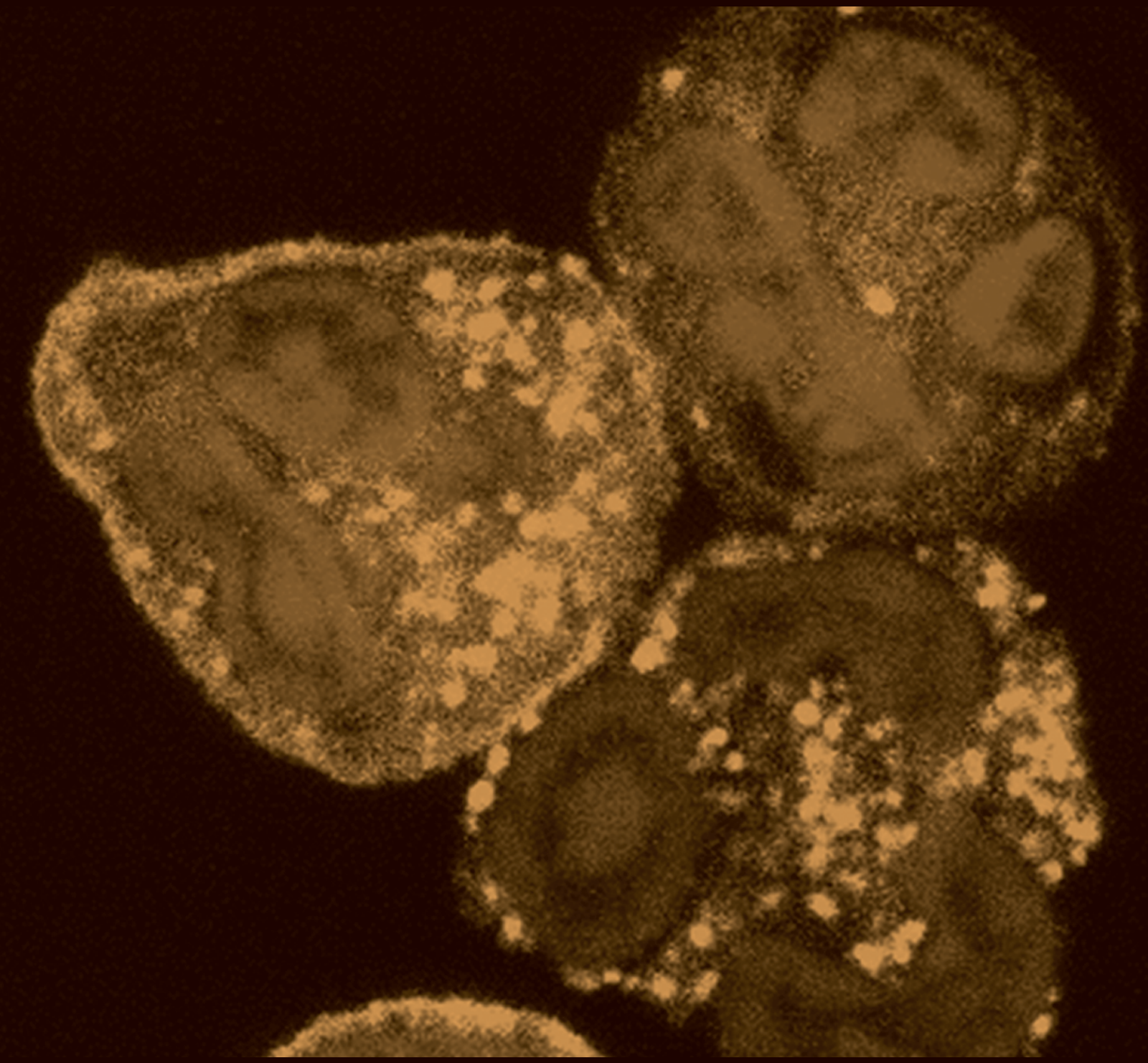
Mediators of Inflammation

# Inflammation in Critical Illness

Guest Editors: David N. Herndon, Celeste C. Finnerty,
and Marc G. Jeschke



# Inflammation in Critical Illness

# Inflammation in Critical Illness

Guest Editors: David N. Herndon, Celeste C. Finnerty, and Marc G. Jeschke

# Editorial Board

# Contents

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 345702, 8 pages
http://dx.doi.org/10.1155/2013/345702

*Research Article*

# Impact of the Body Mass on Complications and Outcome in Multiple Trauma Patients: What Does the Weight Weigh?

**Hagen Andruszkow,[1,2] Juliane Veh,[2] Philipp Mommsen,[2] Christian Zeckey,[2] Frank Hildebrand,[1,2] and Michael Frink[2,3]**

[1] *Department of Trauma and Reconstructive Surgery, University Hospital Aachen, Pauwelsstraße 30, 52074 Aachen, Germany*
[2] *Trauma Department, Hannover Medical School, Carl-Neuberg-Street 1, 30625 Hannover, Germany*
[3] *Department for Trauma, Hand and Reconstructive Surgery, University Medical Center Marburg, Baldingerstraße, 35043 Marburg, Germany*

Correspondence should be addressed to Michael Frink; frink@med.uni-marburg.de

Received 23 July 2012; Revised 24 March 2013; Accepted 22 May 2013

Academic Editor: Celeste C. Finnerty



Obesity is known as an independent risk factor for various morbidities. The influence of an increased body mass index (BMI) on morbidity and mortality in critically injured patients has been investigated with conflicting results. To verify the impact of weight disorders in multiple traumatized patients, 586 patients with an injury severity score >16 points treated at a level I trauma center between 2005 and 2011 were differentiated according to the BMI and analyzed regarding morbidity and outcome. Plasma levels of interleukin- (IL-) 6 and C-reactive protein (CRP) were measured during clinical course to evaluate the inflammatory response to the "double hit" of weight disorders and multiple trauma. In brief, obesity was the highest risk factor for development of a multiple organ dysfunction syndrome (MODS) (OR 4.209, 95%-CI 1.515–11.692) besides injury severity (OR 1.054, 95%-CI 1.020–1.089) and APACHE II score (OR 1.059, 95%-CI 1.001–1.121). In obese patients as compared to those with overweight, normal weight, and underweight, the highest levels of CRP were continuously present while increased systemic IL-6 levels were found until day 4. In conclusion, an altered posttraumatic inflammatory response in obese patients seems to determine the risk for multiple organ failure after severe trauma.

## 1. Introduction

Obesity and overweight represent risk factors for chronic diseases emphasizing diabetes mellitus, hyperlipidemia, heart disease, hypertension, and so forth [1]. While in the United States actually two thirds of the adult population are overweight or obese, obesity is continuously increasing in European citizens with currently approximately 20% [1, 2]. In contrast, the incidences of eating disorders resulting in significant underweight are increasing as well, revealing one of the most common health problems in female adolescents and young women [3, 4]. Physical complications are common among these patients based on endocrinological, electrolyte, hematological, and metabolic abnormalities [3, 4]. Consequently, obesity as well as underweight induce anatomical and physiological changes interfering with the body's response to trauma and emergency surgery [5]. However, consequences of weight disorders on complications and outcome following trauma and emergency surgery are still poorly understood and conflicting results have been reported [6, 7]. Focusing on the relationship between body weight and mortality, a U-shaped correlation between the body mass index and in-hospital mortality is described [2, 8] demonstrating increased mortality in underweight but less markedly in obese patients [2, 7, 8]. Nevertheless, increased morbidity is found in obese patients manifested in impaired hemodynamics and tissue perfusion leading to an increased incidence of multiple organ failure after elective surgery [9]. Consequently, following the literature on critical care medicine, increased mortality in obese patients emphasizing

on critical airway management, difficult surgical exposures, challenging nursing care, and complicated diagnostics is expected [5, 10, 11]. Emerging theories are currently suggesting that trauma- and obesity-induced inflammatory stress plays a pivotal role in increased morbidity and mortality in severe trauma combined with weight disorders [12, 13]. In general, trauma induces an immunological response with a complex acute-phase protein and cytokine release resulting in endothelial cell damage, dysfunction of vascular permeability, microcirculatory disturbances, and necrosis of parenchymal cells [14]. Among inflammatory markers, Interleukin-(IL-) 6 levels currently represent the best correlation with severity of injury and the risk of organ dysfunction [15–18]. In addition, several studies have elucidated increased IL-6 levels in obese patients correlated with insulin resistance [19, 20] and the incidence of diabetes [21].

The present study intends to clarify the relationship between body mass index, complications and outcome after severe multiple trauma. In order to understand the biochemical coherences in these patients in vivo, measurements of relevant inflammatory mediators are ascertained.

## 2. Material and Methods

The present study follows the guidelines of the revised UN declaration of Helsinki in 1975 and its latest amendment in 1996 (42nd general meeting).

*2.1. Study Design and Inclusion Criteria.* A retrospective analysis of all multiple traumatized patients, defined as Injury Severity Score (ISS) ≥16 points, primarily admitted to a level I trauma center between January 1, 2005 and June 30, 2011, was performed. Further inclusion criteria were admission to intensive care unit (ICU) within the first 24 hours and age ≥16 years. Patients with incomplete data referring to weight or height preventing BMI measurements were excluded.

*2.2. Body Mass Index (BMI).* Admission weight and height were used to calculate the body mass index (BMI ($kg/m^2$)). Patients were assigned to four groups based on the BMI using the classification of the World Health Organization as follows: Group I (underweight) (i) BMI < 20.0 $kg/m^2$; Group II (normal weight) (ii) BMI 20.0–24.9 $kg/m^2$; Group III (overweight) (iii) BMI 25.0–29.9 $kg/m^2$; Group IV (obesity) (iv) BMI > 30.0 $kg/m^2$. Demographics, mechanism of injury, injury distribution and severity, clinical course, serum markers, complications, and outcome were analyzed.

*2.3. Injury Severity and Clinical Course.* Injury distribution was determined with the 2005 revised edition of the Abbreviated Injury Scale (AIS) and summarized to the Injury Severity Score (ISS) reflecting the overall injury severity [22]. Each injury is assigned an AIS score according to its relative importance on a six-point scale (1, minor; 2, moderate; 3, serious; 4, severe; 5, critical; 6, unsurvivable) and is allocated to one of six body regions: head and neck; face; thorax; abdomen; extremities (including pelvis); and external. Only the highest AIS score in each body region is used. The three most severely injured body regions have their score squared and added together to the ISS [22].

Clinical course included duration of ventilation (hours), duration of ICU treatment (days), and the overall length of stay (LOS) in days. The duration of initial emergency surgery (minutes) was defined as any surgery performed in the operating room within the first 24 hours after hospital admission.

*2.4. Complications and Outcome.* In order to estimate the risk of mortality, the "Acute Physiology and Chronic Health Evaluation II" (APACHE II) score [23] and its predicted mortality were documented on admission to ICU after emergency surgery was performed.

Complications analyzed included the "systemic inflammatory response syndrome" (SIRS), sepsis, the acute respiratory distress syndrome (ARDS), and the multiple organ dysfunction syndrome (MODS). SIRS and sepsis were classified according to the definitions for sepsis and organ failure [24]. ARDS was assumed following the recommendations of the "The American-European Consensus Conference on ARDS" [25, 26]. The incidence of MODS was categorized as described by Marshall et al. [27] considering multiple organ failure if the score was greater than 12 points on two consecutive days or at least three days during a 14-day observation period [27, 28]. primary outcome was defined as mortality during clinical course.

*2.5. Inflammatory Biomarkers.* C-Reactive protein (CRP) and IL-6 levels were measured with blood samples taken during emergency room management after hospital admission as a standard procedure. The samples represent the immunological response to trauma before emergency surgery was initiated. During the ICU stay, blood samples were taken repetitively every morning at 07:00 a.m. for at least 14 days after admission to ICU.

*2.6. Statistical Methods.* The data were analyzed using the Statistical Package for the Social Sciences (SPSS; version 19; IBM Inc., Somers, NY, USA). Incidences are presented with counts and percentages while continuous values are presented as mean ± standard deviation (SD). Differences between the groups were evaluated with analysis of variance (ANOVA) for continuous data, while Pearson's $\chi^2$-test was used for categorical values. A pairwise comparison was not performed due to an increased type I error. In order to reveal the impact of BMI on complications, a multivariate logistic regression analysis was performed with MODS as a target variable and BMI group, injury severity (ISS), and APACHE II score as potential predictors. Odds ratios with 95% confidence intervals (95%-CI) were noted. The Spearman rank correlation coefficient was used to determine the connection between IL-6 and CRP levels and development of MODS. A two-sided $P$ value < 0.05 was considered to be significant.

## 3. Results

660 adult multiple traumatized patients were treated between January 1, 2005, and June 30, 2011. A total of 74 patients

Table 1: Study population and characteristics according to the BMI.

| | All | Underweight | Normal | Overweight | Obesity | *P* value |
|---|---|---|---|---|---|---|
| Number of patients (%) | 586 (100.0%) | 28 (4.8%) | 265 (45.2%) | 211 (36.0%) | 82 (14.0%) | — |
| Age (years) | **42.0 ± 18.0** | **33.6 ± 19.7** | **37.2 ± 17.4** | **46.7 ± 17.7** | **48.0 ± 14.9** | **<0.001** |
| Male (%) | **410 (70.0%)** | **9 (32.1%)** | **176 (66.4%)** | **162 (76.8%)** | **63 (76.8%)** | **<0.001** |
| GCS | 10.5 ± 4.8 | 9.5 ± 5.1 | 10.1 ± 4.8 | 10.8 ± 4.7 | 11.4 ± 4.7 | 0.066 |
| AIS head | **1.9 ± 1.8** | **2.4 ± 2.0** | **2.0 ± 1.8** | **1.9 ± 1.8** | **1.5 ± 1.5** | **0.034** |
| AIS face | 1.0 ± 1.2 | 1.0 ± 1.2 | 1.0 ± 1.3 | 1.0 ± 1.2 | 0.8 ± 1.0 | 0.337 |
| AIS chest | 2.7 ± 1.5 | 2.6 ± 1.5 | 2.6 ± 1.5 | 2.7 ± 1.6 | 3.0 ± 1.2 | 0.175 |
| AIS abdomen | 1.2 ± 1.6 | 1.5 ± 1.8 | 1.2 ± 1.6 | 1.1 ± 1.5 | 1.3 ± 1.6 | 0.485 |
| AIS extremities | 2.1 ± 1.4 | 1.8 ± 1.4 | 2.2 ± 1.4 | 2.1 ± 1.4 | 2.2 ± 1.3 | 0.285 |
| AIS extern | 1.0 ± 1.1 | 1.0 ± 1.0 | 1.1 ± 1.1 | 1.0 ± 1.1 | 1.1 ± 1.1 | 0.822 |
| ISS | 28.8 ± 10.7 | 29.8 ± 11.2 | 29.1 ± 10.7 | 28.9 ± 11.1 | 27.1 ± 9.7 | 0.502 |

GCS: Glasgow Coma Scale.
AIS: Abbreviated Injury Scale.
ISS: Injury Severity Score.

