\%$; $P = 0.03$) and for 28-day mortality ($I^2 = 74\%$; $P = 0.02$). Because we could not acquire the full text of the study by Jin and Guolong [23], we did not have sufficient information to evaluate its methodological quality. Therefore, we excluded this trial and the heterogeneity for all-cause mortality was resolved ($I^2 = 0\%$; $P = 0.51$). The heterogeneity was still significant for 28-day mortality. We noted that the Cappi trial had a wide confidence interval due to its small sample size [14]. Thus, we identified this trial as an outlier in the 28-day mortality. Even with this adjustment, the results of all-cause mortality (RR = 1.05, 95% CI [0.96, 1.14], $P = 0.33$) and 28-day mortality (RR = 0.95, 95% CI [0.71, 1.27], $P = 0.74$) did not change significantly.

3.4. Secondary Outcomes: Severity of Illness, Length of ICU Stay, and Hypoglycemic Events. The included trials used the SOFA (Sequential Organ Failure Assessment) score, APACHE II (Acute Physiology and Chronic Health Evaluation II) score, SAPS II (Simplified Acute Physiology Score II), and MODS (Multiple Organ Dysfunction Score) to evaluate severity of illness after intensive insulin therapy. Five trials reported SOFA score [10–13, 16], two studies reported APACHE II score, and only the MODS [23] and SAPS II [27] scores were reported in only one study each. The data about SOFA score could only be extracted from two trials that included 578 participants [10, 11]. The pooled estimate in the intensive insulin group was similar to that in the control group (SMD = 0.05, 95% CI [−0.12, 0.21], $P = 0.57$) with no statistical heterogeneity ($I^2 = 0\%$; $P = 1.00$) (Figure 4). Similarly, there was no significant difference in APACHE II score ($P = 0.46$) and SAPS II ($P = 1.00$). The MODS was lower in the intensive insulin group, but its methodological quality was low. This suggests that intensive insulin therapy does not reduce the severity of sepsis.

Five trials reported the length of ICU stay as an outcome [10, 11, 16, 23, 26]. It should be noted that in four of the trials the data were presented as the median (interquartile range) [10, 11, 16, 26], each with no statistically significant difference between the intensive insulin and control groups. The remaining trial with unclear methodological quality found that intensive insulin therapy resulted in a shorter ICU stay, but the result was dubious [23]. We did not merge the medians (interquartile range), because we could not determine whether they had a normal distribution [19].

For the occurrence of hypoglycemia, we extracted data from seven trials including 2,213 participants [10, 11, 13, 14, 16, 25, 27] (Figure 5). There were 196/1,093 (17.9%) hypoglycemic events in the intensive insulin group and 55/1,120 (4.9%) in the control group. The intensive insulin group had a higher rate of hypoglycemia than the control group (RR = 2.93,

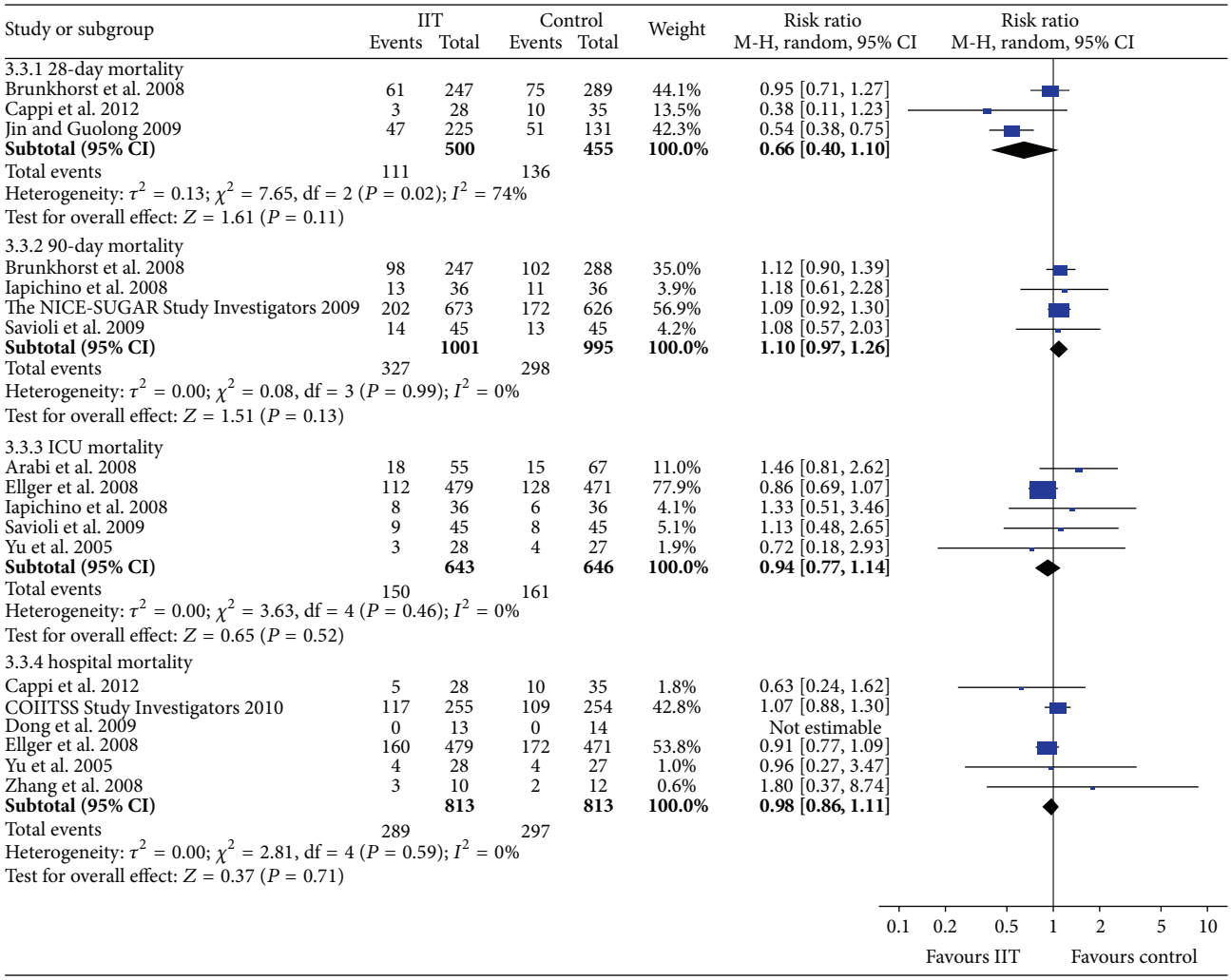

Figure 3: Meta-analysis showing no significant difference between IIT and control groups, with mortality being stratified into 28-day, 90-day, ICU, and hospital mortality. IIT: intensive insulin therapy. CI: confidence interval. M-H: Mantel-Haenszel.

95% CI [1.69, 5.06], $P = 0.0001$), and there was substantial heterogeneity across the trials ($I^2 = 61\%$, $P = 0.02$). We identified the Ellger et al. trial as an outlier [25], and its exclusion resolved the heterogeneity ($I^2 = 19\%$, $P = 0.29$) but did not change the pooled estimate (RR = 2.44, 95% CI [1.59, 3.72], $P < 0.0001$).

3.5. Sensitivity Analysis. Through performing several sensitivity analyses (the trials with "low risk" of "random sequence generation" and "allocation concealment", the trials with >500 patients, the trials with septic shock data, and studies with excluding unclear baseline characteristics) [17, 23, 24], the overall effect size was found to change minimally regardless of mortality or hypoglycemia (Table 3).

3.6. Publication Bias. We were unable to evaluate the publication bias, in part, due to the appearance of heterogeneity and also due to the small number of the included trials in each comparison.

4. Discussion

We conducted a meta-analysis of randomized controlled trials of intensive insulin therapy for septic patients, which showed no reduction of mortality overall nor in any of the subgroups (28-day and 90-day mortality; ICU and hospital mortality). Likewise, in terms of the severity of illness, the intensive insulin group did not show a statistically significant difference from the control. Though one trial found that the intensive insulin therapy group had a shorter ICU stay than the control group, its methodological quality was vague. Due to the data format, we could not merge the other studies with the length of ICU stay. Intensive insulin therapy, however, notably increased the episodes of hypoglycemia. We found substantial heterogeneity in the pooled analysis of mortality and hypoglycemia, but the results remained the same when the heterogeneity was removed using sensitivity analysis.

During the past 30 years, no effective new therapies appeared, despite that our understanding of the pathophysiologic features of sepsis has advanced [28]. A number of

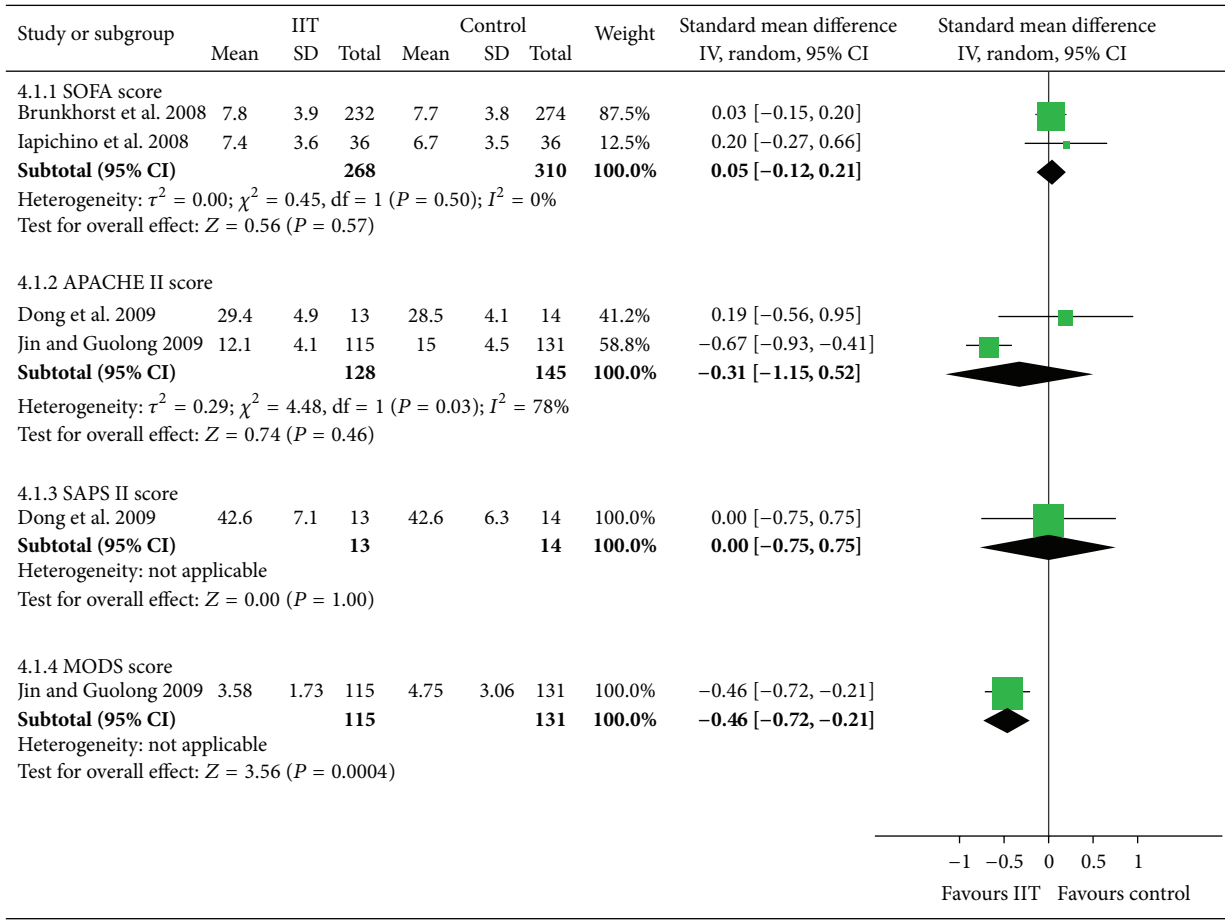

Figure 4: Forest plot with severity of illness showing no significant difference between IIT and control groups in SOFA, APACHE II, and SAPS II scores, with a reduction of MODS score with IIT. IIT: intensive insulin therapy. SD: standard deviation. CI: confidence interval. IV: inverse variance.

observational studies indicate that hyperglycemia is associated with a higher mortality rate, in particular, sepsis-induced hyperglycemia rather than mortality due to preexisting diabetes mellitus [6]. The landmark randomized controlled trial showed that intensive insulin therapy (targeting 80–110 mg/dL) reduced hospital mortality in septic patients [15], so clinicians enthusiastically received it as an effective therapy for these patients. Unfortunately, subsequent randomized controlled trials failed to confirm this beneficial effect.

Several prior reviews investigated the effect of intensive insulin therapy (IIT) for a general population of ICU patients [29–31]. Though we obtained similar results regarding the effect of IIT on mortality and risk of hypoglycemia, there are some differences in our findings compared with the prior reviews. The previous reviews concentrated on a general population of ICU patients. Solyemez Wiener et al. [29], Kansagara et al. [30], and Griesdale et al.'s [31] trials were grouped by type of ICU (medical ICU, surgical ICU, and mixed ICU). Kansagara et al.'s [30] trials were also grouped by type of patients (myocardial infarction and stroke); however, we focused on patients with sepsis. Even though septic patients are intermixed with other ICU patients, they possess distinct treatment and prognostic and clinical outcomes as compared with other ICU patients. Therefore, the effect of intensive insulin therapy for ICU patients is not necessarily congruent with the effect for septic patients. In addition, we classified the outcome of mortality into four subgroups (90-day, 28-day, hospital, and ICU mortality) and added the outcome of severity of illness and the length of ICU stay that were not evaluated in the prior reviews.

We included one specific study [25] that contained a database of two randomized controlled trials [15, 32], which used identical protocols and were implemented at the same center but one year apart. Thus, we treated these studies as a consecutive study. This study [25] showed that intensive insulin therapy reduced the ICU mortality in septic patients who stayed in the ICU for at least three days, but there was no statistically significant difference in septic patients who stayed for less than three days. They performed this subgroup analysis to specifically consider patients whose intensive care was limited or who were withdrawn from intensive care within 3 days of admission to the ICU. Another trial, the Brunkhorst et al. trial [10], showed no effect of intensive insulin on mortality in septic patients who stayed in the ICU at least three or five days. The remaining trials did not stratify by the length of stay in the ICU. Taking

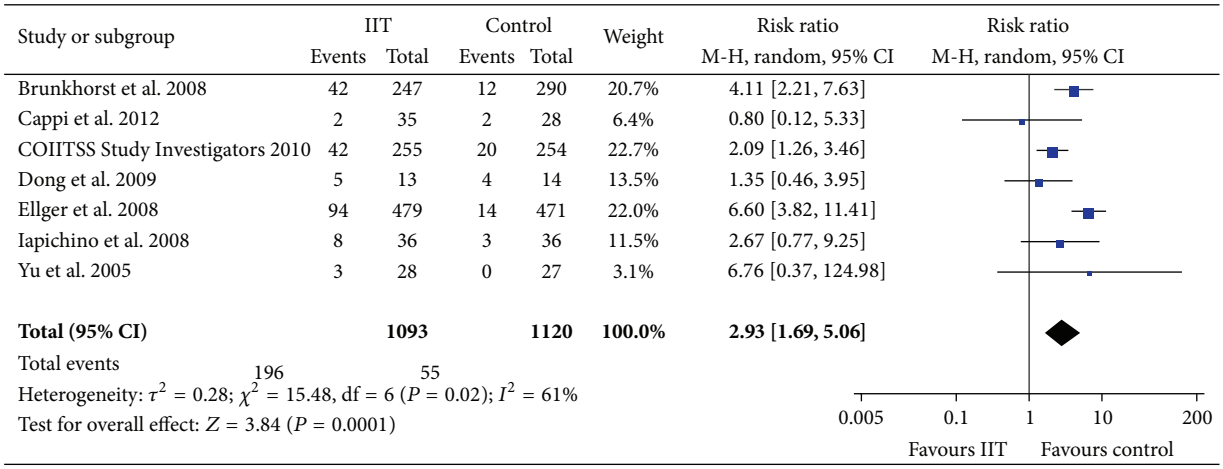

Figure 5: Forest plot showing that IIT increased the risk of hypoglycemia. IIT: intensive insulin therapy. CI: confidence interval. M-H: Mantel-Haenszel.

Table 3: Sensitivity analysis.

Outcome	Number of studies	Number of patients	RR (95% CI)	*P* value
Low risk of random sequence generation and allocation concealment				
Mortality	6	3478	1.04 (0.95–1.15)	0.40
Hypoglycemia	4	2059	3.27 (1.62–6.57)	0.0009
Trials containing >500 patients				
Mortality	4	3293	1.04 (0.94–1.14)	0.44
Hypoglycemia	3	1996	3.81 (1.89–7.69)	0.0002
Patients with similar baseline				
Mortality	9	1910	1.00 (0.89–1.13)	0.97
Hypoglycemia	7	2213	2.93 (1.69–5.06)	0.0001
Patients with septic shock				
Mortality	4	1533	1.03 (0.89–1.18)	0.69
Hypoglycemia	4	1535	2.97 (1.72–5.12)	<0.0001

RR: risk ratio; CI: confidence interval.

the authentic clinical practice environment into consideration, we pooled the overall ICU stay and found no significant difference.

Perhaps the failure to find a benefit with intensive insulin therapy can be attributed to several factors. It remains unclear whether hyperglycemia is a cause of increased mortality or is just a marker of an increased risk of death; it may even be a normal response [6]. Finfer's [33] latest review states that "Until quite recently stress hyperglycemia was seen as a normal and possibly beneficial physiological response to promote cellular glucose uptake" (pp: 1–6).

In agreement with previous studies, our meta-analysis demonstrated that intensive insulin therapy carries a markedly increased risk of hypoglycemia. Hypoglycemia has been reported to have an independent association with increased mortality in patients with sepsis [34, 35]. Considering the increased risk of death due to increased hypoglycemia and the findings that intensive insulin therapy does not reduce mortality, the use of IIT as a strategy to maintain normoglycemia remains unclear.

Septic patients are apt to suffer from a striking increase in blood glucose variability, which causes endothelial dysfunction, oxidative stress, and organ dysfunction [6, 9, 36, 37]. Though the mean blood glucose concentration is similar among trials, the degree of glucose variability may be quite different. Several observational studies have shown that blood glucose variability is independently associated with an increased mortality rate, even more than continuous hyperglycemia [9, 35]. The glycemic lability index, which is calculated from continuous glucose monitoring, reveals the inherent variability better than the standard deviation of the mean blood glucose value [35]. The included trials mainly used sampling at a predefined time, rather than monitoring 24-hour continuous glucose levels, resulting in insufficient data to determine the relationship between glucose variability and mortality rate.

There are several limitations to our meta-analysis. Septic patients who survive to hospital discharge still have a high risk of death in the following months and years, which has been suggested in many studies [38]. The NICE SUGAR trial found that intensive insulin therapy increased mortality compared with the control group at 90 days, but not at 28 days [17]. Furthermore, the VISEP study, which found that intensive insulin therapy did not reduce mortality in septic patients, was discontinued early due to an excess risk for hypoglycemia [10]. Trials included in our meta-analysis mainly contained data gathered before hospital discharge and follow-up was limited. Therefore, our results may not be appropriate for long-term prognosis.

The glucose target range of the most trials in this meta-analysis was 80–110 mg/dL. Perhaps, a result favoring intensive insulin therapy could have been found to occur if a higher concentration range was used in the intensive insulin group, in comparison with uncontrolled hyperglycemia. Restricted by the lack of adequate data, we were not able to stratify trials based on the type of ICU and the proportion of calories provided parenterally or to evaluate secondary outcome measures, such as severity of illness, length of ICU stay, and cost. Also, there is the possibility of publication bias in our review.