Table 2: Clinical course according to the BMI.

| | Underweight | Normal | Overweight | Obesity | *P* value |
|---|---|---|---|---|---|
| Duration of initial emergency surgery (min.) | 94.6 ± 81.7 | 85.0 ± 87.3 | 90.1 ± 89.5 | 105.4 ± 87.7 | 0.329 |
| Duration of ventilation (hours) | **191.1 ± 211.0** | **258.0 ± 304.1** | **329.7 ± 403.7** | **359.3 ± 366.9** | **0.028** |
| Duration of ICU treatment (days) | **11.6 ± 8.9** | **14.3 ± 13.7** | **17.4 ± 18.0** | **18.6 ± 17.5** | **0.044** |
| Length of stay (days) | **20.2 ± 10.5** | **25.7 ± 18.8** | **28.4 ± 22.6** | **34.4 ± 26.0** | **0.005** |

(11.2%) were excluded due to missing body weight or height data preventing BMI measurements.

Including 586 patients, the overall mean BMI was 26.0 ± 4.5 kg/m$^2$ (range 15.1–56.8 kg/m$^2$). The male to female ration was 2.3 : 1 with a mean age of 42.0 ± 18.0 years.

*3.1. BMI, Injury Severity, and Clinical Course.* Diversifying the included trauma collective, 4.8% patients were underweight, 45.2% had normal weight, 36.0% were overweight, and 14.0% were obese. We elucidated significant differences between these four groups with regard to age, gender, and severity of head injuries (Table 1). Obese patients demonstrated the highest age and ratio of males while underweight patients were younger females (Table 1). Analyzing injury distribution, the lowest severity of head injuries was noted in obese patients while no differences were found regarding the overall injury severity as well as the remaining injury distribution (Table 1).

Duration of ventilation, length of ICU treatment and the overall length of stay increased consistently with the BMI. Duration of initial emergency surgery was not influenced by the BMI at admission (Table 2).

*3.2. Complications and Outcome.* According to the measured APACHE II score and its predicted mortality, no differences could be demonstrated between the BMI groups (Table 3). Furthermore, no statistical differences referring to incidence of SIRS, sepsis, and ARDS were evaluated between the BMI groups. However, a strong trend towards an increased rate of MODS in obese patients was proven (Table 4).

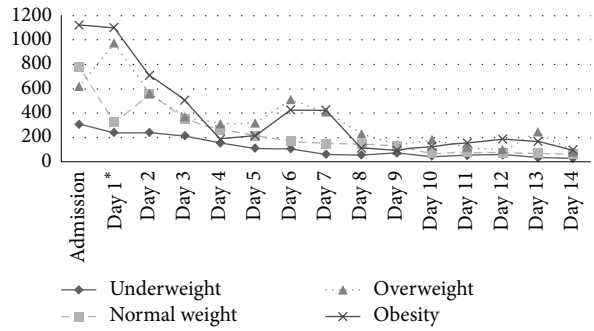



Figure 1: Mean IL-6 courses (pg/mL) evaluated from admission to day 14 according to the BMI groups. Significant differences ($P < 0.05$) are marked with an asterisk referring to the day of distinction.

In a multivariate analysis, obesity was revealed as the highest risk factor for development of multiple organ dysfunction syndrome (OR 4.209, 95%-CI 1.515–11.692; $P = 0.006$) beside injury severity (OR 1.054, 95%-CI 1.020–1.089; $P = 0.001$) and the prognostic APACHE II score (OR 1.059, 95%-CI 1.001–1.121; $P = 0.047$) (Table 5).

Regarding outcome, there was no influence of the nutrition status on mortality although mortality was increased in underweight patients without reaching statistical significance ($P = 0.484$).

*3.3. Inflammatory Biomarkers.* The descriptive course of IL-6 and CRP according to the different BMI groups is illustrated in Figures 1 and 2. Descriptively, obese patients have been

Table 3: Risk profile measured by the APACHE II score, its expected mortality, and outcome.

| | Underweight | Normal | Overweight | Obesity | *P* value |
|---|---|---|---|---|---|
| APACHE II (points) | 14.2 ± 6.8 | 12.7 ± 6.9 | 13.9 ± 7.4 | 14.1 ± 7.0 | 0.228 |
| Expected mortality (%) | 23.0 ± 15.0 | 20.0 ± 14.4 | 23.0 ± 16.8 | 22.7 ± 16.6 | 0.192 |
| Observed mortality (%) | 5 (17.9%) | 25 (9.4%) | 24 (11.4%) | 7 (8.5%) | 0.484 |

Table 4: Clinical complications according to the BMI.

| | Underweight | Normal | Overweight | Obesity | *P* value |
|---|---|---|---|---|---|
| SIRS | 16 (57.1%) | 189 (71.3%) | 156 (73.9%) | 62 (75.6%) | 0.256 |
| Sepsis | 9 (32.1%) | 107 (40.4%) | 91 (43.1%) | 37 (45.1%) | 0.612 |
| ARDS | 10 (35.7%) | 78 (29.4%) | 72 (34.1%) | 31 (37.8%) | 0.463 |
| MODS | 1 (3.6%) | 9 (3.4%) | 12 (5.7%) | 9 (11.0%) | 0.060 |

SIRS: Systemic Inflammatory Response Syndrome.
ARDS: Acute Respiratory Distress Syndrome.
MODS: Multiple Organ Dysfunction Syndrome.

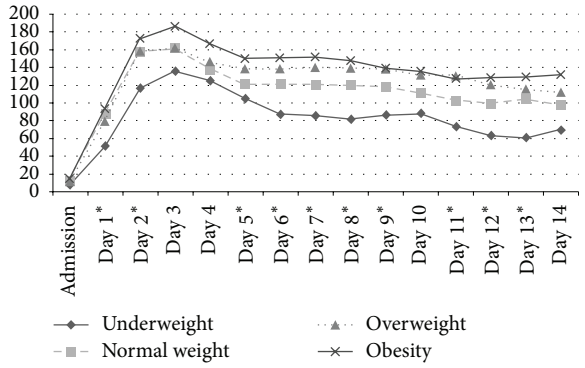



Figure 2: Mean CRP courses (mg/L) evaluated from admission to day 14 according to the BMI groups. Significant differences ($P < 0.05$) are marked with an asterisk referring to the day of distinction.

admitted with increased mean plasma IL-6 levels (1,122.6 ± 4,376.5 pg/mL) followed by patients with normal weight (779.9 ± 1,741.6 pg/mL), overweight (620.9 ± 1,330.0 pg/mL), and underweight (310.0 ± 494.5 pg/ mL) ($P = 0.470$) (Figure 1). However, on day 1 IL-6 values in obese patients were comparable to levels found at admission while in overweight patients an increase was revealed. In contrast, IL-6 levels in normal and underweight patients were decreased at that time ($P = 0.008$). Overall, underweight patients demonstrated the lowest systemic IL-6 values during the observed period (Figure 1). Nevertheless, statistical significant differences of IL-6 levels according to the different BMI groups were only verified on day 1 ($P = 0.008$). Emphasizing on the impact of IL-6, a positive correlation with the incidence of MODS was present at any time during the study period (Table 6).

Regarding CRP values, obese patients demonstrated increased levels after admission without reaching statistical significance (BMI group I 7.5 ± 11.8 mg/L; BMI group II 12.6 ± 24.5 mg/L; BMI group III 11.5 ± 25.1 mg/L; BMI group IV 15.3 ± 25.6 mg/L; $P = 0.452$). A continuous increase with a maximum on day 3 was observed followed by a continuous decrease during the observation period. During the first 14 days highest levels were observed in obese patients followed by overweight, normal weight, and underweight patients. Significant differences between the illustrated CRP values according to the BMI groups were found from day 1 to day 3, from day 4 to 9, and day 11 to day 13 (Figure 2). Increased CRP values were positively correlated with the incidence of MODS from day 3 (Table 6).

## 4. Discussion

The influence of an increased body mass index on morbidity and mortality in critically injured patients has shown conflicting results [5, 29, 30]. However, the present study provides new insights regarding impact of body weight in multiple traumatized patients evaluating the posttraumatic systemic immune response. The main results of the present study could be summarized as follows:

(i) obesity was revealed as highest risk factor for development of MODS while no influence on mortality was shown. Although not reaching statistical significance, underweight patients showed twice the mortality rate as compared to obese patients which may be of clinical significance and should be considered in future diagnostic and therapeutic steps,

(ii) obese patients have been admitted with increased IL-6 levels for the first four days. Overall, underweight patients demonstrated the lowest IL-6 values during the observed period,

(iii) measuring systemic CRP levels, obese patients had significantly increased values for almost the complete study period,

(iv) increased IL-6 levels were positively correlated with the incidence of MODS during the whole study period, while increased CRP levels were positively correlated with MODS after day 3.

The first study investigating the relationship between body mass and trauma was performed in 1991 by Choban et al. [11] including 184 multiple traumatized patients. The authors showed a 42% mortality rate in obese patients compared to 5% in those with normal weight. Due to the remaining question, if this tremendous difference in mortality is based upon a specific injury pattern, Boulanger et al. focused on the body habitus as a predictor for injury pattern [10]. Interestingly, obese trauma victims were more likely to suffer from severe thoracic injuries but incidence of severe head injuries decreased [10]. According to the results of our study with less severe head injuries in obese patients, obesity seems

Table 5: Multivariate regression analysis referring to MODS analyzing BMI, injury severity (ISS), and APACHE II score as potential predictors.

| Predictor | Regression coefficient | Odds ratio (OR) | 95% confidence interval (95%-CI) | *P* value |
|---|---|---|---|---|
| Underweight | 0.254 | 1.289 | 0.152–10.9666 | 0.816 |
| Overweight | 0.606 | 1.832 | 0.718–4.675 | 0.205 |
| Obesity | **1.437** | **4.209** | **1.515–11.692** | **0.006** |
| Injury severity (ISS) | **0.053** | **1.054** | **1.020–1.089** | **0.001** |
| APACHE II | **0.058** | **1.059** | **1.001–1.121** | **0.047** |
| Constant | −5.935 | — | — | <0.001 |

BMI group II (normal weight) was set as a categorical reference group for regression analysis between the BMI groups.

Table 6: Correlation of systemic plasma IL-6 and CRP values with the incidence of MODS during the clinical course.

| | Correlation coefficient IL-6 | Correlation coefficient CRP |
|---|---|---|
| Admission | **0.222*** | 0.054 |
| Day 1 | **0.141*** | 0.045 |
| Day 2 | **0.154*** | 0.036 |
| Day 3 | **0.148*** | **0.093*** |
| Day 4 | **0.170*** | **0.120*** |
| Day 5 | **0.122*** | **0.130*** |
| Day 6 | **0.241*** | **0.129*** |
| Day 7 | **0.186*** | **0.122*** |
| Day 8 | **0.175*** | **0.129*** |
| Day 9 | **0.154*** | **0.156*** |
| Day 10 | **0.184*** | **0.183*** |
| Day 11 | **0.266*** | **0.191*** |
| Day 12 | **0.250*** | **0.239*** |
| Day 13 | **0.261*** | **0.271*** |
| Day 14 | **0.197*** | **0.277*** |

Spearman's rank correlation; $^{*}P < 0.05$.

to influence the injury pattern even 20 years later in the same way despite improving active and passive vehicle safety systems [31].

Several reports were recently performed due to increasing public awareness of the obesity epidemic and its wide influence [30]. Referring to differences in study designs diverse findings according to morbidity and mortality in obese patients were revealed [2, 5–9, 29]. In the current study we were able to identify obesity as the most important risk factor for the development of MODS but interestingly without significantly influencing mortality. In line with these results, obesity has been verified as an isolated risk factor for postinjury multiple organ failure with an odds ratio of 1.8 beside age, massive blood transfusion, and injury severity in a recent study [29]. Moreover, obesity increased length of ICU treatment and in-hospital time [29]. Similar to the presented results no differences were evaluated referring to mortality. The prolonged requirement of ICU treatment and in-hospital time in our as well as other studies may be a direct consequence of increased incidence of MODS [32, 33]. According to Newell et al., obesity in traumatized patients influenced multiple organ failure with a risk of 2.6 odds ratio without effecting mortality (OR 0.81) [30]. In contrast, Neville and colleagues showed an increased incidences of multiple organ failure (13% versus 3%) and increased mortality rates (32% versus 16%) in obese critically injured patients [5]. Contrary to the presented results, the authors assigned their patients only to two groups, obese (BMI ≥ 30) and nonobese (BMI < 30) which might not adequately consider the complexity of weight disorders. One of the largest studies with data of 5,766 traumatized patients from the Trauma Registry of the German Trauma Society showed an increased incidence of multiple organ failure in obese patients while underweight was associated with a lower incidence [7]. Similar to our results no differences between the BMI groups were revealed referring to mortality in a direct comparison. But according to a multivariate analysis, underweight as well as obesity were associated with increased mortality [7]. In brief summary, considering these studies an indisputable association of weight disorders and morbidity and respectively mortality was shown. Although these results are confirmed by data of the present study, to date no potential reasons for the increased morbidity and mortality were proven. One explanatory approach was found by Belzberg et al., who measured reduced tissue perfusion and cardiac output in obese nonsurvivors following severe trauma [9]. Consequently, emerging theories suggested that the cytokine response to trauma as well as surgical interventions might be altered in obesity. Lately, IL-6 as a part of the proinflammatory cascade was verified as the most reliable prognostic marker following trauma [16]. IL-6 not only correlates with injury severity [17] but is highly associated with multiple organ failure and outcome [16, 18]. Congruent to these findings, increased levels of IL-6 were strongly correlated with the incidence of MODS during the whole study period in the presented study. However, increased systemic concentrations of proinflammatory cytokines such as IL-6 are associated with systemic insulin resistance [19, 20] and the incidence of diabetes [21]. Considering that basal systemic IL-6 concentrations are enhanced in obese patients increased plasma levels could be expected following major trauma. Following surgical stress, Gletsu et al. monitored plasma and adipose tissue concentrations of IL-6 after abdominal surgery [13]. The circulating IL-6 concentrations at baseline and after surgery were related to the abdominal

adipose tissue content and exaggerated in obese patients. After surgery, worsening of insulin resistance was correlated with increased systemic as well as adipose tissue content of IL-6 [13]. The effect of surgical interventions on IL-6 release might be similar in major trauma considering the presented results. Comparing systemic IL-6 levels on admission and day one, considerable differences of IL-6 values dependent on the nutrition status were found demonstrating a systemic increase in overweight and obese patients as compared to normal-weight and underweight patients. Since the durations of the initial surgery and the injury severity were comparable between BMI groups, an influence of the nutrition status could be suggested. An increased initial inflammatory state illustrated by systemic IL-6 levels was shown to enhance the sensitivity and vulnerability to trauma [34]. Thus, elevated systemic IL-6 levels in obese trauma victims on admission in the presented study may be from relevance for the further clinical course. In addition, the lowest levels of IL-6 in underweight may result of reduced adipose tissue and fatty acids which release inflammation-related adipokines commonly related to obesity-associated pathologies [35]. Contrary to the proinflammatory adipokines, adiponectin acts as insulin-sensitizing, antiatheroganic hormone with an anti-inflammatory potential [36]. This interaction of pro- and anti-inflammatory mediators is demonstrated to be negatively influenced in obesity which has been characterized as a continuous state of mild inflammation [35, 37, 38].