Unfortunately, none of the included randomized controlled trials used blinding of participants and personnel. Several trials had few mortality events, so we could not detect small differences between groups. Furthermore, the patient characteristics, blood glucose control, and coexisting interventions varied across the included studies.

5. Conclusion

Overall, the results of our meta-analysis suggest that intensive insulin therapy provides no benefit for septic patients. The highly sensitive search strategy, searching multiple databases and clinicaltrials.gov, searching publications written in any language, and performing subgroup analyses and sensitivity analyses, provides strength and rigor for our meta-analysis. Future reviews of septic patients may require individual patient data and more data that captures outcome measures.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, "Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care," *Critical Care Medicine*, vol. 29, no. 7, pp. 1303–1310, 2001.

[2] G. Kumar, N. Kumar, A. Taneja et al., "Nationwide trends of severe sepsis in the 21st century (2000–2007)," *Chest*, vol. 140, no. 5, pp. 1223–1231, 2011.

[3] J. H. Taylor and G. J. Beilman, "Hyperglycemia in the intensive care unit: no longer just a marker of illness severity," *Surgical Infections*, vol. 6, no. 2, pp. 233–245, 2005.

[4] S. E. Inzucchi, "Management of hyperglycemia in the hospital setting," *The New England Journal of Medicine*, vol. 355, no. 18, pp. 1903–1911, 2006.

[5] J. S. Krinsley, "Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients," *Mayo Clinic Proceedings*, vol. 78, no. 12, pp. 1471–1478, 2003.

[6] L. Leonidou, M. Michalaki, A. Leonardou et al., "Stress-induced hyperglycemia in patients with severe sepsis: a compromising factor for survival," *The American Journal of the Medical Sciences*, vol. 336, no. 6, pp. 467–471, 2008.

[7] K. M. Dungan, S. S. Braithwaite, and J.-C. Preiser, "Stress hyperglycaemia," *The Lancet*, vol. 373, no. 9677, pp. 1798–1807, 2009.

[8] K. C. McCowen, A. Malhotra, and B. R. Bistrian, "Stress-induced hyperglycemia," *Critical Care Clinics*, vol. 17, no. 1, pp. 107–124, 2001.

[9] R. M. Waeschle, O. Moerer, R. Hilgers, P. Herrmann, P. Neumann, and M. Quintel, "The impact of the severity of sepsis on the risk of hypoglycaemia and glycaemic variability," *Critical Care*, vol. 12, no. 5, article R129, 2008.

[10] F. M. Brunkhorst, C. Engel, F. Bloos et al., "Intensive insulin therapy and pentastarch resuscitation in severe sepsis," *The New England Journal of Medicine*, vol. 358, no. 2, pp. 125–139, 2008.

[11] G. Iapichino, M. Albicini, M. Umbrello et al., "Tight glycemic control does not affect asymmetric-dimethylarginine in septic patients," *Intensive Care Medicine*, vol. 34, no. 10, pp. 1843–1850, 2008.

[12] M. Savioli, M. Cugno, F. Polli et al., "Tight glycemic control may favor fibrinolysis in patients with sepsis," *Critical Care Medicine*, vol. 37, no. 2, pp. 424–431, 2009.

[13] W.-K. Yu, W.-Q. Li, X.-D. Wang et al., "Influence and mechanism of a tight control of blood glucose by intensive insulin therapy on human sepsis," *Zhonghua Wai Ke Za Zhi*, vol. 43, no. 1, pp. 29–32, 2005.

[14] S. B. Cappi, D. T. Noritomi, I. T. Velasco, R. Curi, T. C. A. Loureiro, and F. G. Soriano, "Dyslipidemia: a prospective controlled randomized trial of intensive glycemic control in sepsis," *Intensive Care Medicine*, vol. 38, no. 4, pp. 634–641, 2012.

[15] G. Van Den Berghe, P. Wouters, F. Weekers et al., "Intensive insulin therapy in critically ill patients," *The New England Journal of Medicine*, vol. 345, no. 19, pp. 1359–1367, 2001.

[16] COIITSS Study Investigators, "Corticosteroid treatment and intensive insulin therapy for septic shock in adults-a randomized controlled trial," *Journal of the American Medical Association*, vol. 303, no. 17, pp. 341–348, 2010.

[17] The NICE-SUGAR Study Investigators, "Intensive versus conventional glucose control in critically ill patients," *The New England Journal of Medicine*, vol. 360, no. 13, pp. 1283–1297, 2009.

[18] R. P. Dellinger, M. M. Levy, A. Rhodes et al., "Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012," *Critical Care Medicine*, vol. 41, no. 2, pp. 580–637, 2013.

[19] S. Green and J. PT Higgins, *Cochrane Handbook for Systematic Reviews of Interventions Version 5. 1. 0*, The Cochrane Collaboration, 2011.

[20] Review Manager (RevMan) [Computer program], Version 5. 2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012.

[21] J. A. Berlin, N. M. Laird, H. S. Sacks, and T. C. Chalmers, "A comparison of statistical methods for combining event rates from clinical trials," *Statistics in Medicine*, vol. 8, no. 2, pp. 141–151, 1989.

[22] F. Polli, M. Savioli, M. Cugno et al., "Effects of recombinant human activated protein C on the fibrinolytic system of patients undergoing conventional or tight glycemic control," *Minerva Anestesiologica*, vol. 75, no. 7-8, pp. 417–426, 2009.

[23] Y. Jin and C. Guolong, "A multicentre study on intensive insulin therapy of severe sepsis and septic shock patients in ICU-collaborative study group on IIT in Zhejiang province, china," *Intensive Care Medicine*, vol. 35, Supplement 1, article 86S, 2009.

[24] Y. M. Arabi, O. C. Dabbagh, H. M. Tamim et al., "Intensive versus conventional insulin therapy: a randomized controlled trial in medical and surgical critically ill patients," *Critical Care Medicine*, vol. 36, no. 12, pp. 3190–3197, 2008.

[25] B. Ellger, M. Westphal, H. D. Stubbe, I. Van Den Heuvel, H. Van Aken, and G. Van Den Berghe, "Glycemic control in sepsis and septic shock. Friend or foe?" *Anaesthesist*, vol. 57, no. 1, pp. 43–48, 2008.

[26] R.-L. Zhang, W. He, T. Li et al., "Evaluation of optimal goal of glucose control in critically ill patients," *Chinese Journal of Clinical Nutrition*, vol. 16, no. 4, pp. 204–208, 2008.

[27] S.-M. Dong, Y.-J. Qin, and Y.-F. Gao, "The influence of intensive insulin therapy on hemodynamics in patients with septic shock," *Chinese Critical Care Medicine*, vol. 21, no. 5, pp. 290–292, 2009 (Chinese).

[28] D. C. Angus, "The search for effective therapy for sepsis: back to the drawing board?" *JAMA—Journal of the American Medical Association*, vol. 306, no. 23, pp. 2614–2615, 2011.

[29] R. Soylemez Wiener, D. C. Wiener, and R. J. Larson, "Benefits and risks of tight glucose control in critically ill adults: a meta-analysis," *JAMA—Journal of the American Medical Association*, vol. 300, no. 8, pp. 933–944, 2008.

[30] D. Kansagara, R. Fu, M. Freeman, F. Wolf, and M. Helfand, "Intensive insulin therapy in hospitalized patients: a systematic review," *Annals of Internal Medicine*, vol. 154, no. 4, pp. 268–282, 2011.

[31] D. E. Griesdale, R. J. de Souza, R. M. van Dam et al., "Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data," *Canadian Medical Association Journal*, vol. 180, no. 8, pp. 821–827, 2009.

[32] G. Van Den Berghe, A. Wilmer, G. Hermans et al., "Intensive insulin therapy in the medical ICU," *The New England Journal of Medicine*, vol. 354, no. 5, pp. 449–461, 2006.

[33] S. Finfer, "Clinical controversies in the management of critically ill patients with severe sepsis-Resuscitation fluids and glucose control," *Virulence*, vol. 4, no. 8, pp. 1–6, 2013.

[34] S. Park, D. G. Kim, G. Y. Suh et al., "Mild hypoglycemia is independently associated with increased risk of mortality in patients with sepsis: a 3-year retrospective observational study," *Critical Care*, vol. 16, no. 5, article R189, 2012.

[35] N. A. Ali, J. M. O'Brien, K. Dungan et al., "Glucose variability and mortality in patients with sepsis," *Critical Care Medicine*, vol. 36, no. 8, pp. 2316–2321, 2008.

[36] H. Hirasawa, S. Oda, and M. Nakamura, "Blood glucose control in patients with severe sepsis and septic shock," *World Journal of Gastroenterology*, vol. 15, no. 33, pp. 4132–4136, 2009.

[37] L. Monnier, E. Mas, C. Ginet et al., "Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes," *Journal of the American Medical Association*, vol. 295, no. 14, pp. 1681–1687, 2006.

[38] D. C. Angus and T. van der Poll, "Severe sepsis and septic shock," *The New England Journal of Medicine*, vol. 369, no. 21, pp. 840–851, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 598654, 8 pages
http://dx.doi.org/10.1155/2014/598654

Review Article

Myeloid-Derived Suppressor Cells in Sepsis

Dengming Lai,[1,2] Chaojin Qin,[1,2] and Qiang Shu[1,2]

[1] *Department of Thoracic & Cardiovascular Surgery, Children's Hospital, Medical College, Zhejiang University, Hangzhou 310003, China*
[2] *Key Laboratory of Reproductive Genetics (Zhejiang University), Ministry of Education, Hangzhou 310003, China*

Correspondence should be addressed to Qiang Shu; shuqiang@zju.edu.cn

Received 6 March 2014; Accepted 3 May 2014; Published 3 June 2014

Academic Editor: Baoli Cheng

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Sepsis is a systemic, deleterious host response to widespread infection. Patients with sepsis will have documented or suspected infection which can progress to a state of septic shock or acute organ dysfunction. Since sepsis is responsible for nearly 3 million cases per year in China and severe sepsis is a common, expensive fatal condition in America, developing new therapies becomes a significant and worthwhile challenge. Clinical research has shown that sepsis-associated immunosuppression plays a central role in patient mortality, and targeted immune-enhancing therapy may be an effective treatment approach in these patients. As part of the inflammatory response during sepsis, there are elevations in the number of myeloid-derived suppressor cells (MDSCs). MDSCs are a heterogeneous population of immature myeloid cells that possess immunosuppressive activities via suppressing T-cell proliferation and activation. The role of MDSCs in sepsis remains uncertain. Some believe activated MDSCs are beneficial to the sepsis host by increasing innate immune responses and antimicrobial activities, while others think expansion of MDSCs leads to adaptive immune suppression and secondary infection. Herein, we discuss the complex role of MDSCs in immune regulation during sepsis, as well as the potential to target these cells for therapeutic benefit.

1. Introduction

Over the last decade, during the emergence of the "host theory," it was first assumed that the clinical features of sepsis were the result of an uncontrolled inflammatory response and an increase in inflammatory cytokines, commonly referred to as a "cytokine storm" [1]. Patients with sepsis may present with hypothermia, shock, elevated heart rate, altered mental status, tachycardia, an elevated white-cell count, and acute organ dysfunction [2, 3]. Recently, researchers have advanced this theory and suggested that infection triggers a much more complex, variable, and prolonged host response in which both proinflammatory and anti-inflammatory cytokines grow rapidly in number. Additionally, some patients with sepsis rapidly produce both categories of cytokines, whereas others have either a predominance of anti-inflammatory cytokines or globally depressed cytokine production [4]. Many investigative agents have been examined in an effort to downregulate cytokine release, which led to the survival of the majority of patients in the early period of this syndrome. Unfortunately, the patients who survive are at risk for developing nosocomial infections with organisms not typically pathogenic in immunocompetent hosts and can have reactivation of latent viruses [5, 6]. The failure of several clinical trials in sepsis has led researchers to be firmly convinced that future research needs to take a new direction [7–9]. Experts have delineated reasons for the failures of new investigative drugs and have presented some advice in the design and conduct of sepsis trials. For example, previous clinical drug studies tested drugs in young and healthy animal models, but patients frequently present with coexisting illness. The outcomes in patients are poorer than those in animals, so animal models of aging and preexisting disease are needed. Torgersen and colleagues have highlighted key immunological defects that impair host immunity including impairment of splenocyte function and depletion of immune effector cells [10]. Additionally, their clinical studies have found that ICU patients whose deaths are sepsis-related have biochemical, flow cytometric, and immunohistochemical findings consistent with

immunosuppression [11]. Therefore, we must pay close attention to the immunosuppression component of sepsis.

MDSCs possess the ability to suppress antigen-specific $CD8^+$ and $CD4^+$ T-cell activation, and several studies have found that MDSCs dramatically increase with sepsis [12]. The role of MDSCs in sepsis is not fully understood. It has been proposed that the overall role of MDSCs involves much more than simply being an immunosuppressive population. Rather, MDSCs expansion is a common response in all inflammatory processes and the expansion of MDSCs population may be protective for the host by increasing immune surveillance and innate immune responses [13]. Exploring the relationship between sepsis and MDSCs will provide insight into how these cells function and guide the development of future treatments for sepsis in clinical trials.

2. Sepsis Etiology: A Central Role of Host Immunity

Sepsis is one of the oldest and most elusive syndromes in medical history. In China, the number of patients with sepsis may exceed 3,000,000 per year with the true incidence being presumably higher, and our group reported an 8.68% occurrence rate of severe sepsis in surgical ICUs in China, with a hospital mortality rate of 48.7% [14]. Studies from the United States show a similar rate of sepsis in the general population [15]. Although many treatments have been applied and many of the treatment successes are owed to antibiotic therapy, the mortality of sepsis still remains at about 20% to 30%. High mortality rates have drawn attention to the incidence of sepsis within populations. The incidence of sepsis depends on both the characteristics of the invasive pathogen and the state of the host immune system. Epidemiologic studies have shown that pneumonia is the most common etiology, followed by intra-abdominal and urinary tract infections [15, 16]. Typically, blood cultures are positive in only one third of cases [15, 17, 18]. *Staphylococcus aureus* and *Streptococcus pneumonia* are the two most common gram-positive isolates, whereas *Escherichia coli*, *Klebsiella* species, and *Pseudomonas aeruginosa* predominate among gram-negative isolates, with a greater total incidence of gram-positive infections [17, 19]. In a recent study, gram-negative bacteria were isolated in 62% of patients who had positive cultures, 47% with gram-positive bacteria and 19% with fungi [20].

When pathogens invade the host, they will activate immune cells through an interaction with pattern-recognition receptors. These receptors recognize structures that are conserved among microbial species, which leads to the upregulation of inflammatory gene transcription and the initiation of innate immunity. Phagocytes like monocytes, macrophages, neutrophil granulocytes, and MDSCs can migrate to the infectious tissues and secrete anti-inflammatory cytokines. They phagocytize the pathogens, promote tissue repair, induce regulatory T-cells, and reduce inflammation. When sepsis initiates, the abilities of immune effector cells are strongly impaired, along with antigen-specific primary antibody production [21]. Some patients may become infected with a pathogen due to a weakened immune system. Researchers have shown that patients who survive early sepsis, but remain dependent on intensive care, have immunosuppression, which is evidenced by a reduced expression of HLA-DR on myeloid cells [11]. These patients have been found to have ongoing infectious foci or reactivation of latent viral infections, despite antimicrobial therapy [6, 10]. Other scholars have found that patients who died of sepsis in the ICU encountered strong functional impairments of splenocytes and the lungs also had increased expression of MDSCs phenotypes from sepsis versus control tissue (47.9% versus 15.7%) [11].

3. The Derivation and Subsets of MDSCs

In the early 1900s, these cells gained increasing appreciation in extramedullary haematopoiesis (EMH) and neutrophilia in tumors, which were later shown to possess abnormal myeloid-cell differentiation. Originally, these abnormal myeloid cells were described as myeloid progenitor cells, which could inhibit lymphocyte numbers and cytotoxic T lymphocyte (CTL) activity [22]. In 1987, hematopoiesis and suppressor bone marrow cells were first observed in patients with Lewis lung carcinoma [23]. In 1996, these cells became known as myeloid-derived suppressor cells. At present, it was clear that these cells lacked membrane markers which express on the surface of mature T-cells, B-cells, and natural killer (NK) cells, as well as macrophages [24]. For several decades, the understanding of MDSCs ranged from "the abnormal myeloid cell" to "immature myeloid cells" to "myeloid-derived suppressor cells." From recent studies, we now know that MDSCs are a heterogeneous population of cells that have an immature state and a remarkable ability to suppress T-cell responses [12]. They are an intrinsic part of the myeloid-cell lineage comprising myeloid-cell progenitors and precursors of myeloid cells. The functional importance of MDSCs in the immune system has received attention over the last decade, and recent spotlights can be attributed to MDSCs's role in the negative regulation of immune responses during cancer, other chronic diseases, and sepsis [13].

Relevant in vivo and in vitro studies have shown that approximately 1–5% of MDSCs form myeloid-cell colonies, and about one-third of this population have the ability to differentiate into mature macrophages and DCs in the presence of appropriate cytokines [25, 26]. In mice, MDSCs are characterized by the coexpression of the myeloid lineage differentiation antigens, Gr-1 and CD11b [27]. Normal murine bone marrow contains 20–30% of cells with this phenotype, but only a small proportion (2–4%) is present in the spleen cells. In sepsis, approximately 40% of the splenocyte population, approximately 90% of cells in bone marrow, and 3–5% cells in peripheral lymph nodes coexpress Gr-1 and CD11b [13]. Conversely, MDSCs in humans do not express this phenotype, rather the cells are most commonly defined as $CD14^-CD11b^+$ cells. Human MDSCs may express the common myeloid marker CD15 or CD33 but lack the expression of markers of mature myeloid and lymphoid cells and the MHC-class-II molecule HLA-DR [28–30]. The cells

described as MDSCs in human studies comprise only 0 to 0.5% of peripheral blood mononuclear cells.

Recently, many studies indicate that MDSCs can be delineated into two types: granulocytic MDSCs and monocytic MDSCs. Granulocytic-MDSCs have a $CD11b^+Ly6G^+Ly6C^{low}$ phenotype, whereas monocytic MDSCs are $CD11b^+Ly6G^-Ly6C^{high}$ [31]. These two different phenotypes possess different functions in the pathophysiology of sepsis. Firstly, only monocytic MDSCs have the ability to differentiate into mature DCs and macrophages in vitro. Furthermore, the granulocytic subset of MDSCs was found to express high levels of reactive oxygen species (ROS) and low levels of nitric oxide (NO), whereas the monocytic subset expressed the opposite pattern. Both subsets expressed arginase 1 (Arg-1) [31]. The abilities of the two cell types to suppress T-cell activity are also different. Monocytic MDSCs produced NO and strongly inhibited T-cell proliferation, while granulocytic-MDSCs produced low levels of NO and did not inhibit T-cell proliferation [32]. The specific difference between the two MDSCs subsets remains to be elucidated.

4. The Activation and Mechanisms of MDSCs

In healthy individuals, immature myeloid cells (IMCs) are generated in bone marrow and quickly differentiate into mature granulocytes, macrophages, or dendritic cells (DCs). In septic conditions, inflammatory factors such as IL-6, IL-10, IL-12, G-CSF, dsRNA, IFN-γ, VEGF, and GM-CSF are elevated, which prevents IMCs from differentiating into mature myeloid cells [33] (Figure 1). In sepsis, MDSC expansion is regulated by many factors, and these factors trigger several different signaling pathways. GM-CSF and IFN-γ have the potential to induce toll-like receptor (TLR) mediated myeloid differentiation primary response gene 88 (MyD88) signaling. Granulocyte-colony stimulating factor (G-CSF) and its receptor initiate the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway [34–36] (Figure 1). These factors not only improve the accumulation of MDSCs, but also initiate their activation.

The most important function of MDSCs is to inhibit immune response via suppressing T-cell proliferation and activation [37]. It has been shown that MDSCs mediate their effect on T lymphocytes in cancer through direct contact and/or through a combination of multiple major mediators such as inducible nitric oxide synthase (iNOS), arginase-1 (Arg1), reactive oxygen species (ROS), transforming growth factor-β (TGF-β), IL-10, regulatory T-cells (Treg), and macrophages [38]. The following is a summary of the mechanisms of action of these mediators.

Arg1 and iNOS are expressed highly in monocytic-MDSCs and utilize L-arginine to produce urea and NO, respectively. Monocytic-MDSCs inhibit T-cell responses through the depletion of L-arginine via the two enzymes. The activation of either of these enzymes inhibits T-cell proliferation by interfering with the transduction of intracellular signals and by inducing T-cell apoptosis [39]. In vitro, iNOS inhibitors (L-NMMA) alone and in combination with Arg1 inhibitors block inhibition of T-cells by MDSCs. Similarly, phosphodiesterase-5 inhibitors delay tumor progression by decreasing Arg1 and iNOS expression and by regulating the suppressive machinery of MDSCs [40]. ROS production has been shown to be a major regulator of the suppressive activity of the granulocytic-MDSCs in both murine models and human cancers [41, 42]. In three different studies, inhibition of ROS production was associated with complete elimination of the suppressive activities of the MDSCs that were isolated from mice and human cancers [28, 41]. In addition, the combination of NO and ROS was associated with the production of peroxynitrite. Peroxynitrite causes protein dysfunctions in target cells and nitration of the T-cell receptor, which in turn, leads to suppression of $CD8^+$ T-cell responses [43].

TGF-β is an immunosuppressive cytokine that has been firmly associated with MDSCs function and with the regulation of tumor induction and expansion [44]. In a study of squamous cell carcinoma of the head and neck, the $CD14^+HLA\text{-}DR^-$ MDSCs subset was noted to be the most predominant and produced higher levels of TGF-β compared with other MDSCs subsets [45]. TGF-β antibody partially restored T-cell proliferation and IFN-γ production. This evidence indicates that MDSCs are likely to be a major source for TGF-β production and their immunosuppressive effect is mediated by factors including TGF-β [45]. In a separate study, Lu et al. reported that TGF-β production promoted tumor cell invasion and metastasis [46]. Yang et al. found that the deletion of TGF-β receptor gene type II resulted in the infiltration of MDSCs in breast cancer and the production of large quantities of TGF-β that led to the promotion of tumor invasion and metastasis [47].

MDSCs may also inhibit T-cell proliferation indirectly by promoting the development of inducible $CD4^+CD25^+Foxp3^+$ Treg. The development of Treg is linked to IL-10 plus TGF-β production [48]. Delano and coworkers have shown that MDSCs can still express several cytokines and chemokines, such as interleukin 10, TNF-α, RANTES, and MIP-1β [34]. High levels of CD80 expression by MDSCs were observed in many cancer tissues. Genetic knockout of CD80 expression in MDSCs alleviated the suppression of antigen-specific immune responses. CD80 itself suppressed antigen-specific immunity via Treg [49]. Another study analyzed the interaction of MDSCs with macrophages in a mouse cancer model and showed that, through IL-10 secretion, MDSCs also induced a type-2 polarization of macrophages which is characterized by a decrease of IL-12 secretion and that promotes tumor growth [50].

5. The Role of MDSCs in Sepsis

Although most of the current information about the function of MDSCs in immune responses has come from studies in the cancer field, there are increasingly more studies that directly investigate the roles of MDSCs in sepsis. Some researchers believe MDSCs are deleterious to the sepsis host. Delano et al. first demonstrated that MDSCs contribute to sepsis-induced T-cell suppression and preferential Th2 polarization [34]. They reported that $GR\text{-}1^+CD11b^+$ MDSCs population was

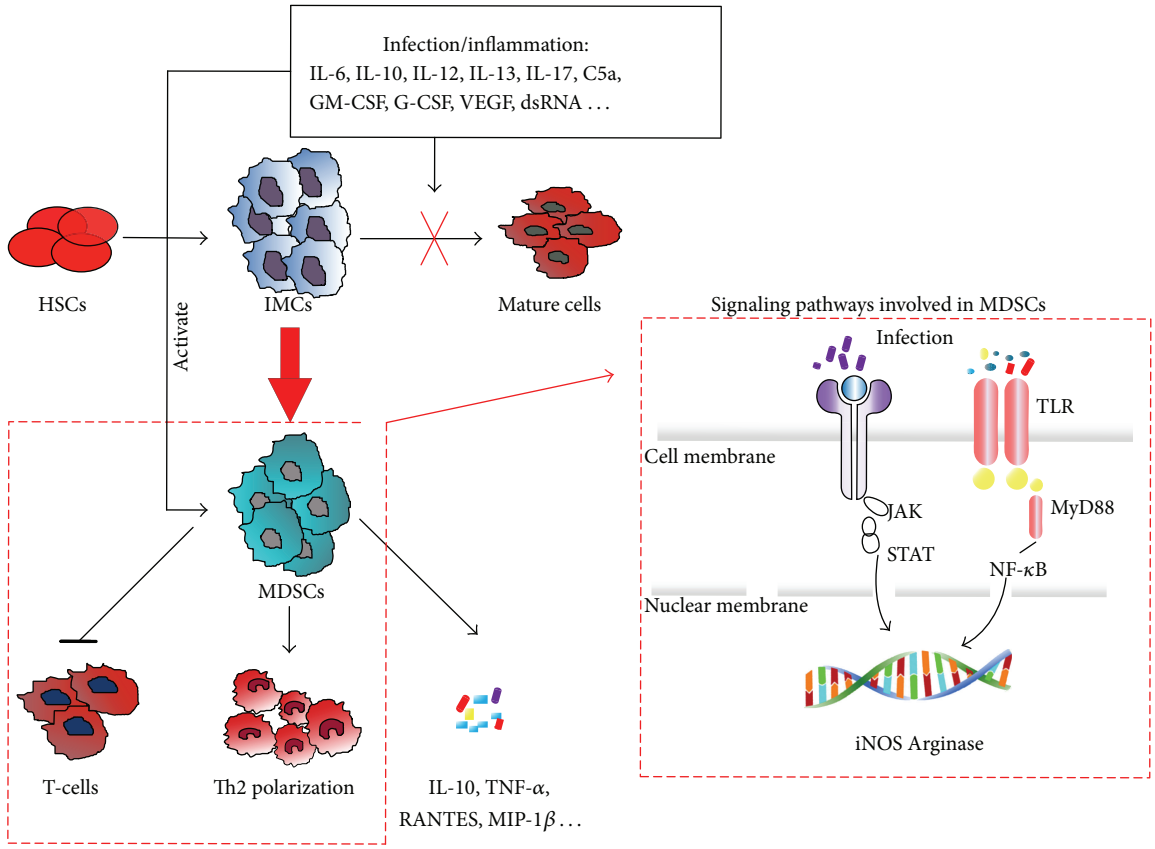

Figure 1: The origin and signaling pathways involved in MDSCs in sepsis. Haematopoietic stem cells (HSCs) differentiate into immature myeloid cells (IMCs) and then quickly differentiate into mature granulocytes, macrophages, or dendritic cells (DCs). In septic conditions, inflammatory factors such as IL-6, IL-10, IL-12, G-CSF, ds RNA, VEGF, and GM-CSF are elevated. They prevent IMCs from differentiating into mature myeloid cells. MDSCs expansion and activation is regulated by many signaling pathways, such as a toll-like receptor (TLR) mediated myeloid differentiation primary response gene 88 (MyD88) signaling and the granulocyte-colony stimulating factor receptor (G-CSFR) mediated the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway. They contribute to the increased production of reactive oxygen species (ROS), inducible nitric oxide synthase (iNOS), and arginase 1 (ARG1). MDSCs in sepsis can reduce the capacity of septic monocytes, macrophages, and neutrophils to respond to bacterial toxins, inhibit the activation of T-cells, and promote Th2 polarization. In addition, MDSCs can secrete several cytokines and chemokines, such as interleukin 10, TNF-α, RANTES, and MIP-1β.

dramatically increased in the spleen, lymph nodes, and bone marrow during polymicrobial sepsis. Phenotypically, these cells were heterogeneous, immature, and predominantly myeloid progenitors that express IL-10 and several other cytokines and chemokines. Splenic GR-1$^+$ cells effectively decreased antigen-specific CD8$^+$ T-cell IFN-γ production but only modestly suppressed antigen-specific and nonspecific CD4$^+$ T-cell proliferation. GR-1$^+$ cell depletion in vivo prevented both the sepsis-induced augmentation of Th2 cell-dependent and depression of Th1 cell-dependent antibody production. They further concluded that signaling through MyD88 is required for complete MDSCs expansion [34]. Martire-Greco and colleagues demonstrated that decreasing the number of viable MDSCs via all-trans retinoic acid improves immunocompetence in a murine model of lipopolysaccharide-induced immunosuppression [51].

Nevertheless, several reports demonstrated that the expansion of activated MDSCs during sepsis may actually be protective. Noel et al. found that when MDSCs were depleted by gemcitabine treatment, burned mice were highly susceptible to secondary pseudomonas aeruginosa infections [52]. Supporting these data, Delano and colleagues showed that lethality to pseudomonas pneumonia was increased early on after the induction of sepsis, but not later, coinciding with repopulation (activation) of Gr1$^+$/CD11b$^+$ cells [53]. Sander and colleagues found that adoptive transfer of MDSCs efficiently protected gp130-deficient mice from sepsis-associated mortality [54]. In addition, hepatic acute-phase proteins control innate immune responses during infection by promoting MDSCs function [54]. In order to be protective against sepsis, MDSCs require prolonged activation [55]. Derive et al. induced septic shock by caecal ligation and puncture in adult mice. They found that polymicrobial sepsis induced a progressive accumulation of MDSCs, mainly CD11b$^+$Ly6G$^+$Ly6C$^-$ granulocytic-MDSCs, in spleens. MDSCs harvested at day 10 after the onset of infection were highly responsive to LPS in terms of cytokines secretion, NF-κB activation, ROS production, and arginase 1 activity. MDSCs collected at day 3 responded poorly to the stimulus. By contrast, both day 3 and day 10 MDSCs were

able to inhibit T-cell proliferation. Furthermore, adoptive transfer of day 10 MDSCs to septic mice attenuated peritoneal cytokine production, increased bacterial clearance, and dramatically improved survival rates [55]. In the same animal model, another research group had similar findings in terms of the duration needed for MDSCs to acquire their protective effect. Adoptive transfer of early (day 3) MDSCs from septic mice into naive mice after caecal ligation and puncture increased proinflammatory cytokine production and early mortality. Conversely, transfer of late (day 12) MDSCs from septic mice had the opposite effect. Early and late MDSCs studied ex vivo also differed in their inflammatory phenotypes. Early MDSCs expressed nitric oxide and proinflammatory cytokines, whereas late MDSCs expressed arginase activity and anti-inflammatory IL-10. They concluded that as the septic inflammatory process progresses, the heterogeneous MDSCs shift from being proinflammatory to anti-inflammatory [56]. Taken together, the role of MDSCs in sepsis is still controversial.

6. Therapy Targeting MDSCs in Sepsis

The treatment of sepsis is challenging and complex, thus, sepsis-related mortality remains high. The principles of the initial management approach are to provide cardiorespiratory resuscitation and to mitigate the immediate threats of uncontrolled infection. Many therapies could be applied to a patient with sepsis, including the use of intravenous fluids and vasopressors, oxygen therapy and mechanical ventilation, and organ function support and other intensive life support. New research has shown that most patients admitted to intensive care units for treatment of sepsis had unresolved septic foci postmortem, suggesting that patients were unable to eradicate invading etiologic pathogens and were highly susceptible to nosocomial organisms, or both [11]. Several clinical trials of drugs that boosted immunity suggested that therapies that improve host immunity might increase survival because immunosuppression has a central role in sepsis-related deaths. Sepsis can be thought of as a battle between the invading microbes and the host's immune response, with each side seeking success. In addition to studying current management approaches, immunotherapies for sepsis must also be examined, especially those focused on the regulation of MDSCs.

Many efforts have been made to target MDSCs in cancer. Both sepsis and cancer share many immunological defects, therefore, some investigators postulate that the recent success of several immunomodulatory drugs in cancer may provide potential immunostimulatory therapies for sepsis [57]. To mitigate the immunosuppressive activities of MDSCs, one of the effective strategies is to differentiate MDSCs into mature cells. All-trans retinoic acid (ATRA) at therapeutic levels has been shown to reduce MDSCs and induce MDSCs differentiation into dendritic cells and macrophages in cancer patients and mice [58]. Nefedova et al. suggested that an upregulation of glutathione synthesis and a reduction in ROS levels were the main mechanisms involved in ATRA-mediated MDSCs differentiation [59]. In a recent randomized clinical trial involving patients with small cell lung cancer ($n = 41$), systemic depletion of MDSCs using ATRA in combination with cancer vaccination led to a statistically significant improvement in the immune response to p53 vaccination in comparison with the vaccination-only group (20%, $P = 0.02$) [60]. In another clinical study, Mirza et al. reported that ATRA administration in patients with metastatic renal cell carcinoma markedly reduced the number of MDSCs ($Lin^{-}HLA\text{-}DR^{-}CD33^{+}$). ATRA also improved the myeloid/dendritic cell ratio and the ability of patients' mononuclear cells to stimulate allogeneic T-cells, increased the dendritic cells/MDSCs ratio in the peripheral blood, and improved the T-cell immune response [61]. In addition, Martire-Greco and colleagues demonstrate that ATRA improves immunocompetence in a murine model of lipopolysaccharide-induced immunosuppression by decreasing the number of viable MDSCs [51].

Inhibition of the signal pathways that regulate the production of the suppressive factors of MDSCs is another promising approach. Sildenafil, a phosphodiesterase-5 inhibitor, reduced arginase 1 and nitric oxide synthase-2 expression in a mouse tumor model. It enhanced intratumoral T-cell infiltration and activation, reduced tumor outgrowth, and improved the antitumor efficacy of adoptive T-cell therapy. Furthermore, sildenafil restored T-cell proliferation of peripheral blood mononuclear cells from multiple myeloma and head and neck cancer patients in vitro [62]. It is not clear whether this favorable effect will be observed clinically in cancer patients. Nitroaspirin is a classic aspirin molecule covalently linked to an NO donor group and is able to release NO. Nitroaspirin does not possess direct antitumor activity. However, by interfering with the inhibitory enzymatic activities of MDSCs, orally administered nitroaspirin normalized the immune status of tumor-bearing hosts, increased the number and function of tumor-antigen-specific T lymphocytes, and enhanced the preventive and therapeutic effectiveness of the antitumor immunity elicited by cancer vaccination [63]. Recently, cimetidine, a histamine type-2 receptor antagonist, was shown to reduce NO production and arginase I expression of MDSCs. MDSCs were prone to apoptosis due to cimetidine treatment resulting in a reversal of MDSCs-mediated T-cell suppression and improved IFN-γ production [64].

Several studies in mice tested a "cell-based therapy" approach using MDSCs or MDSCs-like cells in the treatment of diabetes [65], immunological hepatic injury (IMH) [66], and graft-versus-host disease (GVHD) [39]. Yin et al. found that administration of MDSCs can prolong the survival of diabetic mice transplanted with allogeneic pancreatic cells [65]. Highfill et al. reported that the adoptive transfer of MDSCs significantly improved survival in a model of graft-versus-host disease [67]. In both of these studies, MDSCs were generated ex vivo by culturing BM cells with a combination of colony stimulating factors and interleukins.

Presently, there is no clinical trial targeting MDSCs in sepsis. Some experts believe that the absence of activated MDSCs in patients with sepsis might be the reason why these patients succumb to nosocomial infection [68]. Therefore, pharmacologic agents known to regulate the production of

the suppressive factors of MDSCs or promote the expansion of MDSCs such as growth factors, chemokines, and sildenafil require further study in an effort to improve patient outcomes in sepsis.

7. Conclusions

In recent years, it has become clear that most septic patients do not die from an overwhelming proinflammatory immune response, but rather succumb to their illness in an immunosuppressive state. Although control of the infection and supportive therapies will remain the mainstay for treatment in the early phase of sepsis, there is a developing trend towards immunostimulation for patients in immunosuppressive states. MDSCs are a heterogeneous population of cells that have an immature state and the ability to suppress T-cell responses. The roles of MDSCs in sepsis remain uncertain. Some believe MDSCs are beneficial to the septic host and that the absence of activated MDSCs is the reason why some patients subsequently succumb to nosocomial infection. Others believe that MDSCs are deleterious and the expansion of MDSCs in the host following sepsis leads to global adaptive immune suppression and secondary infection. The roles and mechanisms of MDSCs warrant further exploration and MDSCs could serve as a viable target for sepsis treatment.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (81072416; 81272139; 81130036) and the National Science and Technology Support Program (2012BAI04B05).

References

[1] R. S. Hotchkiss and I. E. Karl, "The pathophysiology and treatment of sepsis," *The New England Journal of Medicine*, vol. 348, no. 2, pp. 138–150, 2003.

[2] A. P. Wheeler and G. R. Bernard, "Treating patients with severe sepsis," *The New England Journal of Medicine*, vol. 340, no. 3, pp. 207–214, 1999.

[3] D. Rittirsch, M. A. Flierl, and P. A. Ward, "Harmful molecular mechanisms in sepsis," *Nature Reviews Immunology*, vol. 8, no. 10, pp. 776–787, 2008.

[4] R. S. Hotchkiss, G. Monneret, and D. Payen, "Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach," *The Lancet Infectious Diseases*, vol. 13, no. 3, pp. 260–268, 2013.

[5] K. E. Kollef, G. E. Schramm, A. R. Wills, R. M. Reichley, S. T. Micek, and M. H. Kollef, "Predictors of 30-day mortality and hospital costs in patients with ventilator-associated pneumonia attributed to potentially antibiotic-resistant gram-negative bacteria," *Chest*, vol. 134, no. 2, pp. 281–287, 2008.

[6] A. P. Limaye, K. A. Kirby, G. D. Rubenfeld et al., "Cytomegalovirus reactivation in critically ill immunocompetent patients," *The Journal of the American Medical Association*, vol. 300, no. 4, pp. 413–422, 2008.

[7] J. Cohen, S. Opal, and T. Calandra, "Sepsis studies need new direction," *The Lancet Infectious Diseases*, vol. 12, no. 7, pp. 503–505, 2012.

[8] D. C. Angus, "The search for effective therapy for sepsis: back to the drawing board?" *Journal of the American Medical Association*, vol. 306, no. 23, pp. 2614–2615, 2011.

[9] R. P. Wenzel and M. B. Edmond, "Septic shock—evaluating another failed treatment," *The New England Journal of Medicine*, vol. 366, no. 22, pp. 2122–2124, 2012.

[10] C. Torgersen, P. Moser, G. Luckner et al., "Macroscopic postmortem findings in 235 surgical intensive care patients with sepsis," *Anesthesia and Analgesia*, vol. 108, no. 6, pp. 1841–1847, 2009.

[11] J. S. Boomer, K. To, K. C. Chang et al., "Immunosuppression in patients who die of sepsis and multiple organ failure," *Journal of the American Medical Association*, vol. 306, no. 23, pp. 2594–2605, 2011.

[12] S. Ostrand-Rosenberg and P. Sinha, "Myeloid-derived suppressor cells: linking inflammation and cancer," *Journal of Immunology*, vol. 182, no. 8, pp. 4499–4506, 2009.

[13] A. G. Cuenca, M. J. Delano, K. M. Kelly-Scumpia et al., "A paradoxical role for myeloid-derived suppressor cells in sepsis and trauma," *Molecular Medicine*, vol. 17, no. 3-4, pp. 281–292, 2011.

[14] B. Cheng, G. Xie, S. Yao et al., "Epidemiology of severe sepsis in critically ill surgical patients in ten university hospitals in China," *Critical Care Medicine*, vol. 35, no. 11, pp. 2538–2546, 2007.