Measuring an inflammatory status, the C-reactive protein has been proven to be one of the most sensitive inflammatory markers [37, 38]. Furthermore, current studies revealed an association with atherosclerotic diseases and diabetes mellitus type II [39, 40]. Within the regulation of the inflammatory cascade, CRP suppresses adiponectin exaggerating to the inflammatory reaction in obesity [37, 38]. This exaggeration of inflammation could be demonstrated according to the presented results illustrating increased systemic CRP levels for almost the complete study period in obese patients. Similar to our suggestions referring the IL-6 values, the highest measurements of CRP in obese patients and, respectively, the lowest in underweight seemed not to be influenced by injury severity, duration of emergency surgery, or the incidences of clinical complications. Similarly, Kraft et al. demonstrated increased CRP and triglyceride levels in overweight pediatric burn patients [41]. The authors explained this finding with the increased proinflammatory, catabolic state of obese patients [41]. Although the prognostic power of CRP referring to complications is debatable [42, 43], increased CRP levels were correlated with the incidence of MODS after day 3 in the present study.

However, the presented study has notable limitations. Apriori, a substantial number of patients had to be excluded due to missing weight and height data preventing the BMI measurement. This limiting aspect is known in comparable studies [2, 5, 7, 8, 30], wherein missing data lead to exclusion up to 17%. In addition, excluded patients in the presented study had the same characteristics as compared to the whole population (data not shown). Therefore, we do not expect this subgroup to compromise the demonstrated results. Nevertheless, one can argue whether the BMI represents an accurate tool to determine weight disorders. Even though a correlation exists between BMI and body fat (%), classification of individual weight disorders may show discrepancies when body fat analyses were performed [44]. Although divergent results have been demonstrated whether BMI or body fat measurements are more accurate to determine potential outcome prognoses [45], we feel safe to use BMI measurement since it represents the mostly accepted parameter in the current literature analyzing trauma populations [2, 5, 7, 8, 30, 41]. Emphasizing our suggestions of adipokines and adiponectin influencing the inflammatory response in multiple trauma, this aspect could not be proven due to the retrospective design of this study. These hormones are not measured as standard laboratory parameters in our multiple trauma patients. To field this limiting aspect, further research is required in order to verify presumable correlations between IL-6 and CRP with these hormones, which have been related to common obesity-associated pathologies.

## 5. Conclusion

According to the presented results, obesity was revealed as an independent risk factor for the development of MODS in severely traumatized patients. In obese patients, systemic IL-6 levels were elevated until day four while CRP presented the highest levels in obese patients followed by overweight, normal-weight, and underweight patients during the whole period. In conclusion, an altered inflammatory reaction following the "double hit" of obesity and multiple trauma seems to determine the risk for multiple organ failure after severe trauma. Thus, the nutrition status seems to play a pivotal role in the posttraumatic clinical course and therefore should be considered in therapeutic strategies in patients suffering from major trauma.

Further research with attention to obese patients might ascertain presumable correlations of IL-6 and CRP with adipokines as well as adiponectin, which are commonly related to obesity-associated pathologies.

## Conflict of Interests

The authors declare no financial conflict of interests.

## References

[1] C. M. Doak, T. M. A. Wijnhoven, D. F. Schokker, T. L. S. Visscher, and J. C. Seidell, "Age standardization in mapping adult overweight and obesity trends in the WHO European Region," *Obesity Reviews*, vol. 13, no. 2, pp. 174–191, 2012.

[2] J. M. O'Brien Jr., G. S. Phillips, N. A. Ali, M. Lucarelli, C. B. Marsh, and S. Lemeshow, "Body mass index is independently associated with hospital mortality in mechanically ventilated adults with acute lung injury," *Critical Care Medicine*, vol. 34, no. 3, pp. 738–744, 2006.

[3] R. D. Grave, "Eating disorders: progress and challenges," *European Journal of Internal Medicine*, vol. 22, no. 2, pp. 153–160, 2011.

[4] C. G. Fairburn and P. J. Harrison, "Eating disorders," *Lancet*, vol. 361, no. 9355, pp. 407–416, 2003.

[5] A. L. Neville, C. V. R. Brown, J. Weng, D. Demetriades, and G. C. Velmahos, "Obesity is an independent risk factor of mortality in severely injured blunt trauma patients," *Archives of Surgery*, vol. 139, no. 9, pp. 983–987, 2004.

[6] R. Maheshwari, C. D. Mack, R. P. Kaufman et al., "Severity of injury and outcomes among obese trauma patients with fractures of the femur and tibia: a crash injury research and engineering network study," *Journal of Orthopaedic Trauma*, vol. 23, no. 9, pp. 634–639, 2009.

[7] M. Hoffmann, R. Lefering, M. Gruber-Rathmann, J. M. Rueger, and W. Lehmann, "The impact of BMI on polytrauma outcome," *Injury*, vol. 43, no. 2, pp. 184–188, 2012.

[8] A. Tremblay and V. Bandi, "Impact of body mass index on outcomes following critical care," *Chest*, vol. 123, no. 4, pp. 1202–1207, 2003.

[9] H. Belzberg, C. C. J. Wo, D. Demetriades, and W. C. Shoemaker, "Effects of age and obesity on hemodynamics, tissue oxygenation, and outcome after trauma," *Journal of Trauma*, vol. 62, no. 5, pp. 1192–1200, 2007.

[10] B. R. Boulanger, D. Milzman, K. Mitchell, and A. Rodriguez, "Body habitus as a predictor of injury pattern after blunt trauma," *Journal of Trauma*, vol. 33, no. 2, pp. 228–232, 1992.

[11] P. S. Choban, L. J. Weireter Jr., and C. Maynes, "Obesity and increased mortality in blunt trauma," *Journal of Trauma*, vol. 31, no. 9, pp. 1253–1257, 1991.

[12] L. Xiang, R. L. Hester, W. L. Fuller et al., "Orthopedic trauma-induced pulmonary injury in the obese Zucker rat," *Microcirculation*, vol. 17, no. 8, pp. 650–659, 2010.

[13] N. Gletsu, E. Lin, J.-L. Zhu et al., "Increased plasma interleukin 6 concentrations and exaggerated adipose tissue interleukin 6 content in severely obese patients after operative trauma," *Surgery*, vol. 140, no. 1, pp. 50–57, 2006.

[14] M. Keel and O. Trentz, "Pathophysiology of polytrauma," *Injury*, vol. 36, no. 6, pp. 691–709, 2005.

[15] F. Hildebrand, P. Giannoudis, C. Kretteck, and H.-C. Pape, "Damage control: extremities," *Injury*, vol. 35, no. 7, pp. 678–689, 2004.

[16] J. Cuschieri, E. Bulger, V. Schaeffer et al., "Early elevation in random plasma IL-6 after severe injury is associated with development of organ failure," *Shock*, vol. 34, no. 4, pp. 346–351, 2010.

[17] F. Gebhard, H. Pfetsch, G. Steinbach, W. Strecker, L. Kinzl, and U. B. Brückner, "Is interleukin 6 an early marker of injury severity following major trauma in humans?" *Archives of Surgery*, vol. 135, no. 3, pp. 291–295, 2000.

[18] R. S. Jawa, S. Anillo, K. Huntoon, H. Baumann, and M. Kulaylat, "Interleukin-6 in surgery, trauma, and critical care Part II: clinical implications," *Journal of Intensive Care Medicine*, vol. 26, no. 2, pp. 73–87, 2011.

[19] A. N. Vgontzas, D. A. Papanicolaou, E. O. Bixler et al., "Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia," *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 3, pp. 1151–1158, 2000.

[20] P. Ziccardi, F. Nappo, G. Giugliano et al., "Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year," *Circulation*, vol. 105, no. 7, pp. 804–809, 2002.

[21] J. Spranger, A. Kroke, M. Möhlig et al., "Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study," *Diabetes*, vol. 52, no. 3, pp. 812–817, 2003.

[22] S. P. Baker, B. O'Neill, W. Haddon Jr., and W. B. Long, "The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care," *Journal of Trauma*, vol. 14, no. 3, pp. 187–196, 1974.

[23] 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.

[24] "American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis," *Critical Care Medicine*, vol. 20, no. 6, pp. 864–874, 1992.

[25] G. R. Bernard, A. Artigas, K. L. Brigham et al., "The American-European Consensus Conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination," *American Journal of Respiratory and Critical Care Medicine*, vol. 149, no. 3, pp. 818–824, 1994.

[26] G. R. Bernard, A. Artigas, K. L. Brigham et al., "Report of the American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes and clinical trial coordination," *Intensive Care Medicine*, vol. 20, no. 3, pp. 225–232, 1994.

[27] J. C. Marshall, D. J. Cook, N. V. Christou, G. R. Bernard, C. L. Sprung, and W. J. Sibbald, "Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome," *Critical Care Medicine*, vol. 23, no. 10, pp. 1638–1652, 1995.

[28] M. Frink, H.-C. Pape, M. Van Griensven, C. Krettek, I. H. Chaudry, and F. Hildebrand, "Influence of sex and age on mods and cytokines after multiple injuries," *Shock*, vol. 27, no. 2, pp. 151–156, 2007.

[29] D. J. Ciesla, E. E. Moore, J. L. Johnson, J. M. Burch, C. C. Cothren, and A. Sauaia, "Obesity increases risk of organ failure after severe trauma," *Journal of the American College of Surgeons*, vol. 203, no. 4, pp. 539–545, 2006.

[30] M. A. Newell, M. R. Bard, C. E. Goettler et al., "Body mass index and outcomes in critically injured blunt trauma patients: weighing the impact," *Journal of the American College of Surgeons*, vol. 204, no. 5, pp. 1056–1061, 2007.

[31] F. Tagliaferri, C. Compagnone, N. Yoganandan, and T. A. Gennarelli, "Traumatic brain injury after frontal crashes: relationship with body mass index," *The Journal of trauma*, vol. 66, no. 3, pp. 727–729, 2009.

[32] M. Talmor, L. Hydo, and P. S. Barie, "Relationship of systemic inflammatory response syndrome to organ dysfunction, length of stay, and mortality in critical surgical illness: effect of intensive care unit resuscitation," *Archives of Surgery*, vol. 134, no. 1, pp. 81–87, 1999.

[33] D. C. Dewar, P. MacKay, and Z. Balogh, "Epidemiology of post-injury multiple organ failure in an australian trauma system," *ANZ Journal of Surgery*, vol. 79, no. 6, pp. 431–436, 2009.

[34] R. C. Bone, "Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation," *Critical Care Medicine*, vol. 24, no. 1, pp. 163–172, 1996.

[35] P. Trayhurn, "Endocrine and signalling role of adipose tissue: new perspectives on fat," *Acta Physiologica Scandinavica*, vol. 184, no. 4, pp. 285–293, 2005.

[36] A. H. Berg, T. P. Combs, and P. E. Scherer, "ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism," *Trends in Endocrinology and Metabolism*, vol. 13, no. 2, pp. 84–89, 2002.

[37] G. Yuan, X. Chen, Q. Ma et al., "C-reactive protein inhibits adiponectin gene expression and secretion in 3T3-L1 adipocytes," *Journal of Endocrinology*, vol. 194, no. 2, pp. 275–281, 2007.

[38] G. Yuan, J. Jia, L. Di et al., "Effects of C-reactive protein on adipokines genes expression in 3T3-L1 adipocytes," *Biochemical and Biophysical Research Communications*, vol. 424, no. 3, pp. 462–468, 2012.

[39] P. Libby, P. M. Ridker, and A. Maseri, "Inflammation and atherosclerosis," *Circulation*, vol. 105, no. 9, pp. 1135–1143, 2002.

[40] A. Festa, R. D'Agostino Jr., R. P. Tracy, and S. M. Haffner, "Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study," *Diabetes*, vol. 51, no. 4, pp. 1131–1137, 2002.

[41] R. Kraft, D. N. Herndon, F. N. Williams, A. M. Al-Mousawi, C. C. Finnerty, and M. G. Jeschke, "The effect of obesity on adverse outcomes and metabolism in pediatric burn patients," *International Journal of Obesity*, vol. 36, no. 4, pp. 485–490, 2012.

[42] Z. Lausevic, M. Lausevic, J. Trbojevic-Stankovic, S. Krstic, and B. Stojimirovic, "Predicting multiple organ failure in patients with severe trauma," *Canadian Journal of Surgery*, vol. 51, no. 2, pp. 97–102, 2008.

[43] M. Meisner, H. Adina, and J. Schmidt, "Correlation of procalcitonin and C-reactive protein to inflammation, complications, and outcome during the intensive care unit course of multiple-trauma patients," *Critical Care*, vol. 10, no. 1, article R1, 2005.

[44] A. De Schutter, C. J. Lavie, K. Arce, S. G. Menendez, and R. V. Milani :, "Correlation and discrepancies between obesity by body mass index and body fat in patients with coronary heart disease," *Journal of Cardiopulmonary Rehabilitation and Prevention*, vol. 33, no. 2, pp. 77–83, 2013.

[45] N. Scheuing, C. Bayer, F. Best et al., "Is there a benefit to use calculated percent body fat or age- and gender-adjusted BMI-SDSLMS to predict risk factors for cardiovascular disease? A German/Austrian multicenter DPV-Wiss analysis on 42 048 type 2 diabetic patients," *Exp Clin Endocrinol Diabetes*, vol. 121, no. 2, pp. 67–74, 2013.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 969875, 9 pages
http://dx.doi.org/10.1155/2013/969875

*Clinical Study*

# Diagnostic Value of Dynamics Serum sCD163, sTREM-1, PCT, and CRP in Differentiating Sepsis, Severity Assessment, and Prognostic Prediction

**Longxiang Su,[1,2,3] Lin Feng,[4] Qing Song,[5] Hongjun Kang,[5] Xingang Zhang,[1] Zhixin Liang,[1] Yanhong Jia,[1] Dan Feng,[6] Changting Liu,[3] and Lixin Xie[1]**

[1] *Department of Respiratory Medicine, Chinese PLA General Hospital, 28 Fuxing Road, Haidian District, Beijing 100853, China*
[2] *Medical College, Nankai University, Tianjin 300071, China*
[3] *Nanlou Respiratory Disease Department, Chinese PLA General Hospital, Beijing 100853, China*
[4] *Department of Respiratory Medicine, Guangzhou Women and Children Medical Care Center, Guangzhou, Guangdong 510623, China*
[5] *Department of Critical Care Medicine, Chinese PLA General Hospital, Beijing 100853, China*
[6] *Department of Medical Statistics, Chinese PLA General Hospital, Beijing 100853, China*

Correspondence should be addressed to Lixin Xie; xielx@263.net

Received 24 September 2012; Accepted 22 May 2013

Academic Editor: Celeste C. Finnerty



*Objective*. To describe the dynamics changes of sCD163, soluble serum triggering receptor expressed on myeloid cells-1 (sTREM-1), procalcitonin (PCT), and C-reactive protein (CRP) during the course of sepsis, as well as their outcome prediction. *Patients and Methods*. An SIRS group (30 cases) and a sepsis group (100 cases) were involved in this study. Based on a 28-day survival, the sepsis was further divided into the survivors' and nonsurvivors' groups. Serum sTREM-1, sCD163, PCT, CRP, and WBC counts were tested on days 1, 3, 5, 7, 10, and 14. *Results*. On the ICU admission, the sepsis group displayed higher levels of sTREM-1, sCD163, PCT, and CRP than the SIRS group ($P < 0.05$). Although PCT and sTREM-1 are good markers to identify severity, sTREM-1 is more reliable, which proved to be a risk factor related to sepsis. During a 14-day observation, sCD163, sTREM-1, PCT, and SOFA scores continued to climb among nonsurvivors, while their WBC and CRP went down. Both sCD163 and SOFA scores are risk factors impacting the survival time. *Conclusion*. With regard to sepsis diagnosis and severity, sTREM-1 is more ideal and constitutes a risk factor. sCD163 is of a positive value in dynamic prognostic assessment and may be taken as a survival-impacting risk factor.