[15] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, "Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care," *Critical Care Medicine*, vol. 29, no. 7, pp. 1303–1310, 2001.

[16] T. Lagu, M. B. Rothberg, M. S. Shieh, P. S. Pekow, J. S. Steingrub, and P. K. Lindenauer, "Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007," *Critical Care Medicine*, vol. 40, no. 3, pp. 754–761, 2012, Erratum in *Critical Care Medicine*, vol. 40, no. 10, p. 2932, 2012.

[17] V. M. Ranieri, B. T. Thompson, P. S. Barie et al., "Drotrecogin alfa (activated) in adults with septic shock," *The New England Journal of Medicine*, vol. 366, no. 22, pp. 2055–2064, 2012.

[18] E. Abraham, K. Reinhart, S. Opal et al., "Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial," *Journal of the American Medical Association*, vol. 290, no. 2, pp. 238–247, 2003.

[19] S. M. Opal, G. E. Garber, S. P. LaRosa et al., "Systemic host responses in severe sepsis analyzed by causative microorganism and treatment effects of drotrecogin alfa (activated)," *Clinical Infectious Diseases*, vol. 37, no. 1, pp. 50–58, 2003.

[20] J.-L. Vincent, J. Rello, J. Marshall et al., "International study of the prevalence and outcomes of infection in intensive care units," *Journal of the American Medical Association*, vol. 302, no. 21, pp. 2323–2329, 2009.

[21] A. Mohr, J. Polz, E. M. Martin et al., "Sepsis leads to a reduced antigen-specific primary antibody response," *European Journal of Immunology*, vol. 42, no. 2, pp. 341–352, 2012.

[22] J. A. Bennett, V. Srinivasa Rao, and M. S. Mitchell, "Systemic bacillus Calmette-Guerin (BCG) activates natural suppressor cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 75, no. 10, pp. 5142–5144, 1978.

[23] M. R. Young, M. Newby, and H. T. Wepsic, "Hematopoiesis and suppressor bone marrow cells in mice bearing large metastatic Lewis lung carcinoma tumors," *Cancer Research*, vol. 47, no. 1, pp. 100–105, 1987.

[24] J. E. Talmadge and D. I. Gabrilovich, "History of myeloid-derived suppressor cells," *Nature Reviews Cancer*, vol. 13, no. 10, pp. 739–752, 2013.

[25] S. Kusmartsev and D. I. Gabrilovich, "Inhibition of myeloid cell differentiation in cancer: the role of reactive oxygen species," *Journal of Leukocyte Biology*, vol. 74, no. 2, pp. 186–196, 2003.

[26] Q. Li, P.-Y. Pan, P. Gu, D. Xu, and S.-H. Chen, "Role of immature myeloid Gr-1+ cells in the development of antitumor immunity," *Cancer Research*, vol. 64, no. 3, pp. 1130–1139, 2004.

[27] S. Kusmartsev, Y. Nefedova, D. Yoder, and D. I. Gabrilovich, "Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species," *Journal of Immunology*, vol. 172, no. 2, pp. 989–999, 2004.

[28] J. Schmielau and O. J. Finn, "Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients," *Cancer Research*, vol. 61, no. 12, pp. 4756–4760, 2001.

[29] B. Almand, J. I. Clark, E. Nikitina et al., "Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer," *Journal of Immunology*, vol. 166, no. 1, pp. 678–689, 2001.

[30] A. C. Ochoa, A. H. Zea, C. Hernandez, and P. C. Rodriguez, "Arginase, prostaglandins, and myeloid-derived suppressor cells in renal cell carcinoma," *Clinical Cancer Research*, vol. 13, no. 2, part 2, pp. 721S–726S, 2007.

[31] J.-I. Youn, S. Nagaraj, M. Collazo, and D. I. Gabrilovich, "Subsets of myeloid-derived suppressor cells in tumor-bearing mice," *Journal of Immunology*, vol. 181, no. 8, pp. 5791–5802, 2008.

[32] T. A. Dietlin, F. M. Hofman, B. T. Lund, W. Gilmore, S. A. Stohlman, and R. C. Van Der Veen, "Mycobacteria-induced Gr-1+ subsets from distinct myeloid lineages have opposite effects on T cell expansion," *Journal of Leukocyte Biology*, vol. 81, no. 5, pp. 1205–1212, 2007.

[33] D. I. Gabrilovich and S. Nagaraj, "Myeloid-derived suppressor cells as regulators of the immune system," *Nature Reviews Immunology*, vol. 9, no. 3, pp. 162–174, 2009.

[34] M. J. Delano, P. O. Scumpia, J. S. Weinstein et al., "MyD88-dependent expansion of an immature GR-1^+CD11b^+ population induces T cell suppression and Th2 polarization in sepsis," *Journal of Experimental Medicine*, vol. 204, no. 6, pp. 1463–1474, 2007.

[35] A. D. Panopoulos and S. S. Watowich, "Granulocyte colony-stimulating factor: molecular mechanisms of action during steady state and "emergency" hematopoiesis," *Cytokine*, vol. 42, no. 3, pp. 277–288, 2008.

[36] A. Ray, K. Chakraborty, and P. Ray, "Immunosuppressive MDSCs induced by TLR signaling during infection and role in resolution of inflammation," *Frontiers in Cellular and Infection Microbiology*, vol. 3, article 52, 2013.

[37] J. C. Ochando and S. H. Chen, "Myeloid-derived suppressor cells in transplantation and cancer," *Immunologic Research*, vol. 54, no. 1–3, pp. 275–285, 2012.

[38] N. Dilek, N. Poirier, C. Usal, B. Martinet, G. Blancho, and B. Vanhove, "Control of transplant tolerance and intragraft regulatory T cell localization by Myeloid-derived suppressor cells and CCL5," *Journal of Immunology*, vol. 188, no. 9, pp. 4209–4216, 2012.

[39] V. Bronte, P. Serafini, A. Mazzoni, D. M. Segal, and P. Zanovello, "L-arginine metabolism in myeloid cells controls T-lymphocyte functions," *Trends in Immunology*, vol. 24, no. 6, pp. 302–306, 2003.

[40] N. Dilek, R. V. de Silly, G. Blancho, and B. Vanhove, "Myeloid-derived suppressor cells: mechanisms of action and recent advances in their role in transplant tolerance," *Frontiers in Immunology*, vol. 3, article 208, 2012.

[41] A. Szuster-Ciesielska, E. Hryciuk-Umer, A. Stepulak, K. Kupisz, and M. Kandefer-Szerszeń, "Reactive oxygen species production by blood neutrophils of patients with laryngeal carcinoma and antioxidative enzyme activity in their blood," *Acta Oncologica*, vol. 43, no. 3, pp. 252–258, 2004.

[42] G. Mantovani, A. Macciò, C. Madeddu et al., "Antioxidant agents are effective in inducing lymphocyte progression through cell cycle in advanced cancer patients: assessment of the most important laboratory indexes of cachexia and oxidative stress," *Journal of Molecular Medicine*, vol. 81, no. 10, pp. 664–673, 2003.

[43] C. A. Corzo, M. J. Cotter, P. Cheng et al., "Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells," *Journal of Immunology*, vol. 182, no. 9, pp. 5693–5701, 2009.

[44] Y. S. Khaled, B. J. Ammori, and E. Elkord, "Myeloid-derived suppressor cells in cancer: recent progress and prospects," *Immunology and Cell Biology*, vol. 91, pp. 493–502, 2013.

[45] K. Chikamatsu, K. Sakakura, M. Toyoda, K. Takahashi, T. Yamamoto, and K. Masuyama, "Immunosuppressive activity of CD14+ HLA-DR- cells in squamous cell carcinoma of the head and neck," *Cancer Science*, vol. 103, no. 6, pp. 976–983, 2012.

[46] Y. Lu, F. Jiang, X. Zheng et al., "TGF-β1 promotes motility and invasiveness of glioma cells through activation of ADAM17," *Oncology Reports*, vol. 25, no. 5, pp. 1329–1335, 2011.

[47] L. Yang, J. Huang, X. Ren et al., "Abrogation of TGFβ signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis," *Cancer Cell*, vol. 13, no. 1, pp. 23–35, 2008.

[48] B. Huang, P.-Y. Pan, Q. Li et al., "Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host," *Cancer Research*, vol. 66, no. 2, pp. 1123–1131, 2006.

[49] R. Yang, Z. Cai, Y. Zhang, W. H. Yutzy IV, K. F. Roby, and R. B. S. Roden, "CD80 in immune suppression by mouse ovarian carcinoma-associated Gr-1 +CD11b + myeloid cells," *Cancer Research*, vol. 66, no. 13, pp. 6807–6815, 2006.

[50] P. Sinha, V. K. Clements, S. K. Bunt, S. M. Albelda, and S. Ostrand-Rosenberg, "Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response," *Journal of Immunology*, vol. 179, no. 2, pp. 977–983, 2007.

[51] D. Martire-Greco, V. I. Landoni, P. Chiarella et al., "all-trans-retinoic acid improves immunocompetence in a murine model of lipopolysaccharide-induced immunosuppression," *Clinical Science*, vol. 126, no. 5, pp. 355–365, 2014.

[52] G. Noel, Q. Wang, A. Osterburg et al., "A ribonucleotide reductase inhibitor reverses burn-induced inflammatory defects," *Shock*, vol. 34, no. 5, pp. 535–544, 2010.

[53] M. J. Delano, T. Thayer, S. Gabrilovich et al., "Sepsis induces early alterations in innate immunity that impact mortality to secondary infection," *Journal of Immunology*, vol. 186, no. 1, pp. 195–202, 2011.

[54] L. E. Sander, S. D. Sackett, U. Dierssen et al., "Hepatic acute-phase proteins control innate immune responses during infection by promoting myeloid-derived suppressor cell function," *Journal of Experimental Medicine*, vol. 207, no. 7, pp. 1453–1464, 2010.

[55] M. Derive, Y. Bouazza, C. Alauzet, and S. Gibot, "Myeloid-derived suppressor cells control microbial sepsis," *Intensive Care Medicine*, vol. 38, no. 6, pp. 1040–1049, 2012.

[56] L. Brudecki, D. A. Ferguson, C. E. McCall, and M. E. Gazzar, "Myeloid-derived suppressor cells evolve during sepsis and can enhance or attenuate the systemic inflammatory response," *Infection and Immunity*, vol. 80, no. 6, pp. 2026–2034, 2012.

[57] F. S. Hodi, S. J. O'Day, D. F. McDermott et al., "Improved survival with ipilimumab in patients with metastatic melanoma," *The New England Journal of Medicine*, vol. 363, no. 8, pp. 711–723, 2010.

[58] S. Kusmartsev, F. Cheng, B. Yu et al., "All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination," *Cancer Research*, vol. 63, no. 15, pp. 4441–4449, 2003.

[59] Y. Nefedova, M. Fishman, S. Sherman, X. Wang, A. A. Beg, and D. I. Gabrilovich, "Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells," *Cancer Research*, vol. 67, no. 22, pp. 11021–11028, 2007.

[60] C. Iclozan, S. Antonia, A. Chiappori, D.-T. Chen, and D. Gabrilovich, "Therapeutic regulation of myeloid-derived suppressor cells and immune response to cancer vaccine in patients with extensive stage small cell lung cancer," *Cancer Immunology, Immunotherapy*, vol. 62, no. 5, pp. 909–918, 2013.

[61] N. Mirza, M. Fishman, I. Fricke et al., "All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients," *Cancer Research*, vol. 66, no. 18, pp. 9299–9307, 2006.

[62] P. Serafini, K. Meckel, M. Kelso et al., "Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function," *Journal of Experimental Medicine*, vol. 203, no. 12, pp. 2691–2702, 2006.

[63] C. De Santo, P. Serafini, I. Marigo et al., "Nitroaspirin corrects immune dysfunction in tumor-bearing hosts and promotes tumor eradication by cancer vaccination," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 11, pp. 4185–4190, 2005.

[64] Y. Zheng, M. Xu, X. Li, J. Jia, K. Fan, and G. Lai, "Cimetidine suppresses lung tumor growth in mice through proapoptosis of myeloid-derived suppressor cells," *Molecular Immunology*, vol. 54, no. 1, pp. 74–83, 2013.

[65] B. Yin, G. Ma, C.-Y. Yen et al., "Myeloid-derived suppressor cells prevent type 1 diabetes in murine models," *Journal of Immunology*, vol. 185, no. 10, pp. 5828–5834, 2010.

[66] G. Liu, Y. Bi, R. Wang et al., "Targeting S1P1 receptor protects against murine immunological hepatic injury through myeloid-derived suppressor cells," *The Journal of Immunology*, vol. 192, no. 7, pp. 3068–3079, 2014.

[67] S. L. Highfill, P. C. Rodriguez, Q. Zhou et al., "Bone marrow myeloid-derived suppressor cells (MDSCs) inhibit graft-versus-host disease (GVHD) via an arginase-1-dependent mechanism that is up-regulated by interleukin-13," *Blood*, vol. 116, no. 25, pp. 5738–5747, 2010.

[68] A. G. Cuenca and L. L. Moldawer, "Myeloid-derived suppressor cells in sepsis: friend or foe?" *Intensive Care Medicine*, vol. 38, no. 6, pp. 928–930, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 712742, 7 pages
http://dx.doi.org/10.1155/2014/712742

Clinical Study

Cortisol Is an Associated-Risk Factor of Brain Dysfunction in Patients with Severe Sepsis and Septic Shock

Duc Nam Nguyen,[1] Luc Huyghens,[1] Haibo Zhang,[2] Johan Schiettecatte,[3] Johan Smitz,[3] and Jean-Louis Vincent[4]

[1] *Department of Critical Care Medicine, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium*
[2] *Department of Anesthesia and Critical Care Medicine and Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Bond Street 30, Toronto, ON, Canada M5B 1W8*
[3] *Department of Immunochemistry, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium*
[4] *Department of Intensive Care, Erasme University Hospital, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium*

Correspondence should be addressed to Duc Nam Nguyen; ducnam_ngyn@yahoo.com

Received 1 February 2014; Accepted 6 April 2014; Published 28 April 2014

Academic Editor: Baoli Cheng

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Objectives. To investigate cortisol levels in brain dysfunction in patients with severe sepsis and septic shock. *Methods*. In 128 septic and sedated patients, we studied brain dysfunction including delirium and coma by the evaluation of Richmond Agitation Sedation Scale (RASS), the Confusion Method Assessment in the ICU (CAM-ICU) after sedation withdrawal and the measurement of serum S100B biomarker of brain injury. Serum cortisol and S100B were measured within 12 hours after ICU admission and daily over the next four days. *Results*. Brain dysfunction was observed in 50% (64/128) before but in 84% (107/128) of patients after sedation withdrawal, and was more common in the patients older than 57 years ($P = 0.009$). Both cortisol ($P = 0.007$) and S100B levels ($P = 0.028$) were higher in patients with than patients without brain dysfunction. Cortisol levels were associated with ICU mortality (hazard ratio = 1.17, $P = 0.024$). Multivariate logistic regression showed that cortisol (odds ratio (OR): 2.34, 95% CI (2.01, 3.22), $P = 0.02$) and the combination effect of cortisol with age (OR: 1.004, 95% CI (1.002, 1.93), $P = 0.038$) but not S100B were associated with brain dysfunction. *Conclusions*. Cortisol was an associated-risk factor of brain dysfunction in patients with severe sepsis and septic shock.

1. Introduction

Cortisol release from the hypothalamic-pituitary-adrenal axis (HPA) is vital for the host survival in stress [1]. In sepsis, high cortisol release could result not only from stress but also from HPA dysfunction and following a metabolism reduction of this hormone at the organs [2–4]. However, an excessive release or chronic exposure to high cortisol levels could be harmful for the host brain, especially at the hippocampus and the frontal cortex where corticoid receptors are highly concentrated. It has been shown in rodents' experimental studies that cortisol reduced the neurons viability to toxic insults (glutamate, hypoxia, ischemia, or hypoglycemia) by ATP energy deprivation in these parts of the brain [5, 6].

Cortisol has been suggested as biomarker for the diagnosis of delirium [7]. High cortisol release associated with delirium was previously reported in stroke [8], in postcardiac surgery [9], in the elderly patients with hip fracture [10], in psychological depression, and in Cushing syndrome [11]. In clinical severe sepsis, high cortisol levels have been reported in patients who developed fatal brain dysfunction [12] but it remained unclear if this could be a sole indicator of severe inflammation and disease severity or was due to brain dysfunction. Also, these results were only reported in a small

number of patients with severe sepsis and remain to be confirmed in a larger study sample.

In this observational prospective study, we investigated the effect of cortisol on the development of brain dysfunction in patients with severe sepsis and septic shock. Brain dysfunction including coma and delirium was evaluated by the Glasgow Coma Score (GCS) or the Richmond Agitation Sedation Scale (RASS) combined with the Confusion Assessment Method in the ICU (CAM-ICU) and with the measurement of serum biomarker of brain injury S100B protein [13].

We also compared the predictive value of cortisol with S100B on the development of brain dysfunction as the measurements of serum biomarkers (S100B protein, neuron-specific enolase, and glial fibrillary acidic protein) have been proposed to help diagnose brain dysfunction regardless of sedation [14].

2. Patients and Methods

We studied 140 patients with severe sepsis and septic shock who were consecutively included in the study from October 2009 to January 2011. The Ethical Committee of our hospital approved the study and the informed consent was obtained from the patient's relatives.

Severe sepsis and septic shock was considered before ICU admission and the inclusion criteria were defined following the international consensus guidelines of sepsis [15]. Exclusion criteria included patients younger than 18 years old, pregnancy, acute cerebral disorder (trauma, stroke, hemorrhage, post-neurosurgery, cardiorespiratory arrest, and meningitis), concomitant treatment with corticoid or etomidate in the previous 24 hours, drugs or alcohol withdrawal, severe psychiatric disorder, dementia with disable neuromuscular disorders, severe chronic liver or renal failure, and nonsurvivors in the first 24 hours from sepsis.

Early goal-directed therapy for sepsis resuscitation and lung protective ventilation strategy were conducted following the international consensus sepsis guidelines. Resuscitation was targeted to obtain a mean arterial pressure ≥ 65 mm Hg and a urine output > 0.5 mL/kg/min with both colloid and crystalloid infusion, combined with norepinephrine (up to 1 μg/kg/min) and dobutamine (up to 10 μg/kg/min). Mechanical ventilation targeted a tidal volume of 6-7 mL/kg ideal body and a plateau pressure < 30 cm H_2O.

Sedation in the ICU was achieved by midazolam (up to 0.05 mg/kg/h) or propofol (up to 10 mg/kg/h) and analgesia was achieved by fentanyl (up to 0.05 mg/kg/min) or remifentanil (up to 0.75 μg/kg/h). Sedation and analgesia doses were daily adapted to obtain a Richmond Agitation Sedation Scale (RASS) score between 0 and −3. Neuromuscular blocking agent administration was interrupted within the first 48 hours after ICU admission.

Organ failure and the critical illness severity were evaluated by the daily Sequential Organ Failure Assessment (SOFA) and the APACHE III scores, respectively.

2.1. Brain Dysfunction Assessment. Before sedation, brain dysfunction was assessed by either GCS or CAM-ICU and it was considered when the GCS ≤ 13 or CAM-ICU was positive. After 24 hours of complete sedation withdrawal and up to ICU discharge, CAM-ICU was assessed together with the RASS score twice per day by the nurse or the physician in charge of the patient.

Delirium was confirmed by the physician in charge of the patient who was not aware about the cortisol and S100B results when RASS score > -3 and CAM-ICU was positive for at least two consecutive days. Hyperactive delirium was defined as the RASS score of 1 to 5 with agitation, irritation, confusion, and emotional labiality. Hypoactive delirium was defined as the RASS of −2 to −1 with apathy, decreased responsiveness or movement, and low consciousness. Coma was defined as the RASS score ≤ -4 throughout the ICU stay. Acquired critical illness neuromyopathy (CINM) was confirmed by electromyography.

2.2. Cortisol and S100B Protein Measurement. After ICU admission and sepsis confirmation, serum cortisol and S100B were simultaneously collected not at fixed time between 6 and 12 hours after hemodynamics stabilization and then once daily in the morning for four consecutive days.

Total serum cortisol concentration was measured in all patients by radioimmunoassay (Diasorin, Stillwater, USA). Normal values in the morning range (7–10 a.m.) were 171– 535 nmol/L without gender difference. The within run coefficient was <5% and the between run coefficients of variation were <10% (range: 27 to 1650 nmol/L).

Serum S100B protein was obtained in 75 patients by radioimmunoassay (immunoradiometric assay, Roche Diagnostics GmbH, Germany) and the normal value provided by the commercial kit was ≤ 0.105 μg/L.

2.3. Statistical Analysis. The parametric statistical methods were used with SPSS version 20.0 (SPSS, Chicago, IL) and SAS version 9.3 (SAS Institute, North Carolina, USA) for analysis.

Chi-square test or Fischer's exact test was used when appropriate for comparisons of categorical variables. Logarithm transformation was used to assume a normal distribution for continuous variables. Student's t-test was used for comparisons of cortisol between groups. Linear mixed models for repeated measures (including a random intercept effect and adjusting with age and gender) were used to compare the global difference of biomarkers over 4 days of measurement between groups. Nonparametric Mann-Whitney U test was used for comparisons of skewed values of S100B between groups.

The Pearson or Spearman correlation test was used to evaluate the correlation between continuous variable when appropriate.

Kaplan-Meier analysis with log-rank test was used to analyze the ICU survival time. The Cox proportional hazards regression model adjusted with stepwise selection of covariates (SOFA score at the first 4 days, age, cortisol levels at admission, S100B levels at day 2, and 2 binary variables: occurrence of brain dysfunction or nosocomial infection)

was used to determine the risk factors associated with ICU mortality.

Multivariate binary logistic regression model adjusted with stepwise selection of covariates (cortisol levels at admission, age, S100B levels at day 2, the SOFA score at the first 4 days, gender, the length of sedation, and one interaction term (age x cortisol levels at admission)) was used to determine the associated-risk factors for the development of brain dysfunction. Variables with univariate chi-square value <0.2 were added and retained at $P \leq 0.05$. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were calculated for the logistic regression model or to determine for the cut-off value of cortisol and age associated with delirium. Smaller Akaike criteria value was used for the selection between different logistic regression models.

Statistical significance was considered at two-sided P value <0.05.

3. Results

Of the 140 patients with severe sepsis and septic shock, 12 (9%) who died under sedation without proper neurological evaluation were excluded from analysis.

Brain dysfunction developed in 50% (64/128) of patients before sedation but up to 84% (107/128) after sedation withdrawal. Delirium developed in 85/107 patients (80%): 41 hypoactive (48%) and 44 hyperactive (52%). Twenty-two patients (20%) remained comatose: brain CT scan showed a hemorrhage in five, a stroke in three and septic emboli in one.

In delirium patients, brain CT scan was negative in 15/44 hyperactive delirium patients but showed a stroke in two and a hemorrhage in one out of 21/41 hypoactive delirium patients.

3.1. The Patients' Characteristics and Comorbidities at ICU Admission (Table 1). Only difference in age was found with regard to brain dysfunction after sedation withdrawal. However, age was not different in regard to brain dysfunction occurring before starting of sedation. Patients who developed brain dysfunction were older than the other patients (67 ± 13 versus 58 ± 18 years, $P = 0.009$). Also comatose patients were older than delirium patients (72 ± 9 versus 65 ± 13 years, $P = 0.007$). As expected, ICU nonsurvivors were older than survivors (68 ± 13 versus 62 ± 14 years, $P = 0.035$).

3.2. Brain Dysfunction and ICU Evolution (Table 2). When compared with non-brain dysfunction, patients who developed brain dysfunction had higher degree of SOFA score at the first four days, higher incidence of nosocomial infection and septic shock recurrence, and higher prevalence of acquired critical illness neuromyopathy (CINM) but a trend of prolonged ICU length of stay (15 ± 10 versus 23 ± 18 days, $P = 0.06$).

Brain dysfunction was associated with higher ICU mortality: death occurred in 41% (18/44) hyperactive delirium, 68% (28/41) hypoactive delirium, and all (100%) of 22 comatose patients ($P < 0.001$) but 14% (3/21) in patients without brain dysfunction. The median (interquartile) ICU survival time was 36 (14, 48) days for delirium and 8 (7, 15) day for comatose patients (all log-rank tests, $P < 0.01$).

The Cox proportional hazards regression model identified three risk factors independently associated with ICU mortality: SOFA score at the first 4 days (hazard ratio (HR) = 1.22, $P = 0.05$), cortisol levels at admission (HR = 1.17, $P = 0.024$), and occurrence of brain dysfunction (HR = 4.89, $P = 0.001$).

3.3. Cortisol and S100B Protein Levels in Brain Dysfunction. Cortisol levels at admission (448 ± 813 versus 211 ± 122 nmol/L, $P = 0.013$) and over four days ($P = 0.007$, Figure 1) were higher in patients who developed brain dysfunction than non-brain dysfunction after adjusting with age and gender. Cortisol levels were higher in ICU nonsurvivors than survivors (510 ± 352 versus 248 ± 187 nmol/L, $P = 0.05$) but were not different between delirium and coma.

Table 1: Characteristics at ICU admission and comorbidities.

Characteristics at ICU admission and comorbidities	$n = 128$
Age, years	65 ± 14
APACHE III score	83 ± 30
SOFA score	8 ± 4
CRP (mg/dL)	208 ± 129
Males/females, *n* (%)	83/45 (65/35)
Medical/surgical patients, *n* (%)	106/22 (83/17)
Bronchopneumonia, *n* (%)	93 (73)
Peritonitis, *n* (%)	22 (17)
Gram negative/positive, *n* (%)	65/42 (51/49)
Active smoking, *n* (%)	33 (26)
Obesity (BMI > 30), *n* (%)	28 (22)
Arterial hypertension, *n* (%)	53 (42)
Diabetes, *n* (%)	24 (19)
Alcohol abuse, *n* (%)	19 (15)
History of brain disorder, *n* (%)	29 (23)
History of Sepsis, *n* (%)	20 (16)

The continuous variables were represented as mean values ± standard deviation.

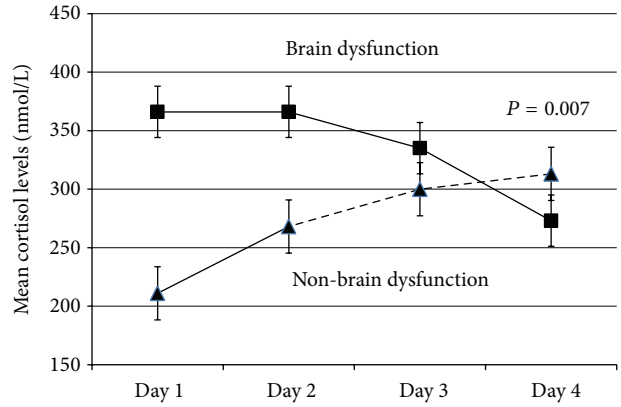

Figure 1: Patients with brain dysfunction released higher cortisol levels than non-brain dysfunction.

Table 2: Biomarkers levels, ICU clinical evolution, and outcome.

	All patients (n = 128)	Non-brain dysfunction (n = 21)	Brain dysfunction (n = 107)	P
Cortisol admission (nmol/L)	413 ± 757	211 ± 122	448 ± 813	0.013
Cortisol day 4 (nmol/L)	279 ± 256	314 ± 352	273 ± 238	0.441
S100B admission (μg/L)	**0.13 (0.07, 0.28)**	0.97 (0.51, 0.18)	0.13 (0.06, 0.49)	0.188
S100B day 4 (μg/L)	**0.13 (0.06, 0.2)**	0.08 (0.04, 0.13)	0.12 (0.08, 0.24)	0.038
Mechanical ventilation, days	16 ± 14	12 ± 11	15 ± 14	0.109
Inotropics length, days	6 ± 5	4 ± 2	6 ± 5	0.191
SOFA score for the first 4 days	8 ± 4	6 ± 4	9 ± 4	0.006
Sedation, days	9 ± 7	8 ± 6	10 ± 8	0.315
Midazolam, n (%)	80 (62)	12 (57)	68 (64)	0.579
Fentanyl, n (%)	75 (59)	13 (62)	62 (58)	0.736
Propofol, n (%)	48 (37)	9 (43)	41 (38)	0.696
Nosocomial infection, n (%)	67 (52)	5 (24)	62 (58)	<0.01
Shock recurrence, n (%)	37 (29)	1 (5)	36 (34)	<0.01
CINM, n (%)	64 (50)	4 (19)	60 (56)	0.003
ICU mortality, n (%)	69 (54)	3 (14)	66 (62)	<0.01

Continuous variables were represented as mean values ± standard deviation.
S100B levels were represented as median (interquartile) due to skewed values.
CNIM: critical illness neuromyopathy.

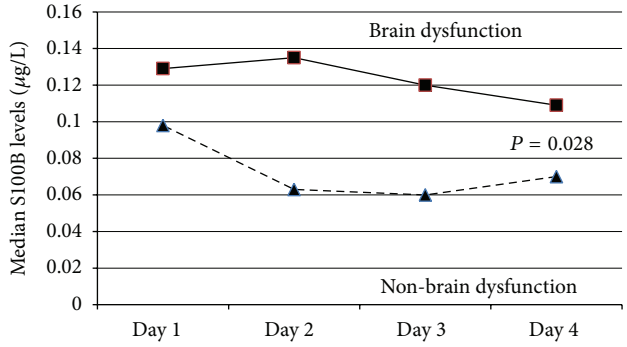

Figure 2: Patients with brain dysfunction released higher S100B levels than non-brain dysfunction.

A cortisol cut-off value of 232 nmol/L determined brain dysfunction after sedation withdrawal with a sensitivity 59% and a specificity 63%. An age cut-off value of 57 years determined brain dysfunction with sensitivity 83% and specificity 63%.

S100B was elevated in brain dysfunction but not in non-brain dysfunction patients (P = 0.028, Figure 2). Comatose patients had higher S100B levels than delirium patients (median (interquartile): 0.14 (0.11, 0.48) versus 0.1 (0.05, 0.37) μg/L, P = 0.007).

Cortisol correlated with S100B levels at ICU admission (r = 0.32, P < 0.01) and with the SOFA score at the first 4 days (r = 0.55, P < 0.01). Neither cortisol nor S100B was correlated with age, serum creatinine, APACHE III or SOFA scores, and C -reactive protein (CRP), (all P value >0.05).

Two risk factors were associated with the development of brain dysfunction in sepsis: cortisol levels at ICU admission (OR: 2.34, 95% CI (2.01, 3.22), P = 0.02) and the combination effect of cortisol with age (OR: 1.004, 95% CI (1.002, 1.93), P = 0.038). The AUC of the ROC curve of this global model to discriminate brain dysfunction was 89% (95% CI: 82, 95%) and remained unchanged when only delirium patients were included in the model.

4. Discussion

In our series of septic patients, brain dysfunction was present in one-half of the patients before sedation was started but increased to 84% of the patients after sedation withdrawal. Hence, sedation and/or other factors aggravated brain dysfunction at a certain time point in a number of patients during the sedation period. Benzodiazepines and opiates administration, although aggravating delirium in the critically ill patients, likely did not significantly influence the results as the number of patients who were sedated with midazolam and fentanyl and the length of sedation were not different in regard to brain dysfunction [16].

Our results suggested that cortisol was an associated-risk factor of brain dysfunction in sepsis. Pfister et al. previously showed that high cortisol levels were associated with S100B elevation and fatal brain dysfunction in a small number of patients with severe sepsis and septic shock [12]. Our results confirmed these observations in a larger cohort of septic patients, as cortisol levels were higher in patients with than patients without brain dysfunction. This could not result from a sole reaction to stress and illness severity because no association was found with the APACHE and SOFA scores or the degree of inflammatory response (as reflected by blood CRP concentrations). Also, cortisol levels decreased over time probably result from deficiency or exhaustion of the stimulating effect of adrenocorticotrophine hormone (ACTH) occurring in these patients [17].

It has been reported that cortisol could be harmful for the brain, especially at the hippocampus [18]. In rodents,

cortisol impaired the neurons viability to insults (hypoxia, ischemia, or hypoglycemia) by inhibiting glucose uptake and utilization, exacerbating energy ATP depletion, and accumulated neurotoxic products over the course of few days [5, 6].

A cortisol cut-off value of 353–550 nmol/L was previously reported to be associated with delirium in stroke [8] and after cardiac surgery [9, 19]. However, we observed a lower cortisol cut-off value of 232 nmol/L associated with brain dysfunction in severe sepsis and this value was still remaining in our normal laboratory reference value (170–535 nmol/L). Hence, other factors should predispose the host brain to be more sensitive to the effect of cortisol. Sepsis with multiple organ dysfunction and aging could contribute to this process as these factors remained the main fatal ICU risk factors.

Sepsis aggravated ongoing brain dysfunction as a component of multiple organ dysfunctions which was reflected by S100B elevation and higher organ dysfunction SOFA scores, respectively. Different regions of the brain were affected in sepsis but the hippocampus was particularly sensitive to hypoxia and inflammation [20]. Moreover, the hippocampus regulates a negative feedback effect of cortisol release from the HPA [6, 21]. The dysfunction and damage of this part of the brain induced aberrant cortisol release, cognitive dysfunction, delirium and was implicated in the development of long term cognitive impairment in the sepsis survivors [17, 22–24]. In addition, the BBB breakdown in sepsis could worsen brain dysfunction by facilitating a crossing of cortisol, toxic amino acids and other proinflammatory mediators from the blood into the brain [25–27]. Using S100B as biomarker of brain injury and the blood-brain barrier (BBB) breakdown [28] we found that S100B elevation correlated with cortisol levels to support this hypothesis. Also, S100B was associated with brain dysfunction severity as higher levels were observed in fatal comatose than other patients.

Sepsis and brain dysfunction commonly targeted the aging patients [29, 30]. Patients who developed brain dysfunction were older and had higher cortisol levels than other patients. Elderly patients with preexisting brain disorder (dementia, atherosclerosis, and degenerative diseases) were more vulnerable to ischemia and to the harmful effect of proinflammatory cytokines or neurotoxic products than younger subjects [17, 31]. However, in our study, sepsis had more impact than preexisting brain disorders as it induced brain dysfunction in more than half of patients at ICU admission even before sedation was starting.

The logistic regression model also confirmed that cortisol levels associated with brain dysfunction were more pronouncing with age (older than 57 years old).

In addition to the above, the aging patients were chronically exposed to higher cortisol levels following an impairment of the negative feedback at the hippocampus with aging [32]. In these patients, cortisol could exacerbate neuronal dysfunction by energy deprivation, particularly at the hippocampus and the cortex, the parts of the brain where corticoid receptors are highly concentrated and sensitive to sepsis [6]. These observations raise concerns if the cortisol treatment should be selected in the aging patients with septic shock to prevent brain dysfunction, especially since it has been reported that a reduction of brain activity and cognitive dysfunction were observed after hydrocortisone administration in healthy volunteers [33].

In contrast with Pfister et al., we found that cortisol was more powerful than S100B to predict brain dysfunction and global ICU mortality. We previously showed that, due the short half-life time, S100B could only predict the early ICU nonsurvivors of sepsis [34]. Unlike S100B, cortisol was not solely a biomarker of brain dysfunction but was related with underlying life-threatening homeostasis disturbance of the host: abnormal HPA stress hormones response, glucose intolerance, immunodepression, and adrenal insufficiency [35–37].

S100B was sensitive to predict coma and severe brain injury (stroke, postanoxic encephalopathy, and head trauma) but more frequent measurements over a longer period than four days were probably necessary to diagnose delirium, which fluctuated over time [38–40]. Lacking of the statistical power with reduction of the predictive value of S100B to determine brain dysfunction could be another explanation as this biomarker was measured in half of patients.

Our study showed some limitations: we did not measure free serum cortisol which reflected its real biological activity although its advantage on total cortisol in sepsis was not confirmed by all authors [41]. Cortisol was not measured in the cerebral-spinal fluid although it was elevated in delirium patients [42]. Also, no direct harmful effect of cortisol on the brain or hippocampus could be demonstrated neither by neurophysiological studies nor brain imaging. We measured cortisol within a time interval but not at fixed time. However, as the circadian cortisol secretion dysfunction frequently occurred in sepsis this did not significantly influence our results [43].

S100B was not brain specific and could be released from sole stress or by other organs than the brain in shock [13, 44]. However, these factors likely did not significantly influence our results as no correlation was found with serum creatinine, APACHE, or SOFA scores. Moreover, it has been recently reported that the extracerebral source of S100B did not affect the levels of this biomarker in the serum after excluding major trauma or severe surgery [45].

5. Conclusion

Cortisol was an associated-risk factor of brain dysfunction in patients with severe sepsis and septic shock.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] G. P. Chrousos, "The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation," *The New England Journal of Medicine*, vol. 332, no. 20, pp. 1351–1362, 1995.

[2] J. I. Webster and E. M. Sternberg, "Role of the hypothalamic-pituitary-adrenal axis, glucocorticoids and glucocorticoid

receptors in toxic sequelae of exposure to bacterial and viral products," *Journal of Endocrinology*, vol. 181, no. 2, pp. 207–221, 2004.

[3] A. Polito, R. Sonneville, C. Guidoux et al., "Changes in CRH and ACTH synthesis during experimental and human septic shock," *PLoS ONE*, vol. 6, no. 16, Article ID e25905, 2011.

[4] E. Boonen, H. Vervenne, P. Meersseman et al., "Reduced cortisol metabolism during critical illness," *The New England Journal of Medicine*, vol. 368, no. 16, pp. 1477–1488, 2013.

[5] R. M. Sapolsky, "A mechanism for glucocorticoid toxicity in the hippocampus: increased neuronal vulnerability to metabolic insults," *Journal of Neuroscience*, vol. 5, no. 5, pp. 1228–1232, 1985.

[6] J. Herbert, I. M. Goodyer, A. B. Grossman et al., "Do corticosteroids damage the brain?" *Journal of Neuroendocrinology*, vol. 18, no. 6, pp. 393–411, 2006.

[7] E. R. Marcantonio, J. L. Rudolph, D. Culley, G. Crosby, D. Alsop, and S. K. Inouye, "Serum biomarkers for delirium," *Journals of Gerontology A: Biological Sciences and Medical Sciences*, vol. 61, no. 12, pp. 1281–1286, 2006.

[8] N. Marklund, M. Peltonen, T. K. Nilsson, and T. Olsson, "Low and high circulating cortisol levels predict mortality and cognitive dysfunction early after stroke," *Journal of Internal Medicine*, vol. 256, no. 1, pp. 15–21, 2004.

[9] D.-L. Mu, D.-X. Wang, L.-H. Li et al., "High serum cortisol level is associated with increased risk of delirium after coronary artery bypass graft surgery: a prospective cohort study," *Critical Care*, vol. 14, no. 6, article R238, 2010.

[10] B. C. van Munster, P. H. Bisschop, A. H. Zwinderman et al., "Cortisol, interleukins and S100B in delirium in the elderly," *Brain and Cognition*, vol. 74, no. 1, pp. 18–23, 2010.

[11] K. Michaud, H. Forget, and H. Cohen, "Chronic glucocorticoid hypersecretion in Cushing's syndrome exacerbates cognitive aging," *The Journal of Neuroscience*, vol. 71, no. 1, pp. 1–8, 2009.

[12] D. Pfister, M. Siegemund, S. Dell-Kuster et al., "Cerebral perfusion in sepsis-associated delirium," *Critical Care*, vol. 12, no. 3, article R63, 2008.