## 1. Introduction

Sepsis is one of the most important causes of morbidity and mortality in the intensive care unit (ICU). Multiple organ dysfunction syndrome (MODS) is common among critical cases of severe sepsis and a primary cause of death [1]. Although mortality is rather variable around the world (the rates between 20% and 63%), 750,000 sepsis cases and 210,000 related deaths are reported annually in the United States during the year 2000 [2, 3]. A directory for sepsis diagnosis and treatment, released by the Surviving Sepsis Campaign (SSC) [4, 5], points out that early identification and effective intervention will significantly improve prognosis and reduce death rate [6]. However, current common clinical indicators of infection include pyrexia, white blood cell counts, C-reactive protein (CRP), and procalcitonin (PCT) are still unsatisfactory. Moreover, at present, without timely identification of etiological evidence, nearly 30% of the relevant diagnoses are not well grounded pathologically [7]. Therefore, some of the patients with infection might have their condition worsened, develop multiple organ dysfunction or failure and die for delayed, ineffective treatment [8]. Currently, it is imperative to identify ideal biomarkers capable of making a clear distinction between sepsis and systemic inflammatory response syndrome (SIRS), sepsis severity assessment, and prognostic prediction.

CD163 is the only type of hemoglobin scavenger receptor, specially expressed on the macrophage membrane [9]. Some

studies report that the release of inflammatory cytokines caused by the oxidation reduction of hemoglobin plays an important role in the development of severe sepsis [10]. Triggering receptor expressed on myeloid cells-1 (TREM-1) is an immunoglobulin superfamily receptor expressed on polymorphonuclear granulocytes and mature monocytes. Bacteria or fungi infections may upregulate its expression, transmit signals downstream, induce the release of proinflammatory cytokines, and bring about relevant inflammatory responses [11]. PCT test has been put to a wide clinical use because it is a related biomarker, indicating infection and severity [12], as well as prognosis in case of infectious diseases [13, 14]. Although PCT is widely used clinically, its value for sepsis diagnosis has also been challenged recently [12, 15]. CRP is a biomarker involved in more than one inflammatory cascade amplification, now widely applied to sepsis diagnosis [16]. It is also faced with a very awkward situation—CRP proves not to be an ideal biomarker in this field [17, 18]. Therefore, the search for a reliable biomarker for sepsis diagnosis is to continue in the days to come. The present study makes a comparison between four biomarkers (sTREM-1, sCD163, PCT, and CRP) and one scoring system (SOFA scoring system) [19], with the purpose of exploring which of these is/are more valuable in sepsis diagnosis, as well as in the prediction of its development and prognosis. Hopefully, our findings could prove to be of some help to clinicians in general.

## 2. Materials and Methods

*2.1. Study Subjects.* All the subjects were selected from inpatients who were hospitalized between September 2009 and July 2011 in the Respiratory ICU, Surgical ICU, and Emergency ICU, Chinese People's Liberation Army (CPLA) General Hospital. Based on the 2001 American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Sepsis Directory [20], patients exhibiting two or more of the following signs during their first 24 h in the ICU were diagnosed as SIRS: (1) temperature of >38°C or <36°C, (2) pulse rate of >90 beats/min, (3) respiratory rate of >20 breaths/min or hyperventilation with a partial pressure of arterial carbon dioxide ($PaCO_2$) of <32 mmHg, or (4) white blood cell (WBC) count of >12,000 $\mu L^{-1}$ or <4000 $\mu L^{-1}$, or >10% immature cells. Patients exhibiting two or more of SIRS signs with proven infections were to be diagnosed as sepsis. Severe sepsis referred to sepsis complicated by organ dysfunction. Septic shock was defined as a state of acute circulatory failure characterized by persistent arterial hypotension unexplained by other causes. Based on the severity of condition, the sepsis group was further divided into subgroups for sepsis, severe sepsis, and septic shock, respectively. With 28-day survival as the demarcation line, the sepsis patients were also divided into a survivors' group (≥28 days survival) and a nonsurvivors' group (<28 days survival). Patients were excluded if they (1) were younger than 18 years of age; (2) acquired immunodeficiency syndrome; (3) had reduced polymorphonuclear granulocyte counts (<500 $\mu L^{-1}$); (4) died within 24 h after admission into the ICU, or refused to participate in the study, quit further treatment on their own will during the period of observation. This study was approved by the Ethics Committee of the Chinese People's Liberation Army (CPLA) General Hospital (Projects no. 20090923-001 and no. 20100701-002) and was registered with the U.S. National Institutes of Health Clinical Trials Register (NCT01388725). Patients or their families were fully informed of the details and signed consent forms in this study.

*2.2. Data Collection.* Upon admission into the ICUs, the following items were recorded for each patient: source of patients, age, gender, chief complaints for admission, symptoms, temperature, Acute Physiology and Chronic Health Evaluation (APACHE) II scores [21], SOFA scores [19], mechanical ventilation, continuous renal replacement treatment (CRRT), etiological factors, pathogens, and underlying diseases. Within 24 h (first day of study) after ICU admission and in the morning of days 3, 5, 7, 10, and 14, intravenous blood samples were obtained and centrifuged at 3,000 rpm for 15 min. The supernatants were transferred to Eppendorf tubes and stored at −80°C.

*2.3. Assays.* All the specimens were renumbered before the experiment. We ensured that each step was blind to researchers. sTREM-1 was determined with a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine Human TREM-1 Immunoassay ELISA Kit, R & D Systems, Minneapolis, Minnesota, the United States, product number DTRM10B); sCD163 was determined with a double antibody sandwich ELISA (soluble CD163 ELISA assay for the measurement of macrophage and monocyte activation, IQ Products, The Netherlands, product number IPQ-383); CRP was determined by scattering turbidimetry (CardioPhase hsCRP, Siemens, Germany); and PCT, and by enzyme-linked fluorescence analysis (ELFA, VIDAS BRAHMS PCT kit, bioMerieux SA, France). ELISA was performed in duplicate and all the other assays were done in strict accordance with the manufacturers' instructions.

*2.4. Statistical Analysis.* Results for continuous variables with normal distributions, including age, temperature, WBC counts, serum CRP, APACHE II scores, and SOFA scores, are given as means ± standard deviations (SDs). Student's $t$-test was performed to compare means between two groups. Analysis of variance (ANOVA) was made to compare means among multiple groups and interpreted based on post hoc comparisons. Results from continuous, abnormally distributed variables, including serum sTREM-1, sCD163, and PCT, are given as medians (interquartile ranges) and were compared by means of nonparametric tests. Results for qualitative variables, such as source of patients, gender, mechanical ventilation (MV), CRRT, etiological factors, pathogens, predisposing factors, and the mortality rate, were denoted as percentages and compared across groups by means of a Chi-square test. Logistic regression analysis was carried out to estimate the odds ratio (OR) and the 95% confidence interval (CI). Stepwise and forward selection procedures were introduced to select iteratively variables

Table 1: Clinical and biological data at admission in ICU according to the diagnosis of sepsis and its severity.

| Characteristics | All SIRS $N = 30$ | All sepsis $N = 100$ | $P$ value | Sepsis $N = 36$ | Severe sepsis $N = 35$ | Septic shock $N = 29$ | $P$ value |
|---|---|---|---|---|---|---|---|
| Age (years) | 52.2 ± 20.4 | 58.9 ± 19.5 | 0.105 | 57.2 ± 19.9 | 55.3 ± 18.5 | 65.4 ± 19.1 | 0.094 |
| Gender (*n*, %) | | | 0.091 | | | | 0.753 |
| Male | 15 (50) | 67 (67) | | 23 (63.9) | 23 (65.7) | 21 (72.4) | |
| Female | 15 (50) | 33 (33) | | 13 (36.1) | 12 (34.3) | 8 (27.6) | |
| Temperature (°C) | 37.2 ± 0.6 | 37.8 ± 1.3 | <0.001 | 37.9 ± 1.1 | 38.0 ± 1.1 | 37.4 ± 1.6 | 0.112 |
| APACHE II score | 11.0 ± 7.0 | 13.4 ± 6.1 | <0.001 | 12.7 ± 6.4 | 18.4 ± 7.2 | 23.1 ± 5.4 | <0.001 |
| SOFA score | — | 7.8 ± 4.4 | — | 4.7 ± 3.0 | 7.3 ± 3.6 | 12.1 ± 3.0 | <0.001 |
| MV (*n*, %) | 23 (76.7) | 80 (80) | 0.693 | 24 (66.7) | 29 (82.9) | 27 (93.1) | 0.026 |
| CRRT (*n*, %) | 1 (3.3) | 22 (22) | 0.019 | 7 (19.4) | 8 (22.9) | 7 (24.1) | 0.892 |
| Possible etiological factors (*n*, %) | | | | | | | |
| Pulmonary infection | — | 83 (83) | — | 30 (83.3) | 32 (91.4) | 21 (74.2) | 0.129 |
| Abdominal infection | — | 18 (18) | — | 6 (16.7) | 3 (8.6) | 9 (31) | 0.064 |
| Urinary tract infection | — | 24 (24) | — | 11 (30.6) | 6 (17.1) | 7 (24.1) | 0.417 |
| Trauma/postoperative infection | — | 31 (31) | — | 12 (33.3) | 10 (28.6) | 9 (31) | 0.154 |
| Bacteremia | — | 23 (23) | — | 12 (33.3) | 7 (20) | 4 (13.8) | 0.091 |
| Catheter-related infections | — | 13 (13) | — | 10 (27.8) | 1 (2.9) | 2 (6.9) | 0.004 |
| Others | — | 4 (4) | — | 0 (0) | 1 (2.9) | 3 (10.3) | 0.074 |
| Pathogens detected | | | | | | | |
| Gram-positive bacteria | — | 37 (37) | — | 15 (41.7) | 8 (22.9) | 14 (48.3) | 0.085 |
| Gram-negative bacteria | — | 81 (81) | — | 31 (86.1) | 28 (80.0) | 22 (75.9) | 0.568 |
| Fungi | — | 62 (62) | — | 25 (69.4) | 21 (60.0) | 16 (55.2) | 0.471 |
| Predisposing factors (*n*, %) | | | | | | | |
| Hypertension | 9 (30) | 41 (41) | 0.277 | 13 (36.1) | 18 (51.4) | 10 (34.5) | 0.295 |
| Diabetes | 2 (6.7) | 16 (16) | 0.319 | 3 (8.3) | 7 (20) | 6 (20.7) | 0.261 |
| COPD | 0 (0) | 14 (14) | 0.067 | 6 (16.7) | 3 (8.6) | 5 (17.2) | 0.493 |
| Coronary heart disease | 3 (10) | 17 (17) | 0.54 | 4 (11.1) | 3 (8.6) | 10 (34.5) | 0.016 |
| Immunosuppressed condition | 0 (0) | 11 (11) | 0.127 | 3 (8.3) | 8 (22.9) | 0 (0) | 0.004 |
| Nervous system disease | 0 (0) | 12 (12) | 0.103 | 5 (13.9) | 2 (5.7) | 5 (17.2) | 0.304 |
| CKD | 1 (3.3) | 8 (8) | 0.636 | 4 (11.1) | 2 (5.7) | 2 (6.9) | 0.687 |
| 28-day mortality rate (*n*, %) | 2 (5.0) | 43 (43.0) | $P < 0.001$ | 6 (16.7) | 17 (48.6) | 20 (69) | $P < 0.001$ |

Quantitative data of normal distribution are presented as mean ± SD. Quantitative data of nonnormal distribution are presented as median (interquartile range). Qualitative data are presented as *n* (%).

RICU: respiratory intensive care unit; SICU: surgical intensive care unit; EICU: emergency intensive care unit; MV: mechanical ventilation; CRRT: continuous renal replacement treatment; APACHE II score: acute physiologic assessment and chronic health evaluation II scores; SOFA score: sequential organ failure assessment scores; CKD: chronic kidney disease.

possibly related to sepsis. To be entered into this model, a $P < 0.05$ from logistic regression model was required. Factors related to survival were explored through Cox regression and calculating the hazard ratios. The AUC (areas under receiver operating characteristic curves) method was employed to evaluate how well the model works in distinguishing sepsis from SIRS, and severe sepsis, and in predicting prognosis. For statistical analysis, SPSS 16.0 (SPSS, Chicago, Illinois, USA) was used, and a two-tailed $P < 0.05$ was considered significant.

## 3. Results

*3.1. Subjects Descriptions.* As this study focused on the dynamics of different biomarkers, patients who died within 24 h after being admitted into the ICU, refused to participate in the study, or quit further treatment on their own within 14 days failed the requirements for continuous observation. A total of 130 patients, selected out of 377 in accordance with relevant criteria, were formally included in this study (see Supplementary Figure 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2013/969875). 30 critical patients with two or more SIRS signs and negative pathologic examination results, who were from the SICU within 24 hours after aseptic surgery, were also selected as SIRS control group in the study. These patients had received a general examination to exclude infection within 24 hours before surgery. In light of the sepsis guidelines, the 100 sepsis patients were further divided into a sepsis subgroup (36 cases), a severe sepsis subgroup (35 cases), and a septic shock subgroup (29 cases). Baseline data at admission into ICU are shown in Table 1. APACHE II and SOFA scores go

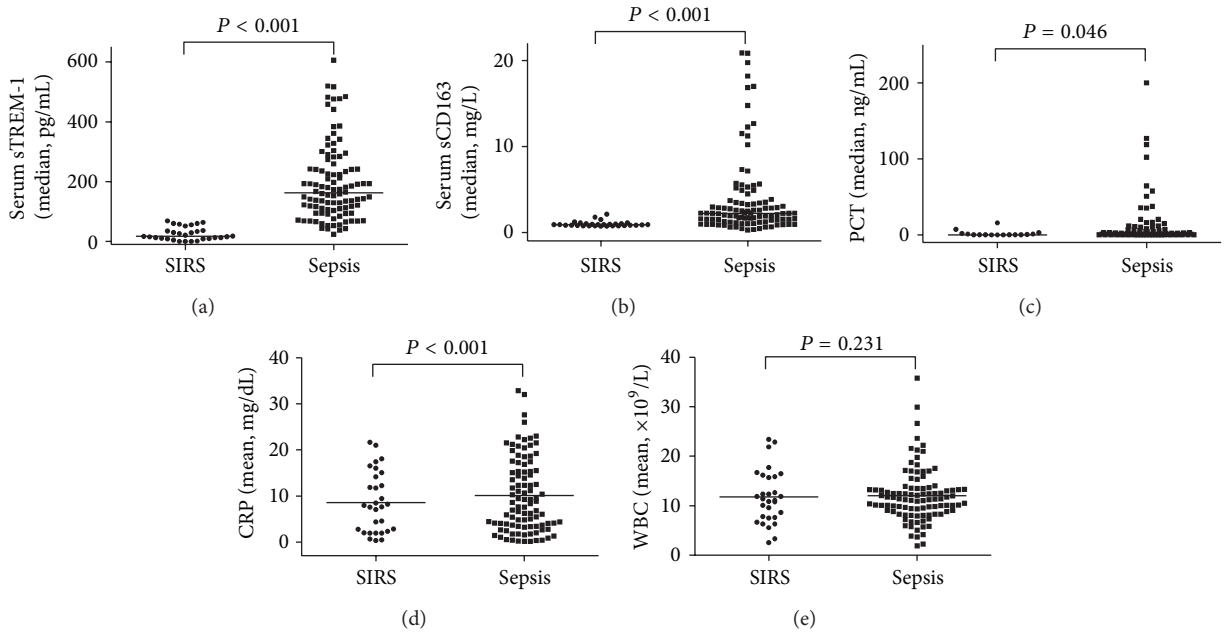



Figure 1: Serum sTREM-1 (a), serum sCD163 (b), PCT (c), CRP (d), and WBC (e) according to the sepsis diagnosis criteria. (SIRS ($n = 30$) versus sepsis ($n = 100$)). The dots denote individual values, and the bars indicate medians or means. Serum sTREM-1, serum sCD163, PCT, and CRP levels come out as 180.92 (150.44) pg/mL versus 29.41 (20.77) pg/mL, $P < 0.001$; 2.22 (2.36) mg/dL versus 0.88 (0.23) mg/dL, $P < 0.001$; 1.65 (10.1) ng/mL versus 0.35 (1.58) ng/mL, $P = 0.046$; 11.76 ± 8.09 mg/dL versus 5.65 ± 4.27 mg/dL, $P < 0.001$, respectively. But a comparison of WBC level between the two groups is devoid of such significance ($12.19 \pm 6.01 \times 10^9$/L versus $11.27 \pm 2.54 \times 10^9$/L, $P = 0.231$).

Table 2: Univariate analysis of dichotomous variables for the purpose of distinguishing sepsis from SIRS.

| Variable | $\beta$ | S.E. | Wald | $P$ | OR | 95% C.I. for OR | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower | Upper |
| sTREM-1 | 0.08 | 0.02 | 17.65 | <0.001 | 1.09 | 1.04 | 1.13 |
| sCD163 | 2.02 | 0.51 | 15.76 | <0.001 | 7.55 | 2.78 | 20.49 |
| WBC | 0.03 | 0.04 | 0.66 | 0.42 | 1.03 | 0.95 | 1.12 |
| CRP | 0.12 | 0.04 | 8.76 | <0.001 | 1.12 | 1.04 | 1.22 |
| PCT | 0.08 | 0.07 | 1.57 | 0.21 | 1.08 | 0.95 | 1.23 |
| APACHE II | 0.09 | 0.03 | 10.18 | <0.001 | 1.10 | 1.04 | 1.16 |

markedly from high to low in the following order: septic shock > severe sepsis > sepsis subgroup ($P < 0.001$). More septic shock patients are in need of mechanical ventilation than sepsis patients ($P = 0.026$). With a 28-day survival as a criterion, mortality rate for the septic shock subgroup is the highest, followed by the severe sepsis subgroup, and the sepsis subgroup ranks the lowest ($P < 0.001$). Statistically, there are no remarkable differences in terms of age, gender, temperature, etiological factors (excluding catheter-related bloodstream infection), pathogens, or accompanying underlying diseases (excluding coronary heart disease and the immunosuppressed condition) between groups. Additionally, it should be explained that some patients had multiple pathogens and/or infection of multiple sites.

*3.2. sTREM-1, sCD163, PCT, CRP, and WBC Counts: Values for Early Sepsis Diagnosis.* On the first day of ICU enrollment, the sepsis group exhibited a higher level in serum sTREM-1, serum sCD163, PCT, and CRP than the SIRS group (Figure 1). Univariate analysis was made to assess possible risk factors to sepsis. The variables taken into account included serum sTREM-1, sCD163, CRP, PCT, WBC counts, and APACHE II score (Table 2). Four variables, sTREM-1, sCD163, CRP, and APACHE II score, were further selected for multivariate regression ($P < 0.001$). Finally, only serum sTREM-1 entered the multivariable regression equation, with OR = 1.089 (95% CI 1.045–1.136, $P < 0.001$). The receiver operating characteristic (ROC) curves were used to calculate serum sTREM-1's performance in sepsis diagnosis (Supplemental Figure 2). AUC turned out to be 0.978 (95% CI 0.958–0.997). With a cut-off point of 64.4 pg/mL for sTREM-1, sensitivity came out as 0.91; specificity, 0.896; PPV, 0.989, and NPV, 0.621.

*3.3. Serum sTREM-1 sCD163, PCT, CRP, and WBC: Values for Severity Assessment of Sepsis.* Figure 2 illustrates a pairwise comparison over serum sTREM-1, WBC counts, serum CRP, serum PCT, and SOFA score between the sepsis, severe sepsis, and septic shock groups, made on the first day of enrollment. It turned out that the sepsis group scored the lowest in sTREM-1, PCT, and SOFA score, which was of statistical significance, compared with any other group. As for the severe sepsis group and the septic shock group, statistically, of all the indicators, only the disparity in the SOFA score between the two deserved attention. So we combined severe sepsis and septic shock groups into a severe sepsis/shock group to express the seriousness of the sepsis condition.

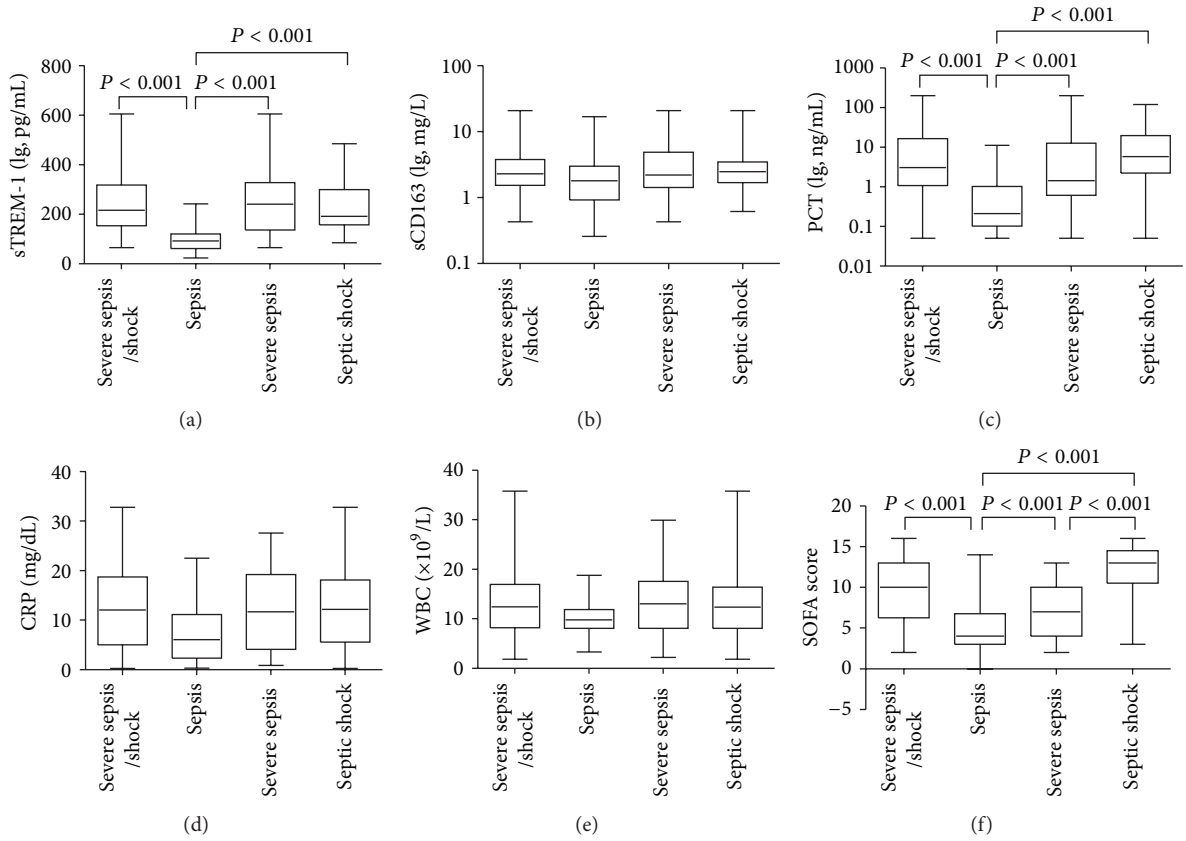



Figure 2: Serum sTREM-1 (a), serum sCD163 (b), PCT (c), CRP (d), WBC (e), and SOFA score (f) on the ICU admission day when sepsis (36 cases), severe sepsis (35 cases), and septic shock (29cases) occurred. Severe sepsis/shock group is defined as a state which represents sepsis severity, including severe sepsis and septic shock (64 cases). $y$-axis of sCD163 and PCT is labeled as logarithmic.

Higher serum sTREM-1, PCT level, and SOFA score in severe sepsis/shock group ($P < 0.05$). Although severe sepsis/shock group had higher sCD163, CRP, and WBC levels, a comparison of such indicators across groups is devoid of such significance. ROCs for serum sTREM-1, PCT, and SOFA score illustrate severe sepsis/shock group, which reflect sepsis severity; see Figure 3.

*3.4. Serum sTREM-1 sCD163, PCT, CRP, and WBC Counts: Values for Dynamic Assessment of Sepsis Prognosis.* Based on the 28-day survival, sepsis patients were also divided into a survivors' group and a nonsurvivors' group. Figure 4 compares these two groups in terms of dynamic changes in serum sTREM-1, sCD163, WBC counts, serum CRP, and serum PCT levels. The curves show that the nonsurvivors' group had higher serum sTREM-1, sCD163, WBC counts, serum PCT levels, and SOFA score during this period of time. For nonsurvivors, their serum sTREM-1, sCD163, serum PCT levels, and SOFA score increased with the passage of time, while their WBC counts and serum CRP levels tended to decline. In contrast, all indicators of the survivors' group revealed a tendency to decline. The serum sCD163, sTREM-1, and PCT levels of nonsurvivors were higher than survivors' at these 6 different time points ($P < 0.05$).

Cox regression was employed to analyze the survival time of sepsis patients, as well as the factors affecting survival. The variables taken into account included sex, age, temperature, serum sTREM-1, sCD163, WBC, CRP, PCT, APACHE II score, SOFA score, use of life support technology (e.g., MV and CRRT), etiological factors, pathogens, and predisposing factors. Within 24 h after the ICU admission, the indicators previously mentioned were derived from the patients. Finally, only sCD163 and SOFA entered the regression equation. For the former, the regression coefficient = 0.09, hazard ratios = 1.09 (95% CI 1.035–1.154, $P < 0.001$), whereas for the latter, the regression coefficient = 0.2, hazard ratios = 1.23 (95% CI 1.126–1.335, $P < 0.001$). The ROC curve denoting these two survival-affecting parameters was drawn on the ICU admission day in order to predict prognosis (Figure 5).

## 4. Discussions

Currently, the exact role of biomarkers in the assessment of septic patients remains obscure [22]. Although 178 related sepsis biomarkers have been identified, it is still controversial which is reliable for sepsis diagnosis [23]. In particular, PCT and CRP, which have been most widely used in clinical treatment, have limited ability to distinguish sepsis from

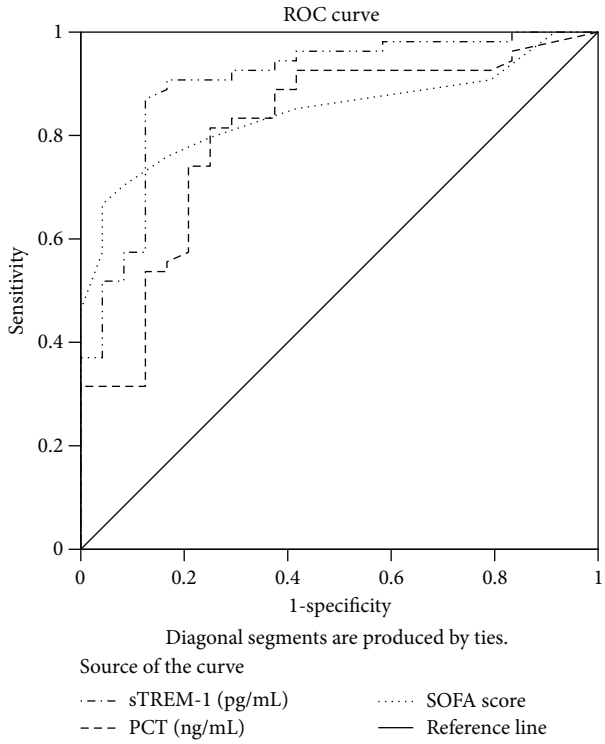



Figure 3: ROC curves for serum sTREM-1 and PCT levels for severe sepsis (severity of sepsis). AUC demonstrates that serum sTREM-1 measures 0.9 (95% CI 0.823–0.977), serum PCT measures 0.806 (95% CI 0.7–0.913), and SOFA score measures 0.846 (95% CI 0.716–0.931). With 136.82 pg/mL as the cut-off point for sTREM-1, sensitivity measures 0.87 and specificity 0.88; with 0.83 ng/mL as the cut-off point for PCT, sensitivity measures 0.82 and specificity 0.75; with 8.5 as the cut-off point for SOFA score, sensitivity measures 0.67 and specificity 0.96.

other inflammatory conditions or to predict outcome. Therefore, the exploration and discovery of sepsis biomarkers still should be paid attention to.

Soluble sTREM-1 is identified as a marker of microbial infection by many studies [24–27]. The upregulation of CD163 at the occurrence of sepsis, caused by activating the waterfall effect from the secretion of anti-inflammatory cytokines, helps scavenge hemoglobin and reduce its oxidative impairment to the body [28, 29]. CD163 is also innately immune and bacterial flora identifying [30]. We found the same phenomenon that, on the admission day, the sepsis group exhibited a higher level of sTREM-1, sCD163, PCT, and CRP than the SIRS group. In addition, the disparity was of statistical significance ($P < 0.05$). That reveals that the indicators previously mentioned are all applicable to early sepsis diagnosis. Multivariate logistic regression displays that serum sTREM-1 is the only risk indicator for sepsis diagnosis. The ROC area for serum sTREM-1 came out as 0.978 (95% CI 0.958–0.997) and both sensitivity and specificity, around 0.9. The diagnostic value of sTREM-1 is obviously higher than sCD163, CRP, and PCT. That is to say, serum sTREM-1 may prove a better indicator for the sepsis diagnosis.

The serum sTREM-1 and PCT levels as well as SOFA score can play a role in severity assessment of sepsis. The value of the three indicators from the severe sepsis group, the septic shock group, and the severe sepsis/shock group all exceeded that from the sepsis group ($P < 0.01$). In a comparison between the severe sepsis group and the septic shock group, only the sofa score possesses a certain significance. To sum up, the sTREM-1 and PCT level and the SOFA score are of diagnostic value for sepsis severity. In addition, sTREM-1 has the highest efficiency, with a ROC area of 0.9; sensitivity turned out to be 0.87, and specificity, 0.88 with 136.82 as the cut-off point for severe sepsis diagnosis. What is interesting is that the severity assessment value of sCD163 is limited. It might be assumed that the expression of sCD163 on the surface of macrophage membrane is regulated by more than one factor. Studies show that interleukin-6 (IL-6) and interleukin-10 (IL-10) stimulate, whereas lipopolysaccharide (LPS) and interferon-$\gamma$ (IFN-$\gamma$) contain, the expression of CD163 molecules on the surface of macrophage membrane [31]. In the meantime, only with the involvement of metalloprotease, LPS, and at least one inflammatory medium, could a drop of CD163 into sCDl63 be possible, by means of activating Toll-like receptors [32]. Therefore, the state and expression of CD163 are governed by the internal environment of the human body. It could also be assumed that the high expression of sCD163 is related to the positive feedback of inflammation. That is to say, the expressive volume of sCD163 is limited right after inflammatory responses are activated.