[13] C.-A. Gonçalves, M. C. Leite, and P. Nardin, "Biological and methodological features of the measurement of S100B, a putative marker of brain injury," *Clinical Biochemistry*, vol. 41, no. 10-11, pp. 755–763, 2008.

[14] K. J. B. Lamers, P. Vos, M. M. Verbeek, F. Rosmalen, W. J. A. van Geel, and B. G. M. van Engelen, "Protein S-100B, neuron-specific enolase (NSE), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) in cerebrospinal fluid (CSF) and blood of neurological patients," *Brain Research Bulletin*, vol. 61, no. 3, pp. 261–264, 2003.

[15] R. P. Dellinger, M. M. Levy, J. M. Carlet et al., "Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2008," *Critical Care Medicine*, vol. 36, no. 1, pp. 296–327, 2008.

[16] M. A. Pisani, T. E. Murphy, K. L. B. Araujo, P. Slattum, P. H. van Ness, and S. K. Inouye, "Benzodiazepine and opioid use and the duration of intensive care unit delirium in an older population," *Critical Care Medicine*, vol. 37, no. 1, pp. 177–183, 2009.

[17] A. Polito, R. Sonneville, C. Guidoux et al., "Changes in CRH and ACTH synthesis during experimental and human septic shock," *PLoS ONE*, vol. 6, no. 11, Article ID e25905, 2011.

[18] R. M. Sapolsky, "Glucocorticoids, stress, and their adverse neurological effects: relevance to aging," *Experimental Gerontology*, vol. 34, no. 6, pp. 721–732, 1999.

[19] J. Kazmierski, A. Banys, J. Latek, J. Bourke, and R. Jaszewski, "Cortisol levels and neuropsychiatric diagnosis as markers of postoperative delirium: a prospective study," *Critical Care*, vol. 17, no. 2, article R38, 2013.

[20] D. Annane, "Hippocampus: a future target for sepsis treatment," *Intensive Care Medicine*, vol. 35, no. 4, pp. 585–586, 2009.

[21] T. W. Buchanan, S. Kern, J. S. Allen, D. Tranel, and C. Kirschbaum, "Circadian regulation of cortisol after hippocampal damage in humans," *Biological Psychiatry*, vol. 56, no. 9, pp. 651–656, 2004.

[22] T. Sharshar, N. S. Hopkinson, D. Orlikowski, and D. Annane, "Science review: the brain in sepsis—culprit and victim," *Critical Care*, vol. 9, no. 1, pp. 37–44, 2005.

[23] F. G. Zampieri, M. Park, F. S. Machado, and L. C. P. Azevedo, "Sepsis-associated encephalopathy: not just delirium," *Clinics*, vol. 66, no. 10, pp. 1825–1831, 2011.

[24] R. O. Hopkins, L. K. Weaver, D. Pope, J. F. Orme Jr., E. D. Bigler, and V. Larson-Lohr, "Neuropsychological sequelae and impaired health status in survivors of severe acute respiratory distress syndrome," *American Journal of Respiratory and Critical Care Medicine*, vol. 160, no. 1, pp. 50–56, 1999.

[25] S. Siami, D. Annane, and T. Sharshar, "The encephalopathy in sepsis," *Critical Care Clinics*, vol. 24, no. 1, pp. 67–82, 2008.

[26] R. Sonneville, F. Verdonck, C. Rauturier et al., "Understanding brain dysfunction in sepsis," *Annals of Intensive Care*, vol. 3, p. 15, 2013.

[27] S. F. Sorrells, J. R. Caso, C. D. Munhoz, and R. M. Sapolsky, "The stressed CNS: when glucocorticoids aggravate inflammation," *Neuron*, vol. 64, no. 1, pp. 33–39, 2009.

[28] N. Marchi, M. Rasmussen, V. Fazio et al., "Peripheral markers of brain damage and blood-brain barrier dysfunction," *Restorative Neurology and Neuroscience*, vol. 21, no. 3-4, pp. 109–121, 2003.

[29] L. McNicoll, M. A. Pisani, Y. Zhang, E. W. Ely, M. D. Siegel, and S. K. Inouye, "Delirium in the intensive care unit: occurrence and clinical course in older patients," *Journal of the American Geriatrics Society*, vol. 51, no. 5, pp. 591–598, 2003.

[30] J. Cerejeira, H. Firmino, A. Vaz-Serra, and E. B. Mukaetova-Ladinska, "The neuroinflammatory hypothesis of delirium," *Acta Neuropathologica*, vol. 119, no. 6, pp. 737–754, 2010.

[31] A. M. J. MacLullich, K. J. Ferguson, T. Miller, S. E. J. A. de Rooij, and C. Cunningham, "Unravelling the pathophysiology of delirium: a focus on the role of aberrant stress responses," *Journal of Psychosomatic Research*, vol. 65, no. 3, pp. 229–238, 2008.

[32] S. Lupien, A. R. Lecours, I. Lussier, G. Schwartz, N. P. V. Nair, and M. J. Meaney, "Basal cortisol levels and cognitive deficits in human aging," *Journal of Neuroscience*, vol. 14, no. 5, part 1, pp. 2893–2903, 1994.

[33] W. R. Lovallo, J. L. Robinson, D. C. Glahn, and P. T. Fox, "Acute effects of hydrocortisone on the human brain: an fMRI study," *Psychoneuroendocrinology*, vol. 35, no. 1, pp. 15–20, 2010.

[34] D. N. Nguyen, H. Spapen, F. Su et al., "Elevated serum levels of S-100β protein and neuron-specific enolase are associated with brain injury in patients with severe sepsis and septic shock," *Critical Care Medicine*, vol. 34, no. 7, pp. 1967–1974, 2006.

[35] G. van den Berghe, "Novel insights into the neuroendocrinology of critical illness," *European Journal of Endocrinology*, vol. 143, no. 1, pp. 1–13, 2000.

[36] V. Maxime, S. Siami, and D. Annane, "Metabolism modulators in sepsis: the abnormal pituitary response," *Critical Care Medicine*, vol. 35, no. 9, pp. S596–S601, 2007.

[37] D. Annane, V. Sébille, G. Troché, J.-C. Raphaël, P. Gajdos, and E. Bellissant, "A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin," *Journal of the American Medical Association*, vol. 283, no. 8, pp. 1038–1045, 2000.

[38] D. L. Nash, M. F. Bellolio, and L. G. Stead, "S100 as a marker of acute brain ischemia: a systematic review," *Neurocritical Care*, vol. 8, no. 2, pp. 301–307, 2008.

[39] K. Shinozaki, S. Oda, T. Sadahiro et al., "S-100B and neuron-specific enolase as predictors of neurological outcome in patients after cardiac arrest and return of spontaneous circulation: a systematic review," *Critical Care*, vol. 13, no. 4, article R121, 2009.

[40] S. M. Bloomfield, J. McKinney, L. Smith, and J. Brisman, "Reliability of S100B in predicting severity of central nervous system injury," *Neurocritical Care*, vol. 6, no. 2, pp. 121–138, 2007.

[41] N. Molenaar, A. B. Johan Groeneveld, H. M. Dijstelbloem et al., "Assessing adrenal insufficiency of corticosteroid secretion using free versus total cortisol levels in critical illness," *Intensive Care Medicine*, vol. 37, no. 12, pp. 1986–1993, 2011.

[42] A. Pearson, A. de Vries, S. D. Middleton et al., "Cerebrospinal fluid cortisol levels are higher in patients with delirium versus controls," *BMC Research Notes*, vol. 3, article 33, 2010.

[43] B. M. Arafah, "Hypothalamic pituitary adrenal function during critical illness: limitations of current assessment methods," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 10, pp. 3725–3745, 2006.

[44] R. Gerlach, G. Demel, H.-G. König et al., "Active secretion of S100B from astrocytes during metabolic stress," *Neuroscience*, vol. 141, no. 4, pp. 1697–1701, 2006.

[45] N. Pham, V. Fazio, L. Cucullo et al., "Extracranial sources of S100B do not affect serum levels," *PLoS ONE*, vol. 5, no. 9, Article ID e12691, 2010.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 807635, 9 pages
http://dx.doi.org/10.1155/2014/807635

Review Article

Hydrogen Gas Presents a Promising Therapeutic Strategy for Sepsis

Keliang Xie,[1,2] Lingling Liu,[1,2] Yonghao Yu,[1,2] and Guolin Wang[1,2]

[1] *Department of Anesthesiology, General Hospital of Tianjin Medical University, Tianjin 300052, China*
[2] *Tianjin Institute of Anesthesiology, Tianjin 300052, China*

Correspondence should be addressed to Yonghao Yu; tougaozy2011@126.com

Received 7 February 2014; Accepted 1 April 2014; Published 16 April 2014

Academic Editor: Baoli Cheng

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Sepsis is characterized by a severe inflammatory response to infection. It remains a major cause of morbidity and mortality in critically ill patients despite developments in monitoring devices, diagnostic tools, and new therapeutic options. Recently, some studies have found that molecular hydrogen is a new therapeutic gas. Our studies have found that hydrogen gas can improve the survival and organ damage in mice and rats with cecal ligation and puncture, zymosan, and lipopolysaccharide-induced sepsis. The mechanisms are associated with the regulation of oxidative stress, inflammatory response, and apoptosis, which might be through NF-κB and Nrf2/HO-1 signaling pathway. In this paper, we summarized the progress of hydrogen treatment in sepsis.

1. Introduction

Sepsis is defined as the presence (probable or documented) of infection together with systemic manifestations of infection [1]. Severe sepsis is defined as sepsis plus sepsis-induced organ dysfunction or tissue hypoperfusion [1]. Sepsis-induced tissue hypoperfusion is defined as infection-induced hypotension, elevated lactate, or oliguria [1]. Septic shock is defined as sepsis-induced hypotension persisting despite adequate fluid resuscitation [1]. Sepsis and its various adverse sequelae, such as septic shock, acute respiratory distress syndrome (ARDS), and multiple organ dysfunction syndrome (MODS), continue to be a leading cause of mortality in intensive care unit (ICU) and a major public health burden throughout the world [2]. With recent dramatic advances in powerful antibiotics and monitoring devices, the mortality rate has been decreased over the past half century [3]. However, the number of people dying from sepsis continues to rise annually owing to the increasing morbidity. It is estimated that about 18 million cases of severe sepsis occur annually worldwide and the incidence rate of sepsis is increased by 1.5% ~ 8% annually [4, 5]. At present, there are more than 1,000,000 cases of severe sepsis among hospitalized patients each year in the USA with a total annual cost of $16.7 billion [6, 7]. In China, the occurrence rate of severe sepsis in surgical ICU is 8.68% with a hospital mortality rate of 48.7% [8, 9].

The pathogenesis and mechanisms of sepsis are complex and not fully understood, which include the excessive release of inflammatory cytokines, the action of oxidative stress (excessive release of reactive oxygen species, ROS), intestinal bacteria and endotoxin translocation, neutrophil dysfunction, microcirculatory impairment, mitochondrial dysfunction, the imbalance between oxygen supply and oxygen consumption, immune and metabolic disorders, and coagulation disorders [10–15]. Our previous studies have found that the uncontrolled inflammatory response and oxidative stress are crucial to the pathogenesis of sepsis, MODS, and ultimately death [16–18]. In addition, it should be noted that genetic variations partially determine individual susceptibility to sepsis. An increasing number of candidate genes have been implicated in sepsis susceptibility, such as macrophage migration inhibitory factor, plasminogen activator inhibitor 1, protein C, and miRNA [19].

However, even when sepsis is timely recognized, there is no effective therapy to sepsis, except antibiotics, fluids, and vasopressors [9, 20]. Disappointedly, undeniable successes in numerous animal studies are not consistent with that

in clinical trials, such as direct anti-inflammatory strategies including anti-TNF-α, IL-1-based therapies, high-dose corticosteroids, and administration of activated protein C, which make the researches about pathogenesis and clinical treatment of sepsis troubled [10, 21–23]. The novel interventional strategies of sepsis should be invented to reduce mortality in sepsis.

Molecular hydrogen (H_2), the smallest, lightest, and most element in the universe, is colorless, odorless, and certain antioxidant. Many years ago, H_2 was regarded as physiological inert gas without more attention from scientists because of relatively low solubility and the difficulty to be absorbed. In 1975, Dole et al. found that exposure to a mixture of 2.5 percent oxygen and 97.5 percent hydrogen at a total pressure of 8 atmospheres for periods up to 2 weeks would cause a regression of squamous cell carcinoma via antioxidant effect [24]. In 1997, Shirahata et al. [25] reported that electrolyzed-reduced water, which dissolved large amounts of H_2, had the ability to protect DNA from oxidative damage, suggesting that it could reduce the risk of life style-related diseases and cancer. H_2 has also been used in medical applications to prevent decompression sickness in deep-sea divers for safety profiles [26]. In 2001, Gharib et al. [27] reported that treatment with 0.7 MPa hydrogen in a hyperbaric chamber for 2 weeks had significantly protective effects towards schistosomiasis-associated chronic liver inflammation, which was associated with antioxidant and anti-inflammatory properties of H_2. It is also proved that molecular hydrogen would directly react with the hydroxyl radical, a highly cytotoxic reactive oxygen species (ROS). In 2007, Ohsawa et al. [28] found that H_2 could exert a therapeutic antioxidant activity by selectively reducing hydroxyl radical and peroxynitrite (another cytotoxic ROS) in vitro, making researches about molecular hydrogen become hot around the world. In recent years, many researchers have found that molecular hydrogen can attenuate multiple organ damage, such as brain, spinal cord, heart, lung, liver, kidney, pancreas, and intestine [28–34]. Besides, it is widely proved that H_2 or H_2-rich saline exerts an effective therapeutic role in many diseases including sepsis, ischemia-reperfusion injury, organ transplantation, stroke, MODS, type 2 diabetes, atherosclerosis, neurodegenerative diseases, and oxygen toxicity [28, 30, 31, 35–40]. However, the mechanisms by which molecular hydrogen provides beneficial effects on many disorders remain unclear, which would be associated with reduction of oxidative stress, inflammation, and apoptosis, as well as regulation of several important signaling pathways.

2. Advances in Hydrogen Treatment of Sepsis

We have made several studies about H_2 treatment in animal models of sepsis. It is well known that cecal ligation and puncture (CLP) causes lethal peritonitis and sepsis due to a polymicrobial infection that is accompanied by multiple organ damage. We firstly investigate the possible therapeutic effects of H_2 on sepsis in a murine model of moderate or severe CLP. For severe CLP (100% lethality), we ligate the distal three-quarters of the cecum and make a single puncture with a 20-gauge needle; for moderate CLP (30–40% survival), we ligate the distal one-half of the cecum and make a single puncture with a 21-gauge needle. We find that H_2 inhalation starting at 1 and 6 h after CLP operation significantly improved the survival rate of septic mice with moderate or severe CLP in a concentration- and time-dependent manner [30]. Moreover, H_2 inhalation at a therapeutic dose (2% and 4%) has no adverse effects on the saturation level of arterial oxygen and hemodynamic parameters. We further find that H_2 treatment provides the beneficial effects on sepsis and sepsis-associated organ damage, including lung, liver, kidney, and brain [30]. Zymosan, a substance derived from the cell wall of the yeast *S. cerevisiae*, can lead to systemic inflammation by inducing a wide range of inflammatory mediators. The zymosan-induced generalized inflammation model has been widely used in many experimental studies for MODS. We also find that H_2 improves survival rate and organ damage in zymosan-induced generalized inflammation model [16]. 2% H_2 inhalation for 1 hour beginning at 1 and 6 hours after zymosan injection significantly improves the 14-day survival rate of zymosan-challenged mice from 10% to 70%. H_2 treatment significantly mitigates the impairments of liver and kidney function in the zymosan-challenged mice [17]. Intratracheal administration of lipopolysaccharide (LPS), the major component of the outer membrane of Gram-negative bacteria, is a well-established model of acute lung injury. Using this model, we find 2% H_2 or hydrogen-rich saline can exert protective effects in a mouse model of acute lung injury [29]. Meanwhile, combination therapy with H_2 (2%) and hyperoxia (98%) increases the 14-day survival rate of moderate sepsis mice to 100% and the 7-day survival rate of severe sepsis mice from 0% to 70% and alleviates injuries of lung, liver, and kidney in moderate and severe sepsis [41]. Similarly, we find that 2% H_2 inhalation significantly ameliorates short- and long-time cognitive function in sepsis survivors (unpublished data). In addition, we find that hydrogen-rich saline can significantly improve the outcome and cardiac function in a rat model of septic shock (unpublished data). Thus, H_2 or hydrogen-rich saline may be an effective therapeutic strategy for patients with sepsis (Table 1).

3. Mechanisms about Hydrogen Treatment of Sepsis

3.1. Anti-Inflammatory Effects. Sepsis is associated with a systemic inflammatory response, mediated by vascular endothelial cells and innate immune cells, including neutrophils, macrophages, and monocytes [48]. The releases of proinflammatory cytokines and chemokines, including tumor necrosis factor- (TNF-) α, interleukin- (IL-) 1, IL-6, high-mobility group box- (HMGB-) 1, and monocyte chemoattractant protein- (MCP-) 1, as the most important cytokines mediating the acute phase of inflammatory response, normally triggers beneficial host innate immune response to confine the infection and tissue damage. However, in sepsis, the excessive and prolonged production of these cytokines can produce overwhelming inflammatory response, which is even more deadly than the original infection. These inflammatory

Table 1: Effects of molecular hydrogen treatment on sepsis.

Drugs	Authors	Animal or cell type	Animal or cell model	Administration dose and way	Effect
2% hydrogen	Liu et al. (2013) [42]	Wister rats	15 mg/kg LPS (septic shock)	Inhalation for 2 h after LPS injection	Anti-inflammatory (IL-6, IL-8, TNF-α, and MPO) Antioxidant (·OH, MDA, and SOD) Inhibition of apoptosis (Fas, Bcl-2)
Hydrogen-rich saline	Chen et al. (2013) [43]	RAW 264.7 macrophages	1 mg/mL LPS	Incubation with hydrogen-rich saline	Anti-inflammatory (TNF-α, IL-1β, HMGB1, and IL-10) Nrf2/HO-1 signaling pathway
Hydrogen-rich saline	Xie (2013) [44]	Wister rats	CLP	5 mL/Kg or 10 mL/Kg	Anti-inflammatory (MPO) Antioxidant (iNOS)
2% H_2 and 98% hyperoxia	Xie et al. (2012) [41]	C57BL/6 mice	CLP	2% H_2 and 98% hyperoxia for 3 h and 6 h after CLP	Anti-inflammatory (TNF-α, HMGB1, IL-10, and MPO) Antioxidant (CAT, SOD, and 8-iso-PGF2α)
Hydrogen-rich saline	Li et al. (2013) [45]	Sprague-Dawley rats	CLP	5 mL/Kg hydrogen-rich saline at 0, 6, and 18 h after CLP	Anti-inflammatory (TNF-α, IL-6, HMGB1, IL-10, HMGB1, and MPO) Antioxidant (MDA, SOD)
2% hydrogen	Xie et al. (2012) [46]	C57BL/6 mice	CLP	2% H_2 inhalation for 1 h and 6 h after CLP	Nrf2/HO-1 signaling pathway
2% hydrogen or hydrogen-rich saline	Xie et al. (2012) [29]	C57BL/6 mice	25 ug LPS (acute lung injury)	2% H_2 inhalation for 1 h and 6 h after LPS given or 10 mL/Kg hydrogen-rich saline	Anti-inflammatory (TNF-α, IL-1β, IL-6, IL-10, KC, MIP-1α, MIP-2/MCP-1, and MPO) NF-κB signaling pathway Inhibition of apoptosis (TUNEL, caspase-3)
2% hydrogen	Xie et al. (2010) [30, 47]	C57BL/6 mice	CLP	2% H_2 inhalation for 1 h and 6 h after CLP	Anti-inflammatory (HMGB1, MPO) Antioxidant (CAT, SOD, and 8-iso-PGF2α)
2% hydrogen	Xie et al. (2010) [16]	ICR mice	1 g/Kg zymosan (inflammation model)	2% H_2 inhalation for 1 h and 6 h after zymosan injection	Anti-inflammatory (TNF-α, HMGB1) Antioxidant (SOD, 8-iso-PGF2α)

cytokines can result in a variety of pathologic phenomena, including priming of the vascular endothelium by synthesis of adhesion molecules, activation of neutrophils, synthesis of cyclooxygenase products, generation of nitrous oxide, ROS, apoptosis, and induction of hypotension and shock-like state [49–52]. It is well known that the excessive production of proinflammatory cytokines causes capillary leakage, tissue injury, and lethal multiple organ failure in severe sepsis [10, 53, 54]. It is also reported that elevated proinflammatory cytokine levels directly correlate with severity and mortality in human sepsis [55, 56]. The proinflammatory cytokines also lead to activation of the complement and coagulation cascades [57]. Furthermore, proinflammatory cytokines can upregulate the expression of inflammatory mediators via positive feedback loop and, consequently, induce further detrimental phenomena [58].