Dynamic changes in serum sTREM-1 may prove helpful for prognostic assessment [33, 34]. More than one study reports that sCD163 is more valuable for earlier prognostic assessment [35, 36]. We found that, seen from the dynamic tendency of the curve denoting sepsis prognosis, the differences in serum sTREM-1, CRP, and PCT level as well as in SOFA score at these six different time points were statistically significant, with the nonsurvivors' group having higher values all the time, and showing a higher CRP and WBC level even at the final stage, which was also statistically significant. For the nonsurvivors, sTREM-1, sCD163, and PCT level as well as SOFA score went up with the passage of time, whereas for the survivors, these indicators tended to decline. This demonstrates that sTREM-1, sCD163, and PCT, as well as SOFA score, have their value for clinical application in dynamic assessment of sepsis prognosis. Relevant factors affecting survival within the first 24 h of ICU stay were analyzed and sorted out using Cox regression. It turned out that only the sCD163 level and SOFA score entered the equation and served as the independent risk factors affecting survival. Further analysis was made of sCD163 level and SOFA score by means of ROC curve to determine the cut-off point. Therefore, these two factors are likely to function as an index for reference in terms of early prognosis assessment. sTREM-1, however, did not work well in prognostic assessment as a risk factor. The reason may lie in the fact that it is protective to inflammation at the initial stage. At the onset, sTREM-1 may combine with membrane-bound TREM-1 by competitive, ligand binding, or with DAP-12, an inhibitive receptor, by specific binding, thus containing human body's

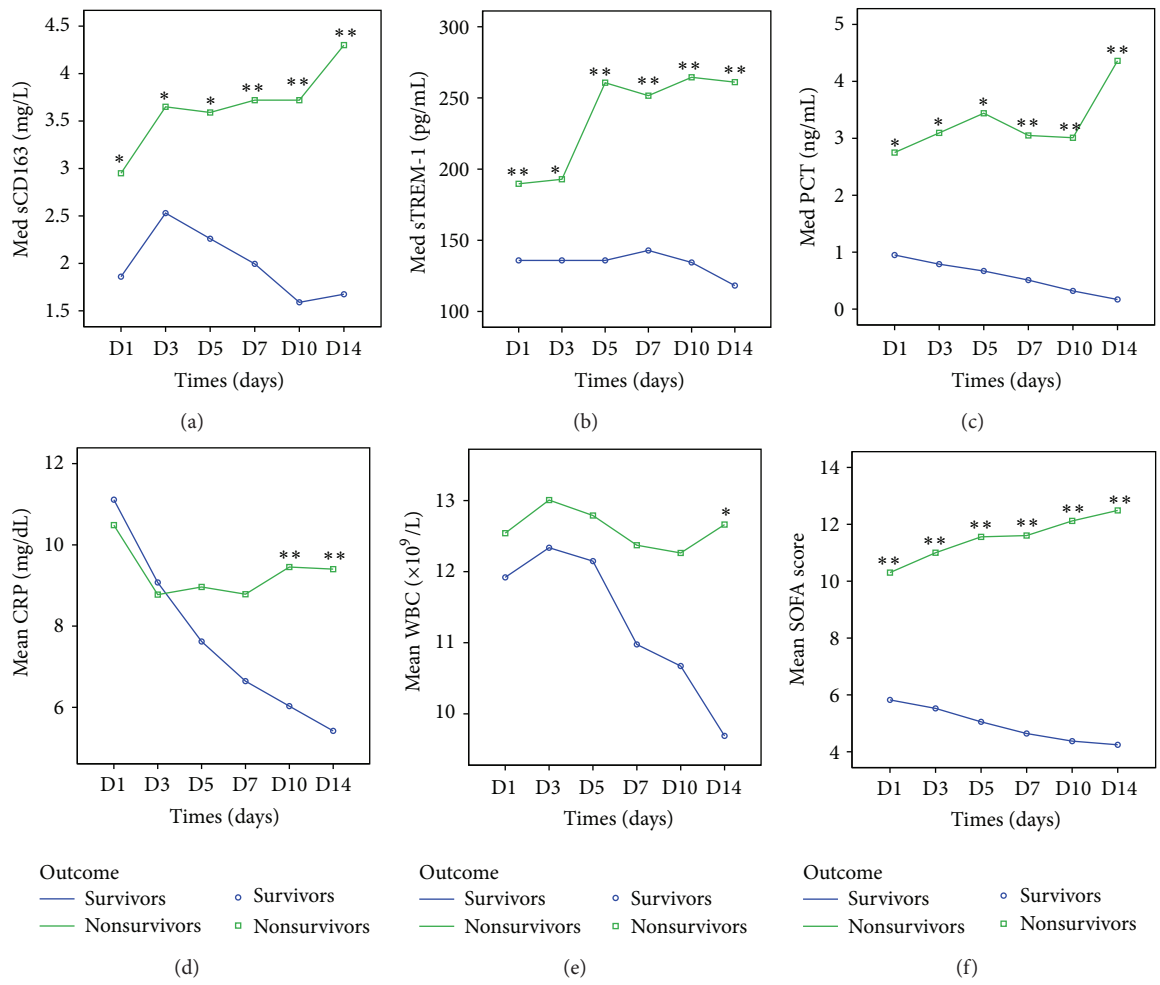



Figure 4: Serum sCD163 level (a), Serum sTREM-1 level (b), PCT level (c), CRP level (d), and WBC counts (e) measured over 14 days in patients diagnosed with sepsis, based on 28-day survival. The differences in serum sCD163, sTREM-1, and PCT levels at these 6 different time points were statistically significant, with the nonsurvivors group having higher values at all time points and also showing a higher CRP level on days 10 and 14, which were also statistically significant. WBC counts in the nonsurvivors group were also higher than those of the survivors group, but only one time point (day 14) registered difference statistically significant. Survivors = 57; nonsurvivors = 43; $^{*}P < 0.05$; $^{**}P < 0.01$.

excessive responses to inflammation. With the development of the disease and the inflammatory cascade amplification, the above combinations reach a saturation point. sTREM-1 accumulates and is then released into the blood in large quantities. For these reasons, sTREM-1 level at the initial stage may be fairly low and insensitive in early prognostic assessment and could only play a better role in later stage, dynamic prognostic assessment.

The present study, however, has its own limitations. First, central tendency values were used to describe the dynamics of different biomarkers. It is very helpful to draw conclusions about decisions for individual patients and their values. However, owing to the limitations of our sample size, prospective clinical studies are still wanted to provide further proof for the clinical diagnostic value of these biomarkers. Second, the internal environment of the human body is an important contributor to the expression of the markers identified in this study. The internal environment can be influenced by clinical care (drugs, timing, dose, mechanical ventilation technique, CRRT, etc.). Clinical care environment may also have impact on the responses of their patients and therefore on their measurements. We cannot negate the impact of such factors. Third, the purpose of this study is to observe the dynamic changes of the various indicators. For this, it precludes a considerable portion of candidates according to the exclusion criteria. At the same time, the SIRS patients, without both prior surgery and 24 h postsurgery infections were selected as control group. This study also does not rule out impact of the specific conditions on the enrollees.

In summary, it may be concluded from the study that sTREM-1 is more ideal than PCT and CRP for early sepsis diagnosis and severity assessment and constitutes an independent risk diagnostic parameter. sCD163 and SOFA scores possess positive clinical values in dynamic, prognostic assessment and function as independent, survival-affecting risk factors. Future studies with larger subject populations

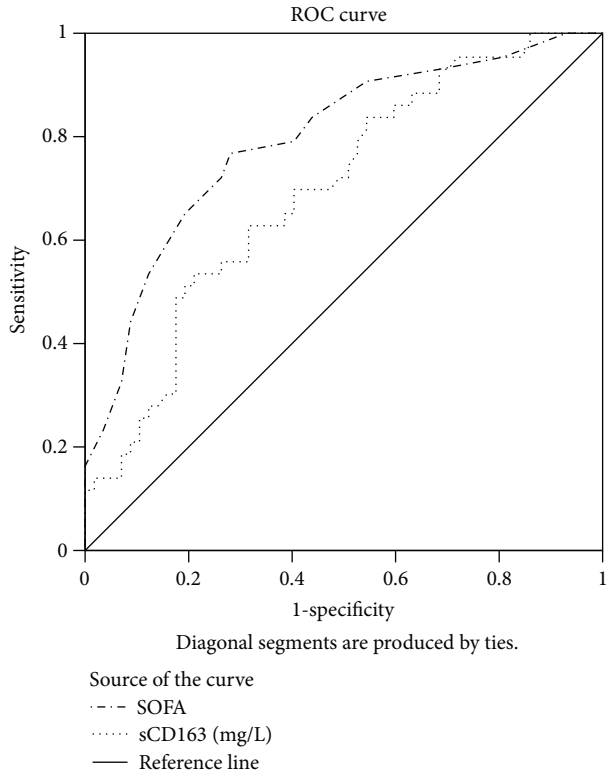



Figure 5: ROC curves for serum sCD163 and SOFA score for sepsis prognosis. AUC demonstrates that serum sCD163 measures 0.696 (95% CI 0.593–0.799) and SOFA score measures 0.794 (95% CI 0.705–0.833). With 2.84 mg/L as the cut-off point for sCD163, sensitivity measures 0.535 and specificity 0.789, positive predictive value (PPV) 0.657, and negative predictive value (NPV) 0.692; with 7.5 as the cut-off point for SOFA score, sensitivity measures 0.767, specificity 0.719, PPV 0.673, and NPV 0.804.

and with attention to the clinical care environment are expected to define the application of these parameters in clinical decision making.

## Conflict of Interests

All the authors declare that they have no conflict of interests.

## Authors' Contribution

Dr. Longxiang Su and Dr. Lin Feng have contributed equally to this work.

## Acknowledgments

The work was supported by the Chinese National Science and Technology Pillar Program (no. 2009BAI86B03). The authors express their thanks to the staff members of RICU, SICU, and EICU of the CPLA General Hospital for their assistance in the project.

## References

[1] K. D. Kochanek, S. L. Murphy, R. N. Anderson, and C. Scott, "Deaths: final data for 2002," *National Vital Statistics Reports*, vol. 53, no. 5, pp. 1–115, 2004.

[2] 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.

[3] 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.

[4] R. P. Dellinger, J. M. Carlet, H. Masur et al., "Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock," *Critical Care Medicine*, vol. 32, no. 3, pp. 858–873, 2004.

[5] 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," *Intensive Care Medicine*, vol. 34, no. 1, pp. 17–60, 2008.

[6] R. M. Otero, H. B. Nguyen, D. T. Huang et al., "Early goal-directed therapy in severe sepsis and septic shock revisited: concepts, controversies, and contemporary findings," *Chest*, vol. 130, no. 5, pp. 1579–1595, 2006.

[7] S. Gibot and A. Cravoisy, "Soluble form of the triggering receptor expressed on myeloid cells-1 as a marker of microbial infection," *Clinical Medicine & Research*, vol. 2, no. 3, pp. 181–187, 2004.

[8] N. Shapiro, M. D. Howell, D. W. Bates, D. C. Angus, L. Ngo, and D. Talmor, "The association of sepsis syndrome and organ dysfunction with mortality in emergency department patients with suspected infection," *Annals of Emergency Medicine*, vol. 48, no. 5, pp. 583.e1–590.e1, 2006.

[9] C. Buechler, M. Ritter, E. Orsó, T. Langmann, J. Klucken, and G. Schmitz, "Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli," *Journal of Leukocyte Biology*, vol. 67, no. 1, pp. 97–103, 2000.

[10] R. Larsen, R. Gozzelino, V. Jeney et al., "A central role for free heme in the pathogenesis of severe sepsis," *Science Translational Medicine*, vol. 2, no. 51, Article ID 51ra71, 2010.

[11] A. Bouchon, F. Facchetti, M. A. Weigand, and M. Colonna, "TREM-1 amplifies inflammation and is a crucial mediator of septic shock," *Nature*, vol. 410, no. 6832, pp. 1103–1107, 2001.

[12] B. M. Tang, G. D. Eslick, J. C. Craig, and A. S. McLean, "Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis," *Lancet Infectious Diseases*, vol. 7, no. 3, pp. 210–217, 2007.

[13] F. Bloos, J. C. Marshall, R. P. Dellinger et al., "Multinational, observational study of procalcitonin in ICU patients with pneumonia requiring mechanical ventilation: a multicenter observational study," *Critical Care*, vol. 15, no. 2, article R88, 2011.

[14] E. J. Giamarellos-Bourboulis, I. Tsangaris, T. Kanni et al., "Procalcitonin as an early indicator of outcome in sepsis: a prospective observational study," *Journal of Hospital Infection*, vol. 77, no. 1, pp. 58–63, 2011.

[15] E. J. Giamarellos-Bourboulis, P. Giannopoulou, P. Grecka, D. Voros, K. Mandragos, and H. Giamarellou, "Should procalcitonin be introduced in the diagnostic criteria for the systemic inflammatory response syndrome and sepsis?" *Journal of Critical Care*, vol. 19, no. 3, pp. 152–157, 2004.

[16] P. Póvoa, "C-reactive protein: a valuable marker of sepsis," *Intensive Care Medicine*, vol. 28, no. 3, pp. 235–243, 2002.

[17] D. H. Wyllie, I. C. J. W. Bowler, and T. E. A. Peto, "Bacteraemia prediction in emergency medical admissions: role of C reactive protein," *Journal of Clinical Pathology*, vol. 58, no. 4, pp. 352–356, 2005.

[18] C. C. Lee, M. Y. Hong, N. Y. Lee, P. L. Chen, C. M. Chang, and W. C. Ko, "Pitfalls in using serum C-reactive protein to predict bacteremia in febrile adults in the ED," *The American Journal of Emergency Medicine*, vol. 30, no. 4, pp. 562–569, 2012.

[19] 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.

[20] M. M. Levy, M. P. Fink, J. C. Marshall et al., "2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference," *Critical Care Medicine*, vol. 31, no. 4, pp. 1250–1256, 2003.

[21] 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.

[22] 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, no. 4, pp. 296–327, 2008.

[23] C. Pierrakos and J.-L. Vincent, "Sepsis biomarkers: a review," *Critical Care*, vol. 14, no. 1, article R15, 2010.

[24] S. Gibot, A. Cravoisy, B. Levy, M.-C. Bene, G. Faure, and P.-E. Bollaert, "Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia," *The New England Journal of Medicine*, vol. 350, no. 5, pp. 451–458, 2004.

[25] R. M. Determann, M. Weisfelt, J. de Gans, A. van der Ende, M. J. Schultz, and D. van de Beek, "Soluble triggering receptor expressed on myeloid cells 1: a biomarker for bacterial meningitis," *Intensive Care Medicine*, vol. 32, no. 8, pp. 1243–1247, 2006.

[26] R. M. Determann, J. W. Olivier van Till, O. van Ruler, S. Q. van Veen, M. J. Schultz, and M. A. Boermeester, "sTREM-1 is a potential useful biomarker for exclusion of ongoing infection in patients with secondary peritonitis," *Cytokine*, vol. 46, no. 1, pp. 36–42, 2009.

[27] L.-X. Su, L. Feng, J. Zhang et al., "Diagnostic value of urine sTREM-1 for sepsis and relevant acute kidney injuries: a prospective study," *Critical Care*, vol. 15, no. 5, article R250, 2011.

[28] M. Kristiansen, J. H. Graversen, C. Jacobsen et al., "Identification of the haemoglobin scavenger receptor," *Nature*, vol. 409, no. 6817, pp. 198–201, 2001.

[29] J. Zuwała-Jagiełło, "Haemoglobin scavenger receptor: function in relation to disease," *Acta Biochimica Polonica*, vol. 53, no. 2, pp. 257–268, 2006.

[30] B. O. Fabriek, R. V. Bruggen, D. M. Deng et al., "The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria," *Blood*, vol. 113, no. 4, pp. 887–892, 2009.

[31] M. M. J. Polfliet, B. O. Fabriek, W. P. Daniëls, C. D. Dijkstra, and T. K. van den Berg, "The rat macrophage scavenger receptor CD163: expression, regulation and role in inflammatory mediator production," *Immunobiology*, vol. 211, no. 6-8, pp. 419–425, 2006.

[32] L. K. Weaver, P. A. Pioli, K. Wardwell, S. N. Vogel, and P. M. Guyre, "Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors," *Journal of Leukocyte Biology*, vol. 81, no. 3, pp. 663–671, 2007.

[33] S. Gibot, A. Cravoisy, M.-N. Kolopp-Sarda et al., "Time-course of sTREM (soluble triggering receptor expressed on myeloid cells)-1, procalcitonin, and C-reactive protein plasma concentrations during sepsis," *Critical Care Medicine*, vol. 33, no. 4, pp. 792–796, 2005.

[34] J. Zhang, D. She, D. Feng, Y. Jia, and L. Xie, "Dynamic changes of serum soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) reflect sepsis severity and can predict prognosis: a prospective study," *BMC Infectious Diseases*, vol. 11, article 53, 2011.

[35] S. Gaïni, S. S. Pedersen, O. G. Koldkjær, C. Pedersen, S. K. Moestrup, and H. J. Møller, "New immunological serum markers in bacteraemia: anti-inflammatory soluble CD163, but not proinflammatory high mobility group-box 1 protein, is related to prognosis," *Clinical and Experimental Immunology*, vol. 151, no. 3, pp. 423–431, 2008.

[36] H. J. Møller, S. K. Moestrup, N. Weis et al., "Macrophage serum markers in pneumococcal bacteremia: prediction of survival by soluble CD163," *Critical Care Medicine*, vol. 34, no. 10, pp. 2561–2566, 2006.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 165974, 16 pages
http://dx.doi.org/10.1155/2013/165974

*Review Article*

# Cytokines in Sepsis: Potent Immunoregulators and Potential Therapeutic Targets—An Updated View

**Wibke Schulte,[1,2] Jürgen Bernhagen,[2] and Richard Bucala[1]**

[1] *Department of Internal Medicine, Yale University School of Medicine, The Anlyan Center, S525, P.O. Box 208031, 300 Cedar Street, New Haven, CT 06520-8031, USA*
[2] *Institute of Biochemistry and Molecular Cell Biology, University Hospital of RWTH Aachen University, Pauwelsstraße 30, 52074 Aachen, Germany*

Correspondence should be addressed to Wibke Schulte; wibke.k.schulte@gmail.com

Received 5 October 2012; Accepted 22 May 2013

Academic Editor: Celeste C. Finnerty



Sepsis and septic shock are among the leading causes of death in intensive care units worldwide. Numerous studies on their pathophysiology have revealed an imbalance in the inflammatory network leading to tissue damage, organ failure, and ultimately, death. Cytokines are important pleiotropic regulators of the immune response, which have a crucial role in the complex pathophysiology underlying sepsis. They have both pro- and anti-inflammatory functions and are capable of coordinating effective defense mechanisms against invading pathogens. On the other hand, cytokines may dysregulate the immune response and promote tissue-damaging inflammation. In this review, we address the current knowledge of the actions of pro- and anti-inflammatory cytokines in sepsis pathophysiology as well as how these cytokines and other important immunomodulating agents may be therapeutically targeted to improve the clinical outcome of sepsis.

## 1. Introduction

Sepsis, or the invasion of microbial pathogens into the bloodstream, is characterized by a systemic proinflammatory response, which can lead to severe sepsis and septic shock [1]. Sepsis, severe sepsis, and septic shock are major healthcare problems worldwide; they affect millions of people each year, and their incidence increases annually [2, 3]. Despite significant advances in intensive care treatment over the last years, septic shock remains associated with high mortality rates [4]. An epidemiologic study reported that septic shock is the most common cause of death in noncoronary intensive care units, and the tenth leading cause of death overall in high-income countries [2]. The outcome of sepsis is particularly unfavorable in elderly, immunocompromised, and critically ill patients [5]. Reasons for the anticipated increase in sepsis incidence and its associated mortality include the increasing number of immunocompromised patients, emerging antibiotic resistance in microorganisms, and the aging population [6].

Besides its clinical challenge, the treatment of sepsis imposes a large economic burden on healthcare systems worldwide [7]. With an estimated 750,000 cases occurring in the United States alone each year, the annual total costs have been estimated to be approximately $16.7 billion nationally [8]. Sepsis was identified as one of the five conditions that account for the most expensive hospital stays in the United States [7].

## 2. Definition of Sepsis

The word "sepsis" is derived from the word "*σηψις*," which in the original Greek means "decomposition" or "putrefaction," and was first mentioned in Homer's poems approximately 2700 years ago [9]. Only relatively recently have studies led to detailed descriptions of the clinical findings in septic patients and to an understanding of the underlying pathophysiology. These findings in turn have led to redefinitions of sepsis and its sequelae. Generally, sepsis is viewed as the response of the host toward invading pathogens or its toxins and is

Table 1: Diagnostic criteria for the systemic inflammatory response syndrome (SIRS).

| Defined by the presence of two or more of the following clinical findings |
|---|
| (1) Body temperature >38°C or <36°C |
| (2) Heart rate >90 $min^{-1}$ |
| (3) Respiratory rate >20 $min^{-1}$ or $PaCO_2$ <32 mmHg |
| (4) White blood cell count >12,000 cells $\mu L^{-1}$ or <4,000 cells $\mu L^{-1}$ or >10% immature (band) forms |

Table adapted from [11].

a syndrome that consists of multiple clinical and biochemical findings [10]. In 1991, a consensus conference was held by the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) to develop a single and universally accepted definition of sepsis to improve the early diagnosis and treatment of the disease and facilitate research. A key result of this consensus conference was the introduction of the term "systemic inflammatory response syndrome" (SIRS) which was defined as a combination of clinical signs without the existence of an underlying infection [1, 11] (Table 1). SIRS can be triggered by a variety of non-infectious conditions, such as trauma, burns, hemorrhagic or hypovolemic shock, pancreatitis, and other disease states. In contrast, the diagnosis of sepsis requires clinical evidence of infection along with an underlying SIRS disease state. Severe sepsis is characterized as sepsis complicated by acute organ dysfunction, hypoperfusion, or hypotension [1]. It may lead to "multiple organ dysfunction syndrome" (MODS), or septic shock. Septic shock refers to a state of acute circulatory failure that is characterized by persistent arterial hypotension (systolic pressure <90 mmHg or a mean arterial pressure <60 mmHg) despite adequate fluid resuscitation and in the absence of other causes of hypotension [1].

Following the 1991 consensus conference, the SIRS criteria were rapidly adopted by many clinicians and scientists and were widely used to select patients for clinical trials. However, many authors criticized the SIRS diagnostic criteria for their poor specificity and lack of prognostic value, as these criteria are broad and limited in number [12–14]. In 2001, an International Sepsis Definition Conference convened aiming to evaluate the previous definitions of SIRS, sepsis, severe sepsis, and septic shock [11]. Following this conference, an expanded list of clinical and biochemical diagnostic criteria for sepsis was released, which better reflected this complex disease state. In 2004, a committee of international sepsis experts published clinical practice guidelines for the management of severe sepsis and septic shock [15]. These guidelines were widely disseminated as part of the "Surviving Sepsis Campaign" and are regularly updated, with the last revision made in 2013 [10].

## 3. Pathophysiology of Sepsis

In recent years, a significant body of literature has been published in an attempt to understand the complex and dynamic pathophysiologic mechanisms that underlie the heterogeneous sepsis syndrome. Sepsis has been shown to develop when the initial, appropriate host response to an infection becomes amplified and subsequently dysregulated [16], leading to an imbalance between proinflammatory and anti-inflammatory responses. It has been reported that the innate immune response, which unlike the adaptive immune response, is able to immediately respond to invading pathogens, plays a major role in the initiation of sepsis pathophysiology [17]. The activation of this "first line of cellular defense" results in an excessive release of cytokines, chemokines, and other inflammatory regulators. Cytokines regulate a variety of inflammatory responses, including the migration of immune cells to the locus of infection, which is a crucial step in containing a localized infection and preventing it from becoming systemic. However, a dysregulated cytokine release may lead to endothelial dysfunction, characterized by vasodilation and increased capillary permeability. The resulting leakage syndrome is clinically associated with hypotension, hemoconcentration, macromolecular extravasation, and edema, which are frequent findings in septic patients [18]. The dysfunctional epithelial barriers enable pathogens and their products to further invade the host organism, to disturb regulatory mechanisms, and ultimately, to cause remote organ dysfunctions [19]. Moreover, increasing evidence has indicated that immune and inflammatory responses are tightly interwoven with different physiologic processes within the human host, such as coagulation [20], metabolism [21, 22], and neuroendocrine activation [23, 24]. An inflammation-induced dysregulation of the coagulation system, for instance, significantly aggravates the deleterious effects of sepsis and can result in lethal disseminated intravascular coagulation [25].

Traditionally, sepsis was viewed as an excessive systemic proinflammatory reaction to invasive microbial pathogens. More recently, it has been proposed that the early phase of hyperinflammation is followed or overlapped by a prolonged state of immunosuppression [26–28], referred to as sepsis-induced immunoparalysis [29]. This immunoparalytic state is characterized by impaired innate and adaptive immune responses and, may play a central role in the pathogenesis of tissue damage, multiple organ failure, and death induced by sepsis.

## 4. Initiation of the Immune Response

The innate immune system detects invading microorganisms via pathogen recognition receptors (PRRs), which are expressed on epithelial barriers as well as on immune cells such as dendritic cells and macrophages [30] (Figure 1). A specific family of PRRs named Toll-like receptors (TLRs) recognizes conserved macromolecular motives from microorganisms, called pathogen-associated molecular patterns (PAMPs). Examples of bacterial PAMPs include lipopolysaccharide (LPS; the main virulence factor of Gram-negative bacteria), peptidoglycan, lipoteichoic acid (a cell wall component of Gram-positive bacteria), flagellin, and bacterial DNA [6, 31]. The stimulation of TLRs or the NOD-like receptor

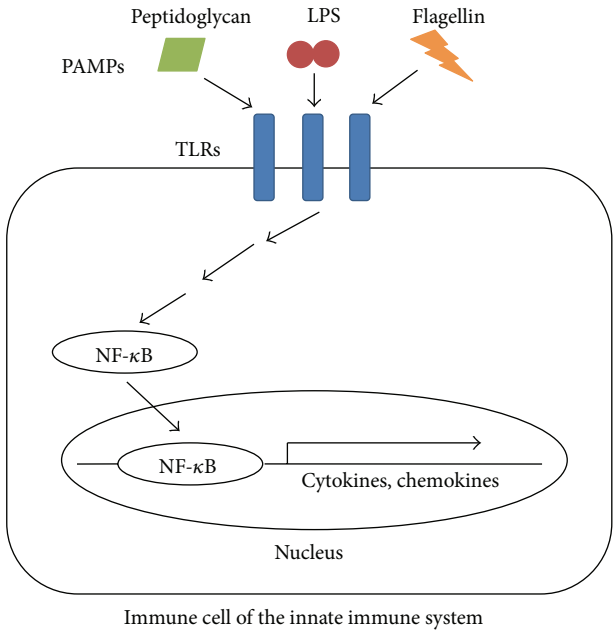



Figure 1: Initiation of the immune response following infection. Immune cells of the innate immune system recognize invading pathogens via Toll-like receptors (TLRs). The binding of pathogen-associated molecular patterns (PAMPs), such as peptidoglycan, lipopolysaccharide (LPS), or flagellin, to TLRs initiates signal transduction cascades that lead to the activation of nuclear factor κB (NF-κB). NF-κB is subsequently translocated into the nucleus where it induces the expression of cytokines and chemokines.

(NLR) family of intracellular PRRs results in the triggering of downstream signaling cascades. Depending on the particular receptor engaged, this process leads to the activation of a transcriptional response program that includes nuclear factor κB (NF-κB), followed by the production and secretion of cytokines, chemokines, and nitric oxide (NO) [32–34].

## 5. Cytokines in Sepsis Pathophysiology

The term cytokine describes a functional class of small protein mediators with low molecular weights (mostly <40 kDa), which are produced in a regulated fashion to affect the activation and differentiation of the immune response. Once released, proinflammatory cytokines lead to an ensuing activation of the innate or the adaptive immune response, characterized by the further production of immunoregulatory or effector cytokines [97]. The sequential release of specific cytokines is referred to as a "cytokine cascade" [98]. In the 1990s, sepsis was believed to be associated with an exacerbated release of mainly proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-12, interferon (IFN)-γ, and macrophage migration inhibitory factor (MIF). The term "cytokine storm" thus arose [99]. However, recent research on the pathophysiologic mechanisms underlying sepsis indicates that the profound proinflammatory response is counteracted by certain anti-inflammatory cytokines, including IL-10, transforming growth factor (TGF)-β, and IL-4, which attempt to restore immunological equilibrium [16, 100]. Lately, efforts have been made to identify unifying mechanisms by employing genome-wide expression data in early and late sepsis. Tang et al. reported that sepsis leads to the immediate upregulation of PRRs and the activation of signal transduction cascades [101]. However, important inflammatory markers, such as TNF-α, IL-1, or IL-10, did not show any consistent pattern in their gene expression and are highly variable in individuals. These findings suggest that the host response to sepsis is not a simple model with an initial proinflammatory phase followed by an anti-inflammatory response, but rather a highly interactive and dynamic process that may reflect heterogeneous genome-specific pathways. A tightly regulated balance in the cytokine network, which comprises proinflammatory cytokines, anti-inflammatory cytokines, and soluble inhibitors of proinflammatory cytokines, such as soluble TNF receptors (sTNFRs), IL-1 receptor antagonist (IL-1Ra), and IL-1 receptor type II (IL-1R2), is crucial for eliminating invading pathogens on the one hand and restricting excessive, tissue-damaging inflammation on the other [102, 103].

This review summarizes current knowledge of the role of cytokines in the regulation of the immune response in sepsis. The actions of individual pro- and anti-inflammatory cytokines are described in more detail and are directly associated with sepsis pathophysiology (see Table 2 for a summary). Along with the increasing knowledge of cytokine actions in recent years, a number of therapeutic strategies targeting cytokines and other immunomodulating agents have been proposed for clinical use in septic patients. Their current role in the treatment of sepsis is discussed later in this review.