HMGB1 is a member of the high-mobility group protein superfamily that has been widely studied as nuclear proteins that bind DNA, stabilize nucleosomes, and facilitate gene transcription [59]. HMGB-1 has been implicated in the pathogenesis of inflammatory diseases and proposed to be a crucial mediator in the pathogenesis of many diseases including sepsis, arthritis, cancer, autoimmunity diseases, and diabetes [60]. HMGB1 can interact with various receptors including RAGE, Toll-like receptor- (TLR-) 2, and TLR-4 to mediate chemotaxis and release of proinflammatory cytokines in monocytes/macrophages and delayed endotoxin lethality, which is required for the full expression of inflammation in animal models of endotoxemia, sepsis, and arthritis [59, 61]. Furthermore, targeting of HMGB1 with antibodies or specific antagonists has been found to have protective effects in established preclinical inflammatory disease models, including lethal endotoxemia and sepsis [62]. As the late inflammatory cytokine, HMGB1 plays a central role in the inflammatory response, becoming a key therapy to resolve inflammation [63, 64].

Our studies show that H_2 inhalation can decrease the early proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and late proinflammatory cytokine (HMGB1) in serum and tissues (lung, liver, and kidney) of preclinical animal models of sepsis [16, 29, 30, 41]. Furthermore, H_2 treatment reduces the levels of chemokines (KC, MIP-1α, MIP-2, and MCP-1) in the bronchoalveolar lavage fluid of LPS-induced ALI mice. In addition, H_2 treatment decreases LPS-induced neutrophils recruitment into the lungs [29]. All results demonstrate that H_2 treatment downregulates the cytokines and chemokines

in the different mouse models of sepsis. Therefore, it has been suggested to use molecular hydrogen as a new anti-inflammatory strategy.

Recently, some studies have shown that neuroinflammation in the central nervous system can cause brain damage in sepsis. The vast release of cytokines, such as TNF-α, IL-1β, and HMGB1, leads to alterations of cell function, the blood-brain barrier disruption, and brain dysfunction [65–67]. In particular, HMGB1, released from necrotic neurons via a NR2B-mediated mechanism, promotes cerebral edema via activation of microglial TLR4 and the subsequent expression of the astrocytic water channel, aquaporin-4 in traumatic brain injury [68]. We find that the blood-brain barrier disruption and cognitive dysfunction of sepsis animals are significantly alleviated by H_2 treatment, which are associated with the decrease of the levels of proinflammatory cytokines (TNF-α, IL-1β, and HMGB1) in the cerebral tissue (unpublished data).

With the accumulation of knowledge regarding proinflammatory cytokines produced by the immune system, investigators focus on the physiological mechanisms that maintain homeostasis. So far, various endogenous anti-inflammatory mediators are proved to have a capacity to prevent proinflammatory cytokine-mediated diseases [48]. IL-10, as an important anti-inflammatory and immunosuppressant, can mediate the downregulation of inflammatory response by a variety of mechanisms. Importantly, IL-10 can inhibit the generation and release of a variety of cytokines in monocytes/macrophages, such as TNF-α, IL-1, IL-8, GM-CSF, and G-CSF [69]. IL-10 plays a protective role in the systemic inflammatory response. Combination therapy with H_2 (2%) and hyperoxia (98%) significantly increases the IL-10 level in serum and tissues (lung, liver, and kidney) of septic mice with moderate or severe CLP [41].

3.2. Antioxidant Effects. Oxidative stress defines disequilibrium between the levels of produced ROS and the ability of a biological system to detoxify the reactive intermediates [70]. ROS can be generated through several pathways such as direct interactions between redox-active metals and oxygen species via reactions including the Fenton and Haber-Weiss reactions, or by indirect pathways involving the activation of enzymes such as nitric oxide synthase (NOS) or NADPH oxidases. Intracellular accumulation of ROS, such as superoxide anion, hydrogen peroxide, singlet oxygen, hydroxyl radical, and peroxy radical, can arise from toxic insults or normal metabolic processes. These species may perturb the cell's natural antioxidant defense systems, resulting in damage to all of the major classes of biological macromolecules, including nucleic acids, proteins, carbohydrates, and lipids [70]. Furthermore, hydroxyl radical is one of the strongest oxidant species and reacts indiscriminately with nucleic acids, lipids, and proteins [71]. One type of ROS can be converted into another type via antioxidant enzymes in vivo. For example, superoxide dismutase (SOD) converts superoxide anion radical into H_2O_2, which is detoxified into H_2O by either glutathione peroxidase or catalase (CAT) [72]. Besides ROS, reactive nitrogen species (RNS) can mediate nitrosative stress. RNS are generated by the quick reaction of superoxide with nitric oxide (NO), which results in the production of large amount of peroxynitrite [73, 74].

In excess, ROS and their by-products that are capable of causing oxidative damage may be detrimental to tissues and organs [75]. Recently, some studies demonstrated that H_2 would exert a therapeutic antioxidant activity by selectively reducing hydroxyl radicals (the most cytotoxic ROS) and effectively reverse tissue damage such as transient cerebral ischemia, neonatal cerebral hypoxia-ischemia, liver injury, lung injury, and myocardial injury induced by ischemia and reperfusion [31, 32, 76–78]. A growing number of studies have found that excessive production of ROS and RNS plays important roles in the pathogenesis of sepsis [79–81]. Therefore, scavenging ROS, RNS, and their by-products is a critical antioxidant process, which may be a good and critical measure for treating sepsis. We report that H_2 treatment significantly decreases the levels of 8-iso-prostaglandin-F2α (8-iso-PGF2α) in serum, lung, liver, and kidney tissue, which could exactly reflect the level of oxidative stress [16, 29, 30]. The levels of 8-iso-PGF2α in serum and tissues are also reduced by combination therapy with H_2 and O_2 [41]. In addition, our researches find that H_2 treatment can significantly improve the activities of antioxidant enzymes (SOD and CAT) in serum and organ tissues of mice of moderate and severe sepsis models [16, 30, 41]. These outcomes suggest that H_2 treatment provides beneficial effects on sepsis and sepsis-associated organ damage, which are associated with downregulation of oxidative stress.

3.3. Antiapoptosis Effects. Apoptosis, the regulated destruction of a cell, is a complicated process [82]. Many pathways can lead to activation of cell death. Death proteases are homologous to each other and are part of a large protein family known as the caspases, and blocking caspases can rescue condemned cells from their apoptotic fate. Besides the caspases, mitochondria sequester is a potent cocktail of proapoptotic proteins. Most prominent among these is cytochrome C, the humble electron carrier. Cytochrome C is one of the components required for activation of caspase-9 in the cytosol. Bcl-2 family is intimately involved in the regulation of cytochrome C crossing the mitochondria.

In addition, a role for oxidative stress in apoptosis has been shaped by several independent observations. For many years, direct treatment of cells with oxidants like hydrogen peroxide or redox-active quinones was thought to exclusively cause necrosis, but more recent studies have shown that lower doses of these agents can trigger apoptosis [83].

Apoptosis is a common pathological basis of many diseases, which plays an important role in the development of various diseases. Xiang et al. [84] detect that 2% H_2 inhalation markedly attenuates morphological liver injury and apoptosis by reducing lipid peroxidation such as MDA. Cai et al. [77] find that 2% H_2 therapy in a duration-dependent manner significantly reduces the number of positive TUNEL cells and suppresses caspase-3 and caspase-12 activities in neonatal hypoxia-ischemia rat model. We find that H_2 inhalation markedly inhibits pulmonary cell

apoptosis by TUNEL staining in LPS-challenged mice. Similarly, the caspase-3 activity is significantly increased in the lungs of LPS-challenged animals, which is prevented by H_2 treatment [29]. Moreover, 1.3% H_2 can reduce the number of apoptotic positive cells and infarct sizes due to opening of mitochondrial K_{ATP} channels followed by inhibition of mPTP in the acute myocardial infarction and reperfusion model [85]. Besides, H_2-rich saline may effectively decrease the degree of necrosis, apoptosis, and cell autophagy in rats with acute CO poisoning, which could be related to decrease in the content of Fe and increase in the content of serum Cu associated with free radical metabolism [86].

3.4. Signaling Pathways. NF-κB transcription factors also regulate the expression of hundreds of genes that are involved in regulating cell growth, differentiation, development, inflammation, and apoptosis [87]. In quiescent cells, NF-κB activity is principally regulated by the IκB proteins, which possess ankyrin repeats and are generally inhibitory to DNA binding. The activity of the typical IκBs is controlled through phosphorylation by upstream IκB kinases (IKKs). The canonical NF-κB pathway is activated mostly by the stimulation of proinflammatory receptors, such as the TNF receptor superfamily, the Toll-like receptor family (TLRs), and cytokine receptors for the interleukins [88]. It is also activated by genotoxic agents as well. Phosphorylation of IκBα on serines 32 and 36 by the IKK complex (primarily IKKβ) targets it for ubiquitination. Subsequently the ubiquitinated IκBα is degraded by the proteosome and this unmasks the DNA-binding activity of the p50/RelA heterodimer and also allows it to translocate to the nucleus where it can bind to κB sites and activate gene transcription. It is well known that NF-κB regulates gene expression of cytokines, chemokines, and adhesion molecules. Therefore, NF-κB is increasingly recognized as a crucial player in many steps of regulation of inflammatory responses. Noncanonical NF-κB activation is stimulated by specific TNF receptor family members that signal through the recruitment of TRAF2 and TRAF3 [88]. In addition, different target genes are differentially induced by distinct NF-κB dimers. Furthermore, NF-κB subunits also contain sites for phosphorylations and other posttranslational modifications which are important for activation and crosstalk with other signaling pathways [87]. Heme oxygenase-1 (HO-1) and apoptosis-associated factors, including TRAF-1 and Bcl-XL, are also mediated by NF-κB [89]. A previous study indicates that H_2 inhalation reduces epithelial apoptosis in ventilator-induced lung injury via NF-κB activation [90]. H_2 inhibits TNF-α-induced lectin-like oxidized LDL receptor-1 expression by inhibiting the phosphorylation of IκB-α and activation of NF-κB in endothelial cells [91]. Moreover, H_2 can indirectly activate the NF-κB signaling through reducing oxygen free radical [28]. However, in our study, H_2 treatment inhibits the lung NF-κB p65 nuclear translocation and DNA-binding activity in LPS-challenged mice [29].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important cytoprotective transcription factor [92]. Nrf2 controls the coordinated expression of important antioxidant and detoxification genes (Phase II genes) through a promotor sequence termed the antioxidant response element (ARE). Phase II genes, including heme oxygenase-1 (HO-1), glutathione S-transferases (GSTs), and NAD(P)H quinine oxidoreductase, work in synergy to constitute a pleiotropic cellular defense that scavenges reactive oxygen/nitrogen species (ROS/RNS), detoxifies electrophiles and xenobiotics, and maintains intracellular reducing potential. HO-1 is an ubiquitous and redox-sensitive inducible stress protein that degrades heme to CO, iron, and biliverdin [93]. Some studies have found that Nrf2 is a novel regulator of the innate immune response that dramatically improves survival during experimental sepsis by protecting against dysregulated inflammation [94]. Heme oxygenase-1 (HO-1) and the product of its enzymatic reaction, CO, not only have beneficial anti-inflammatory properties, but also enhance bacterial clearance by increasing phagocytosis and the endogenous antimicrobial response [95]. Some researchers found that hydrogen treatment during exposure to hyperoxia significantly improved blood oxygenation, reduced inflammatory events, and induced HO-1 expression, which did not mitigate hyperoxic lung injury or induce HO-1 in Nrf2-deficient mice [96]. Previous studies investigate that hydrogen gas inhalation significantly promotes the expression of Nrf2 in septic organs including lung, liver, and kidney. However, hydrogen gas did not improve the survival rate in Nrf2-deficient mice [46, 97]. Moreover, H_2 treatment dose-dependently attenuates the increased levels of proinflammatory cytokines and further increases the level of anti-inflammatory cytokine IL-10 with the increase of HO-1 protein expression and activity in LPS-stimulated RAW 264.7 macrophages [43]. Therefore, downstream molecules of Nrf2 signaling pathway play an important role in the pathophysiological process of sepsis.

4. Hydrogen-Rich Saline Exerts the Same Therapeutic Effect with H_2

Hydrogen-rich saline, in which the concentration of hydrogen is more than 0.6 mmol/L, is easily and safely manufactured [29]. Hydrogen-rich saline can alleviate inflammatory response, inhibit cell apoptosis, and reverse oxidative stress to reduce organ injuries, which would be a good method for clinical application. Currently, it is generally accepted that hydrogen-rich saline exerts an effective therapeutic role in many disorders including sepsis, ischemia-reperfusion injury, allergy, and degenerative diseases [29, 98–100].

In our study, we find that hydrogen-rich saline has similar beneficial effects on LPS-induced lung injury as hydrogen inhalation, which are also associated with inhibition of infiltration of inflammatory cell and activation of NF-κB [29]. In addition, hydrogen-rich saline effectively ameliorates hemodynamics, vascular reactivity in a dose-dependent manner in rat model of peritonitis-induced septic shock. Meanwhile, vital organ dysfunction, such as heart, lung, liver, and kidney, is significantly mitigated via resolving inflammatory responses and decreasing the iNOS expression [44]. Similarly, hydrogen-rich saline has potential protective effects against

sepsis by decreasing proinflammatory responses, oxidative stress, and apoptosis in a rat model of polymicrobial sepsis [45]. In addition, hydrogen-rich saline markedly reverses cognitive impairment and mortality in a dose-dependent manner in rats submitted to sepsis by cecal ligation and puncture, which are relative to the suppression of oxidative stress and cell apoptosis [101].

5. Advantages of H_2 or Hydrogen-Rich Saline Treatment of Sepsis

It is obvious that hydrogen is electronically neutral and has favorable distribution characteristics: it can penetrate biomembranes and diffuse into the cytosol, mitochondria, and nucleus [102]. Despite the moderate reduction activity of H_2, its rapid gaseous diffusion might make it highly effective for reducing cytotoxic radicals. Besides, it stands to reason that H_2 will react with only the strongest oxidants. H_2 is mild enough not to disturb metabolic oxidation reduction reactions or to disrupt ROS involved in cell signaling—unlike some antioxidant supplements with strong reductive reactivity, which increase mortality, possibly by affecting essential defensive mechanisms. Thus, H_2 treatment is advantageous for medical procedures without serious unwanted side effects [28]. Furthermore, H_2 is neither inflammable nor explosive at low concentrations (<4.6% in air and 4.1% in pure oxygen) [28]. Moreover, only 2% hydrogen gas can have obvious protective effects on sepsis. Meanwhile, hydrogen-rich saline is also available and safe for medical applications.

6. Conclusion

Although recent treatment modalities and interventions have contributed to the improvement for sepsis patients, the high mortality rate of severe sepsis suggests the necessity for additional therapies. Recently, vigorous experimental studies have been undergone to identify effective therapy of molecular hydrogen for sepsis. What is more, this novel therapy may be tested in clinical situation in the future. However, we should deeply proceed to more experimental researches to investigate the plausible and comprehensive mechanisms of hydrogen to treat sepsis.

Conflict of Interests

The authors have declared that no conflict of interests exists.

Authors' Contribution

Keliang Xie and Lingling Liu contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 81071533 to Yonghao Yu and 81101409 to Keliang Xie), the Natural Science Foundation of the Tianjin Science Committee (Nos. 11JCYBJC12900 to Yonghao Yu and 13JCQNJC11400 to Keliang Xie), and the Foundation of Tianjin Bureau of Public Health (2011KZ108 to Keliang Xie).

References

[1] J. Briegel and P. Mohnle, "International guidelines of the Surviving Sepsis Campaign: update 2012," *Der Anaesthesist*, vol. 62, no. 4, pp. 304–309, 2013.

[2] F. B. Mayr, S. Yende, and D. C. Angus, "Epidemiology of severe sepsis," *Virulence*, vol. 5, no. 1, pp. 4–11, 2014.

[3] Y. Okazaki and A. Matsukawa, "Pathophysiology of sepsis and recent patents on the diagnosis, treatment and prophylaxis for sepsis," *Recent Patents on Inflammation and Allergy Drug Discovery*, vol. 3, no. 1, pp. 26–32, 2009.

[4] G. S. Martin, "Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes," *Expert Review of Anti-Infective Therapy*, vol. 10, no. 6, pp. 701–706, 2012.

[5] A. Perner, N. Haase, A. B. Guttormsen et al., "Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis," *The New England Journal of Medicine*, vol. 367, no. 2, pp. 124–134, 2012.

[6] G. S. Martin, D. M. Mannino, S. Eaton, and M. Moss, "The epidemiology of sepsis in the United States from 1979 through 2000," *The New England Journal of Medicine*, vol. 348, no. 16, pp. 1546–1554, 2003.

[7] J. A. Russell, "Management of sepsis," *The New England Journal of Medicine*, vol. 355, no. 16, pp. 1699–1713, 2006.

[8] B. Cheng, G. Xie, S. Yao et al., "Epidemiology of severe sepsis in critically ill surgical patients in ten university hospitals in China," *Critical Care Medicine*, vol. 35, no. 11, pp. 2538–2546, 2007.

[9] H. B. Nguyen and D. Smith, "Sepsis in the 21st century: recent definitions and therapeutic advances," *American Journal of Emergency Medicine*, vol. 25, no. 5, pp. 564–571, 2007.

[10] R. S. Hotchkiss and I. E. Karl, "The pathophysiology and treatment of sepsis," *The New England Journal of Medicine*, vol. 348, no. 2, pp. 138–150, 2003.

[11] P. Q. Eichacker, C. Natanson, and R. L. Danner, "Surviving sepsis—practice guidelines, marketing campaigns, and Eli Lilly," *The New England Journal of Medicine*, vol. 355, no. 16, pp. 1640–1642, 2006.

[12] Y. Oba and K. Iwata, "Treatment of neonatal sepsis with immune globulin," *The New England Journal of Medicine*, vol. 366, no. 1, p. 91, 2012.

[13] W. L. Lee and A. S. Slutsky, "Sepsis and endothelial permeability," *The New England Journal of Medicine*, vol. 363, no. 7, pp. 689–691, 2010.

[14] E. Abraham, P.-F. Laterre, R. Garg et al., "Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death," *The New England Journal of Medicine*, vol. 353, no. 13, pp. 1332–1341, 2005.

[15] R. S. Hotchkiss, G. Monneret, and D. Payen, "Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach," *The Lancet Infectious Diseases*, vol. 13, no. 3, pp. 260–268, 2013.

[16] K. Xie, Y. Yu, Z. Zhang et al., "Hydrogen gas improves survival rate and organ damage in zymosan-induced generalized inflammation model," *Shock*, vol. 34, no. 5, pp. 495–501, 2010.

[17] J. Mu, K. Xie, L. Hou et al., "Subanesthetic dose of isoflurane protects against zymosan-induced generalized inflammation and its associated acute lung injury in mice," *Shock*, vol. 34, no. 2, pp. 183–189, 2010.

[18] L. Hou, K. Xie, N. Li et al., "100% oxygen inhalation protects against zymosan-induced sterile sepsis in mice: the roles of inflammatory cytokines and antioxidant enzymes," *Shock*, vol. 32, no. 4, pp. 451–460, 2009.

[19] L. P. Chung and G. W. Waterer, "Genetic predisposition to respiratory infection and sepsis," *Critical Reviews in Clinical Laboratory Sciences*, vol. 48, no. 5-6, pp. 250–268, 2011.

[20] S. N. Stehr and K. Reinhart, "Sepsis as a global health problem-why we need a global sepsis alliance," *Shock*, vol. 39, supplement 1, pp. 3–4, 2013.

[21] R. P. Dellinger, M. M. Levy, A. Rhodes et al., "Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012," *Intensive Care Medicine*, vol. 39, no. 2, pp. 165–228, 2013.

[22] P. Brocklehurst, B. Farrell, A. King et al., "Treatment of neonatal sepsis with intravenous immune globulin," *The New England Journal of Medicine*, vol. 365, no. 13, pp. 1201–1211, 2011.

[23] A. Mackenzie, "Activated protein C for sepsis," *The New England Journal of Medicine*, vol. 362, no. 12, pp. 1150–1151, 2010.

[24] M. Dole, F. R. Wilson, and W. P. Fife, "Hyperbaric hydrogen therapy: a possible treatment for cancer," *Science*, vol. 190, no. 4210, pp. 152–154, 1975.

[25] S. Shirahata, S. Kabayama, M. Nakano et al., "Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage," *Biochemical and Biophysical Research Communications*, vol. 234, no. 1, pp. 269–274, 1997.

[26] P. Fontanari, M. Badier, C. Guillot et al., "Changes in maximal performance of inspiratory and skeletal muscles during and after the 7.1-MPa hydra 10 record human dive," *European Journal of Applied Physiology*, vol. 81, no. 4, pp. 325–328, 2000.

[27] B. Gharib, S. Hanna, O. M. S. Abdallahi, H. Lepidi, B. Gardette, and M. de Reggi, "Anti-inflammatory properties of molecular hydrogen: investigation on parasite-induced liver inflammation," *Comptes Rendus de l'Academie des Sciences III*, vol. 324, no. 8, pp. 719–724, 2001.

[28] I. Ohsawa, M. Ishikawa, K. Takahashi et al., "Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals," *Nature Medicine*, vol. 13, no. 6, pp. 688–694, 2007.

[29] K. Xie, Y. Yu, Y. Huang et al., "Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis," *Shock*, vol. 37, no. 5, pp. 548–555, 2012.

[30] K. Xie, Y. Yu, Y. Pei et al., "Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release," *Shock*, vol. 34, no. 1, pp. 90–97, 2010.

[31] K. Hayashida, M. Sano, I. Ohsawa et al., "Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury," *Biochemical and Biophysical Research Communications*, vol. 373, no. 1, pp. 30–35, 2008.

[32] K.-I. Fukuda, S. Asoh, M. Ishikawa, Y. Yamamoto, I. Ohsawa, and S. Ohta, "Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress," *Biochemical and Biophysical Research Communications*, vol. 361, no. 3, pp. 670–674, 2007.

[33] L. Xiang, J. W. Tan, L. J. Huang et al., "Inhalation of hydrogen gas reduces liver injury during major hepatotectomy in swine," *World Journal of Gastroenterology*, vol. 18, no. 37, pp. 5197–5204, 2012.

[34] T. Hayashi, T. Yoshioka, K. Hasegawa et al., "Inhalation of hydrogen gas attenuates left ventricular remodeling induced by intermittent hypoxia in mice," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 301, no. 3, pp. H1062–H1069, 2011.

[35] J. Zheng, K. Liu, Z. Kang et al., "Saturated hydrogen saline protects the lung against oxygen toxicity," *Undersea and Hyperbaric Medicine*, vol. 37, no. 3, pp. 185–192, 2010.

[36] B. M. Buchholz, D. J. Kaczorowski, R. Sugimoto et al., "Hydrogen inhalation ameliorates oxidative stress in transplantation induced intestinal graft injury," *American Journal of Transplantation*, vol. 8, no. 10, pp. 2015–2024, 2008.

[37] S. Kajiyama, G. Hasegawa, M. Asano et al., "Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance," *Nutrition Research*, vol. 28, no. 3, pp. 137–143, 2008.

[38] Y. Fu, M. Ito, Y. Fujita et al., "Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease," *Neuroscience Letters*, vol. 453, no. 2, pp. 81–85, 2009.

[39] J. Cai, Z. Kang, K. Liu et al., "Neuroprotective effects of hydrogen saline in neonatal hypoxia-ischemia rat model," *Brain Research*, vol. 1256, pp. 129–137, 2009.

[40] X. Ji, W. Liu, K. Xie et al., "Beneficial effects of hydrogen gas in a rat model of traumatic brain injury via reducing oxidative stress," *Brain Research*, vol. 1354, pp. 196–205, 2010.

[41] K. Xie, W. Fu, W. Xing et al., "Combination therapy with molecular hydrogen and hyperoxia in a murine model of polymicrobial sepsis," *Shock*, vol. 38, no. 6, pp. 656–663, 2012.

[42] W. Liu, L. P. Shan, X. S. Dong et al., "Combined early fluid resuscitation and hydrogen inhalation attenuates lung and intestine injury," *World Journal of Gastroenterology*, vol. 19, no. 4, pp. 492–502, 2013.

[43] H. G. Chen, K. L. Xie, H. Z. Han et al., "Heme oxygenase-1 mediates the anti-inflammatory effect of molecular hydrogen in LPS-stimulated RAW 264.7 macrophages," *International Journal of Surgery*, vol. 11, no. 10, pp. 1060–1066, 2013.

[44] K. Xie, "Therapeutic effects of hydrogen-rich saline on peritonitis-induced septic shock in rats," pp. 148-149, 2013.

[45] G. M. Li, M. H. Ji, X. J. Sun et al., "Effects of hydrogen-rich saline treatment on polymicrobial sepsis," *Journal of Surgical Research*, vol. 181, no. 2, pp. 279–286, 2013.

[46] K. Xie, Y. Yu, L. Hou et al., "Nrf2 is critical in the protective role of hydrogen gas against murine polymicrobial sepsis," *British Journal of Anaesthesia*, vol. 108, no. 3, pp. 538–539, 2012.

[47] K.-L. Xie, L.-C. Hou, G.-L. Wang, and L.-Z. Xiong, "Effects of hydrogen gas inhalation on serum high mobility group box 1 levels in severe septic mice," *Zhejiang da Xue Xue Bao. Yi Xue Ban*, vol. 39, no. 5, pp. 454–457, 2010.

[48] A. Matsuda, A. Jacob, R. Wu et al., "Novel therapeutic targets for sepsis: regulation of exaggerated inflammatory responses," *Journal of Nippon Medical School*, vol. 79, no. 1, pp. 4–18, 2012.

[49] D. Beasley, J. H. Schwartz, and B. M. Brenner, "Interleukin 1 induces prolonged L-arginine-dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells," *The Journal of Clinical Investigation*, vol. 87, no. 2, pp. 602–608, 1991.

[50] P. B. Chapman, T. J. Lester, E. S. Casper et al., "Clinical pharmacology of recombinant human tumor necrosis factor in patients with advanced cancer," *Journal of Clinical Oncology*, vol. 5, no. 12, pp. 1942–1951, 1987.

[51] C. A. Dinarello, "Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock," *Chest*, vol. 112, supplement 6, pp. 321S–329S, 1997.

[52] S. Okusawa, J. A. Gelfand, T. Ikejima, R. J. Connolly, and C. A. DInarello, "Interleukin 1 induces a shock-like state in rabbits. Synergism with tumor necrosis factor and the effect of cyclooxygenase inhibition," *The Journal of Clinical Investigation*, vol. 81, no. 4, pp. 1162–1172, 1988.

[53] B. Cai, E. A. Deitch, and L. Ulloa, "Novel insights for systemic inflammation in sepsis and hemorrhage," *Mediators of Inflammation*, vol. 2010, Article ID 642462, 10 pages, 2010.

[54] N. C. Riedemann and P. A. Ward, "Anti-inflammatory strategies for the treatment of sepsis," *Expert Opinion on Biological Therapy*, vol. 3, no. 2, pp. 339–350, 2003.

[55] J.-P. Mira, A. Cariou, F. Grall et al., "Association of TNF2, a TNF-α promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study," *The Journal of the American Medical Association*, vol. 282, no. 6, pp. 561–568, 1999.

[56] T. van der Poll and S. F. Lowry, "Tumor necrosis factor in sepsis: mediator of multiple organ failure or essential part of host defense?" *Shock*, vol. 3, no. 1, pp. 1–12, 1995.

[57] H. Moshage, "Cytokines and the hepatic acute phase response," *The Journal of Pathology*, vol. 181, no. 3, pp. 257–266, 1997.

[58] R. E. Kast, "Tumor necrosis factor has positive and negative self regulatory feed back cycles centered around cAMP," *International Journal of Immunopharmacology*, vol. 22, no. 11, pp. 1001–1006, 2000.

[59] U. Andersson and K. J. Tracey, "HMGB1 in sepsis," *Scandinavian Journal of Infectious Diseases*, vol. 35, no. 9, pp. 577–584, 2003.

[60] J. A. Nogueira-Machado and V. C. de Oliveira, "HMGB-1 as a target for inflammation controlling," *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*, vol. 6, no. 3, pp. 201–209, 2012.

[61] H. Yang and K. J. Tracey, "Targeting HMGB1 in inflammation," *Biochimica et Biophysica Acta*, vol. 1799, no. 1-2, pp. 149–156, 2010.

[62] J. S. Bae, "Role of high mobility group box 1 in inflammatory disease: focus on sepsis," *Archives of Pharmacal Research*, vol. 35, no. 9, pp. 1511–1523, 2012.

[63] W. Huang, Y. Tang, and L. Li, "HMGB1, a potent proinflammatory cytokine in sepsis," *Cytokine*, vol. 51, no. 2, pp. 119–126, 2010.

[64] S. Karlsson, V. Pettilä, J. Tenhunen, R. Laru-Sompa, M. Hynninen, and E. Ruokonen, "HMGB1 as a predictor of organ dysfunction and outcome in patients with severe sepsis," *Intensive Care Medicine*, vol. 34, no. 6, pp. 1046–1053, 2008.

[65] A. Fung, M. Vizcaychipi, D. Lloyd, Y. Wan, and D. Ma, "Central nervous system inflammation in disease related conditions: mechanistic prospects," *Brain Research*, vol. 1446, pp. 144–155, 2012.

[66] J. I. Granger, P. L. Ratti, S. C. Datta et al., "Sepsis-induced morbidity in mice: effects on body temperature, body weight, cage activity, social behavior and cytokines in brain," *Psychoneuroendocrinology*, vol. 38, no. 7, pp. 1047–1057, 2013.

[67] C. M. Comim, M. C. Vilela, L. S. Constantino et al., "Traffic of leukocytes and cytokine up-regulation in the central nervous system in sepsis," *Intensive Care Medicine*, vol. 37, no. 4, pp. 711–718, 2011.

[68] M. D. Laird, J. S. Shields, S. Sukumari-Ramesh et al., "High mobility group box protein-1 promotes cerebral edema after traumatic brain injury via activation of toll-like receptor 4," *Glia*, vol. 62, no. 1, pp. 26–38, 2014.

[69] A.-F. Petit-Bertron, C. Fitting, J.-M. Cavaillon, and M. Adib-Conquy, "Adherence influences monocyte responsiveness to interleukin-10," *Journal of Leukocyte Biology*, vol. 73, no. 1, pp. 145–154, 2003.

[70] H. Sies and E. Cadenas, "Oxidative stress: damage to intact cells and organs," *Philosophical Transactions of the Royal Society of London B: Biological sciences*, vol. 311, no. 1152, pp. 617–631, 1985.

[71] S.-S. Sheu, D. Nauduri, and M. W. Anders, "Targeting antioxidants to mitochondria: a new therapeutic direction," *Biochimica et Biophysica Acta*, vol. 1762, no. 2, pp. 256–265, 2006.

[72] J. F. Turrens, "Mitochondrial formation of reactive oxygen species," *The Journal of Physiology*, vol. 552, no. 2, pp. 335–344, 2003.

[73] A. C. Carr, M. R. McCall, and B. Frei, "Oxidation of LDL by myeloperoxidase and reactive nitrogen species: reaction pathways and antioxidant protection," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 7, pp. 1716–1723, 2000.

[74] C. Szabó, H. Ischiropoulos, and R. Radi, "Peroxynitrite: biochemistry, pathophysiology and development of therapeutics," *Nature Reviews Drug Discovery*, vol. 6, no. 8, pp. 662–680, 2007.

[75] W.-J. Liaw, T.-H. Chen, Z.-Z. Lai et al., "Effects of a membrane-permeable radical scavenger, tempol, on intraperitoneal sepsis-induced organ injury in rats," *Shock*, vol. 23, no. 1, pp. 88–96, 2005.

[76] S. Ohta, "Hydrogen gas and hydrogen water act as a therapeutic and preventive antioxidant with a novel concept," *Nihon Ronen Igakkai Zasshi*, vol. 45, no. 4, pp. 355–362, 2008.

[77] J. Cai, Z. Kang, W. W. Liu et al., "Hydrogen therapy reduces apoptosis in neonatal hypoxia-ischemia rat model," *Neuroscience Letters*, vol. 441, no. 2, pp. 167–172, 2008.

[78] Y.-F. Mao, X.-F. Zheng, J.-M. Cai et al., "Hydrogen-rich saline reduces lung injury induced by intestinal ischemia/reperfusion in rats," *Biochemical and Biophysical Research Communications*, vol. 381, no. 4, pp. 602–605, 2009.

[79] S. Biswal and D. G. Remick, "Sepsis: redox mechanisms and therapeutic opportunities," *Antioxidants & Redox Signaling*, vol. 9, no. 11, pp. 1959–1961, 2007.

[80] A. Nunomura, P. I. Moreira, H. G. Lee et al., "Neuronal death and survival under oxidative stress in Alzheimer and Parkinson diseases," *CNS & Neurological Disorders—Drug Targets*, vol. 6, no. 6, pp. 411–423, 2007.

[81] W. Hoetzenecker, B. Echtenacher, E. Guenova et al., "ROS-induced ATF3 causes susceptibility to secondary infections during sepsis-associated immunosuppression," *Nature Medicine*, vol. 18, no. 1, pp. 128–134, 2012.

[82] M. O. Hengartner, "The biochemistry of apoptosis," *Nature*, vol. 407, no. 6805, pp. 770–776, 2000.

[83] J. Chandra, A. Samali, and S. Orrenius, "Triggering and modulation of apoptosis by oxidative stress," *Free Radical Biology and Medicine*, vol. 29, no. 3-4, pp. 323–333, 2000.

[84] L. Xiang, J. W. Tan, L. J. Huang et al., "Inhalation of hydrogen gas reduces liver injury during major hepatotectomy in swine," *World Journal of Gastroenterology*, vol. 18, no. 37, pp. 5197–5204, 2012.

[85] A. Yoshida, H. Asanuma, H. Sasaki et al., "H_2 mediates cardioprotection via involvements of K_{ATP} channels and permeability transition pores of mitochondria in dogs," *Cardiovascular Drugs and Therapy*, vol. 26, no. 3, pp. 217–226, 2012.

[86] W. Wang, L. Tian, Y. Li et al., "Effects of hydrogen-rich saline on rats with acute carbon monoxide poisoning," *The Journal of Emergency Medicine*, vol. 44, no. 1, pp. 107–115, 2013.

[87] B. Hoesel and J. A. Schmid, "The complexity of NF-κB signaling in inflammation and cancer," *Molecular Cancer*, vol. 12, article 86, 2013.

[88] M. J. Morgan and Z.-G. Liu, "Crosstalk of reactive oxygen species and NF-κB signaling," *Cell Research*, vol. 21, no. 1, pp. 103–115, 2011.

[89] Z. Zhuang, X. J. Sun, X. Zhang et al., "Nuclear factor-κB/Bcl-XL pathway is involved in the protective effect of hydrogen-rich saline on the brain following experimental subarachnoid hemorrhage in rabbits," *Journal of Neuroscience Research*, vol. 91, no. 12, pp. 1599–1608, 2013.

[90] C.-S. Huang, T. Kawamura, X. Peng et al., "Hydrogen inhalation reduced epithelial apoptosis in ventilator-induced lung injury via a mechanism involving nuclear factor-κB activation," *Biochemical and Biophysical Research Communications*, vol. 408, no. 2, pp. 253–258, 2011.

[91] G. Song, H. Tian, J. Liu, H. Zhang, X. Sun, and S. Qin, "H_2 inhibits TNF-α-induced lectin-like oxidized LDL receptor-1 expression by inhibiting nuclear factor κB activation in endothelial cells," *Biotechnology Letters*, vol. 33, no. 9, pp. 1715–1722, 2011.

[92] M. Kobayashi and M. Yamamoto, "Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation," *Antioxidants & Redox Signaling*, vol. 7, no. 3-4, pp. 385–394, 2005.

[93] T. Satoh, S.-I. Okamoto, J. Cui et al., "Activation of the Keap1/Nrf2 pathway for neuroprotection by electrophillic phase II inducers," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 3, pp. 768–773, 2006.

[94] R. K. Thimmulappa, H. Lee, T. Rangasamy et al., "Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis," *The Journal of Clinical Investigation*, vol. 116, no. 4, pp. 984–995, 2006.

[95] S. W. Chung, X. Liu, A. A. Macias, R. M. Baron, and M. A. Perrella, "Heme oxygenase-1-derived carbon monoxide enhances the host defense response to microbial sepsis in mice," *The Journal of Clinical Investigation*, vol. 118, no. 1, pp. 239–247, 2008.

[96] T. Kawamura, N. Wakabayashi, N. Shigemura et al., "Hydrogen gas reduces hyperoxic lung injury via the Nrf2 pathway in vivo," *American Journal of Physiology: Lung Cellular and Molecular Physiology*, vol. 304, no. 10, pp. L646–L656, 2013.

[97] A. Y. Shih, D. A. Johnson, G. Wong et al., "Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress," *The Journal of Neuroscience*, vol. 23, no. 8, pp. 3394–3406, 2003.

[98] X. Zheng, Y. Mao, J. Cai et al., "Hydrogen-rich saline protects against intestinal ischemia/reperfusion injury in rats," *Free Radical Research*, vol. 43, no. 5, pp. 478–484, 2009.

[99] C. Chen, Q. Chen, Y. Mao et al., "Hydrogen-rich saline protects against spinal cord injury in rats," *Neurochemical Research*, vol. 35, no. 7, pp. 1111–1118, 2010.

[100] C. Wang, J. Li, Q. Liu et al., "Hydrogen-rich saline reduces oxidative stress and inflammation by inhibit of JNK and NF-κB activation in a rat model of amyloid-beta-induced Alzheimer's disease," *Neuroscience Letters*, vol. 491, no. 2, pp. 127–132, 2011.

[101] J. Zhou, Y. Chen, G.-Q. Huang et al., "Hydrogen-rich saline reverses oxidative stress, cognitive impairment, and mortality in rats submitted to sepsis by cecal ligation and puncture," *Journal of Surgical Research*, vol. 178, no. 1, pp. 390–400, 2012.

[102] A. M. James, H. M. Cochemé, and M. P. Murphy, "Mitochondria-targeted redox probes as tools in the study of oxidative damage and ageing," *Mechanisms of Ageing and Development*, vol. 126, no. 9, pp. 982–986, 2005.