## 6. Proinflammatory Cytokines

*6.1. TNF-α and IL-1.* TNF-α and IL-1 (a term used for a family of proteins, including IL-1α and IL-1β [104]) are among the most extensively studied cytokines in sepsis pathophysiology. Both are powerful proinflammatory cytokines that have been implicated in a large number of infectious and noninfectious inflammatory diseases, the latter including atherosclerosis [35], rheumatoid arthritis [36], osteoarthritis [105], and Alzheimer's disease [38]. TNF-α is a 17 kDa protein that is not only derived predominantly from activated immune cells (macrophages) but also from nonimmune cells (fibroblasts) in response to invasive, infectious, or inflammatory stimuli [37, 40]. The release of TNF-α from macrophages begins within 30 minutes after the inciting event, following gene transcription and RNA translation, which established this mediator to be an early regulator of the immune response. TNF-α acts via specific transmembrane receptors, TNF receptor (TNFR)1, and TNFR2 [106], leading to the activation of immune cells and the release of an array of downstream immunoregulatory mediators. Likewise, IL-1 is released primarily from activated macrophages in a timely manner similar to TNF-α, signals through two distinct receptors, termed IL-1 receptor type I (IL-1R1) and IL-1R2, and has comparable downstream effects on immune cells [44, 47]. The injection of

Table 2: Summary of the main features of pro- and anti-inflammatory cytokines.

| Cytokine | Main sources | Main functions | Interactions with other cytokines | Alteration/involvement in diseases | Physiologic inhibitors and therapeutic targeting strategies | References |
|---|---|---|---|---|---|---|
| Proinflammatory | | | | | | |
| TNF-$\alpha$ | Immune cells of the innate and adaptive immune system (mainly macrophages and lymphocytes); fibroblasts | Differentiation and activation of immune cells; induction of fever and coagulation; cachexia; apoptosis | Promote the release of downstream proinflammatory effector molecules | Role in atherosclerosis, RA, Alzheimer's disease, autoimmune diseases, and cancer | sTNFRs; anti-TNF Ab; TNFR inhibitors | [35–43] |
| IL-1 | " | Induction of fever and coagulation; hematopoiesis; promotes the extravasation of inflammatory cells | " | Role in autoinflammatory diseases, heart failure, and diabetes | IL-1R2; IL-1Ra; anti-IL-1$\beta$ mAb | [16, 38, 39, 44–48] |
| IL-6 | " | Activation of B and T lymphocytes; modulation of hematopoiesis and acute phase response; induction of fever | Released in response to TNF-$\alpha$ and IL-1 but inhibits their release; promotes anti-inflammatory responses (sTNFRs, IL-1Ra, and TGF-$\beta$) | $\uparrow$ Serum levels following burns, major surgery, in sepsis, RA, and Crohn's disease | sIL-6R, anti-IL-6 Ab, and anti-IL-6R Ab | [49–63] |
| IL-12 | Monocytes/macrophages; Neutrophils; dendritic cells | Promotes type 1 adaptive immune response and differentiation of $T_H1$ T lymphocytes; induces antitumor immune response | Induces IFN-$\gamma$ production | Role in cancer | Anti-IL-12 mAb | [64–67] |
| IFN-$\gamma$ | NK cells; $T_H1$ and $CD8^+$ cytotoxic T-cells | Antiviral activity; potentially reverses immunoparalysis in sepsis | Released in response to TNF-$\alpha$, IL-12, and IL-18 | $\uparrow$ Serum levels in sepsis | rIFN-$\gamma$ | [68–73] |
| MIF | Pituitary cells; monocytes/macrophages | Activation of macrophages and T-cells; overrides the anti-inflammatory effect of glucocorticoids | Released in response to infection, inflammation, and proinflammatory cytokines; promotes the release of proinflammatory effector molecules | $\uparrow$ Serum levels in acute and chronic inflammatory diseases; role in cancer | Small molecule inhibitors (ISO-1, benzoxazol-2-ones); human anti-MIF Ab; MIF-derived peptide sequences | [69, 74–84] |
| Anti-inflammatory | | | | | | |
| IL-10 | Immune cells of the innate and adaptive immune system | Immunosuppressive properties, such as the impairment of antigen presentation and phagocytosis | Suppress the release of proinflammatory cytokines; stimulate production of sTNFRs and IL-1Ra | Dysregulated in autoimmune diseases | rIL-10 | [85–88] |
| TGF-$\beta$ | Macrophages; smooth muscle cells | Involved in tissue repair, fibrosis, and sepsis-induced immunosuppression | " | $\uparrow$ Serum levels in sepsis; upregulated in cancer and fibrosis | Small molecule inhibitors; anti-TGF-$\beta$ mAb | [89–94] |
| IL-4 | $T_H2$ T lymphocytes; mast cells; basophils; eosinophils | Promotes differentiation of $T_H2$ T lymphocytes | Induces release of IL-4 and IL-13 from macrophages | Role in scleroderma, asthma, and tuberculosis | Anti-IL-4R$\alpha$ mAb | [95, 96] |

RA: rheumatoid arthritis; sTNFRs: soluble TNF receptors; mAb: monoclonal antibody; IL-1Ra: IL-1 receptor antagonist; rIFN-$\gamma$: recombinant IFN-$\gamma$; ISO-1: (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester.

TNF-$\alpha$ into experimental animals causes a syndrome that is largely indistinguishable from septic shock [107] and infusion of recombinant TNF-$\alpha$ into humans results in SIRS [108–110]. Similar results were reported for IL-1 [111–113]. TNF-$\alpha$ and IL-1 act synergistically to induce a shock-like state characterized by vascular permeability, severe pulmonary edema, and hemorrhage [113]. Importantly, TNF-$\alpha$ and IL-1 were also identified as pivotal mediators for the development of fever and, thus, belong to a group of pyrogenic cytokines [39].

A role for TNF-$\alpha$ and IL-1 in sepsis was demonstrated in numerous reports, including both experimental animal models of septic shock and studies in humans with sepsis. The administration of bacterial endotoxin results in the production and release of TNF-$\alpha$ and IL-1 into the systemic circulation, where peak concentrations are detected 60–90 min after LPS administration [114–117]. Once released, TNF-$\alpha$ and IL-1 act on different target cells, such as macrophages, endothelial cells, and neutrophils. TNF-$\alpha$ leads to an enhanced production of macrophages from progenitor cells [118], promotes the activation and differentiation of macrophages [43], and prolongs their survival [119]. All these effects enhance proinflammatory responses in sepsis. In endothelial cells, TNF-$\alpha$ enhances the expression of adhesion molecules, such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, and chemokines [120, 121]. TNF-$\alpha$ also increases integrin adhesiveness in neutrophils and promotes their extravasation into tissues. TNF-$\alpha$ and IL-1 were identified as the main mediators of inflammation-induced activation of coagulation, with TNF-$\alpha$ having a potent upregulating action on endothelial expression of procoagulant [42]. In addition, TNF-$\alpha$ and IL-1 amplify inflammatory cascades in an autocrine and paracrine manner by activating macrophages to secrete other proinflammatory cytokines (IL-6, IL-8, and MIF), lipid mediators, and reactive oxygen and nitrogen species [16, 46], leading to sepsis-induced organ dysfunction. Because of its unique ability to orchestrate downstream cytokine cascade, TNF-$\alpha$ is considered to be a "master regulator" of inflammatory cytokine production [37], while the important regulatory role of IL-1 in inflammation is widely accepted as well.

Soluble cytokine receptors and receptor antagonists, termed sTNFRs, IL-1R2, and IL-1Ra, were identified for TNF-$\alpha$ and IL-1, which modulate the actions of these cytokines. Elevated levels of sTNFRs and IL-1Ra were measured in the systemic circulation of healthy volunteers administered endotoxin [122, 123], and in septic patients, in whom sTNFRs and IL-1Ra plasma concentrations also correlated with disease severity, and in the case of sTNFRs, with mortality [124–126]. In different murine models of septic shock, the administration of IL-1Ra increased survival, suggesting a therapeutic effect for IL-1Ra [48, 127]. For sTNFRs, it was proposed that the ratio between TNF-$\alpha$ and sTNFRs, rather than the absolute plasma concentration of TNF-$\alpha$ or sTNFRs alone, has prognostic value in septic patients [41]. This indicates that a tight balance between cytokines and their soluble inhibitors is crucial for a positive outcome of sepsis. However, the exact mechanisms underlying this balance remain incompletely understood.

*6.2. IL-6.* IL-6 is a 21 kDa glycoprotein produced by a wide variety of cells, especially macrophages, dendritic cells, lymphocytes, endothelial cells, fibroblasts, and smooth muscle cells in response to stimulation with LPS, IL-1, and TNF-$\alpha$ [53–56]. Elevated IL-6 concentrations are measured in many acute conditions, such as burns, major surgery and sepsis [52], and peak subsequent to TNF-$\alpha$ and IL-1 concentrations [123, 128]. Plasma levels of IL-6 are stably elevated in these conditions and correlate with many indicators of disease severity such as clinical scores [129], stress after surgery [130] and trauma [131], the occurrence of multiple organ failure and septic shock [132, 133], and the overall mortality [134].

IL-6 has a variety of biological effects, including the activation of B and T lymphocytes and the coagulation system, and the modulation of hematopoiesis [49, 61]. In contrast to TNF-$\alpha$ and IL-1, the injection of IL-6 by itself does not produce a sepsis-like state [135]. A key function of IL-6 is the induction of fever [50] and the mediation of the acute phase response [51, 59], a systemic reaction to an inflammatory stimulus that is characterized by fever, leukocytosis, and the release of hepatic acute phase proteins such as C-reactive protein, complement components, fibrinogen, and ferritin [136]. *In vivo* studies in *Il-6*-knockout mice demonstrated that the deletion of the *Il-6* gene decreases lung inflammation in a model of acute lung injury [137] and protects from mortality and the development of organ failure in a zymosan-induced acute peritoneal inflammation [138]. More recently, Pathan et al. showed that IL-6 causes myocardial depression in meningococcal disease [139]. Myocardial dysfunction in septic shock leads to impaired tissue perfusion, multiorgan failure, and death.

Despite its proinflammatory properties, IL-6 also has been shown to promote anti-inflammatory responses. IL-6 inhibits the release of TNF-$\alpha$ and IL-1 [57] and enhances the circulation levels of anti-inflammatory mediators, such as IL-1Ra, sTNFRs, IL-10, TGF-$\beta$, and cortisol [58, 60, 62]. A protective effect of IL-6 was shown in experimental endotoxemia [140, 141], whereas the genetic deletion of IL-6 did not alter the mortality in a model of polymicrobial sepsis induced by cecal ligation and puncture (CLP) [142].

*6.3. IL-12.* Phagocytes (monocytes/macrophages and neutrophils) and dendritic cells are the major sources of the heterodimeric cytokine IL-12 [66, 67], which is structurally related to the IL-6 cytokine family [143]. IL-12 regulates innate immune responses and promotes the development of a type 1 adaptive immune response, which is characterized by enhanced mononuclear phagocyte responses. Thus, IL-12 links early, nonspecific, and later, specific immune responses. Upon release, IL-12 induces T-cells and natural killer (NK) cells to produce IFN-$\gamma$, which directly activates macrophages to enhance their bactericidal activity and produce additional T helper 1 ($T_H1$) cytokines [64]. Additionally, IL-12 stimulates the differentiation of naive $CD4^+$ T-cells into $T_H1$ cells and protects them from antigen-induced apoptotic death [65]. IL-12 also increases the proliferation and colony formation of hematopoietic progenitors.

Despite many years of research, the role of IL-12 in sepsis remains controversial. Initially, animal models of sepsis were employed to investigate its role in sepsis. Increased plasma IL-12 concentrations were measured in animals following the administration of LPS or *Escherichia coli*, and after polymicrobial sepsis induced by CLP [144–146]. The immunoneutralization or genetic deletion of IL-12 resulted in an increased mortality of mice undergoing CLP, with a subsequent decrease in IFN-$\gamma$ and an increase in IL-10 levels [144, 147]. However, in a different animal model, an increase in LPS-induced mortality was observed in mice transiently overexpressing IL-12 and the neutralization of IL-12 improved survival following LPS challenge [145]. Clinically, a prospective study in patients undergoing major visceral surgery suggested that a selective defect in preoperative monocyte IL-12 production impairs the host defense against postoperative infections and, thus, increases the risk of lethal sepsis [148]. Likewise, it was reported that survivors from severe sepsis produce more IL-12 from LPS-stimulated peripheral blood mononuclear cells (PBMCs) than nonsurvivors [149], and that they show serial increases in their IL-12 response from PBMCs [150].

*6.4. IFN-$\gamma$.* IFN-$\gamma$ is mainly produced by activated NK cells, $T_H1$, and $CD8^+$ cytotoxic T cells [68]. Its production is tightly regulated and stimulated by macrophage-derived cytokines, especially TNF-$\alpha$, IL-12, and IL-18 [72]. IFN-$\gamma$ was discovered due to its antiviral activity [73]. Subsequently, the important immunoregulatory role of IFN-$\gamma$ to a wider range of pathogens became evident. Mice lacking IFN-$\gamma$ were shown to be more susceptible to intracellular pathogens, such as *Leishmania major* [151], *Listeria monocytogenes* [152], Mycobacteria [153], and different viruses [154]. The neutralization of IFN-$\gamma$ or its receptor makes mice more resistant to an LPS-induced shock [155, 156]. IFN-$\gamma$ is normally not detectable in the plasma of healthy humans, but its levels can be elevated in patients with sepsis [69]. Plasma levels of IFN-$\gamma$ do not correlate with sepsis severity or mortality. Recently, a role for IFN-$\gamma$ in the reversal of sepsis-induced immunoparalysis was reported. During the immunoparalytic state, macrophages were shown to display impaired phagocyte functions and to release reduced amounts of $T_H1$-promoting cytokines upon stimulation with bacterial products [157, 158]. Flohé et al. showed that IFN-$\gamma$, as well as granulocyte-macrophage colony-stimulating factor (GM-CSF), was able to restore macrophage function in macrophages taken from septic mice upon bacterial stimulation *ex vivo* [70]. Likewise, a recently published *in vivo* study in humans demonstrated that IFN-$\gamma$ partially reverses immunoparalysis, identifying IFN-$\gamma$ as a potential new treatment option for sepsis [71].

*6.5. MIF.* MIF is a pleiotropic proinflammatory cytokine, which is responsible for the first cytokine activity to be discovered [159]. MIF is released by pituitary cells in response to LPS and stress [74, 77] and by immune cells (most importantly monocytes and macrophages) after exposure to various infectious and inflammatory stimuli, including LPS, TNF-$\alpha$, and IFN-$\gamma$ [78, 160]. Uniquely among innate cytokines, MIF is present in preformed pools within cells and is rapidly released upon proinflammatory and stress stimulation [161]. This release response of the preformed protein is followed by MIF gene transcription and RNA translation, which replenishes intracellular stores. The Golgi complex-associated protein p115 was identified as an intracellular binding partner for MIF that is essential for its secretion [161]. Once secreted, MIF increases macrophage antimicrobial responses by increasing macrophage survival [83], elevating TLR4 expression on macrophages [162] and promoting macrophage inflammatory recruitment [75]. MIF also promotes the secretion of downstream cytokines, such as TNF-$\alpha$, IFN-$\gamma$, and IL-1, and it promotes the activation of T cells [80]. MIF activates immune cells by binding to CD74 (the cell surface form of the class II-associated invariant chain), which leads to the recruitment of CD44 into a signaling complex and the downstream initiation of the ERK1/2 MAP kinase pathway [163, 164]. Additionally, MIF engages the chemokine receptors CXCR2 and CXCR4 in a high affinity, noncognate interaction [75]. While the precise signaling mechanisms of MIF through these receptors are yet to be clarified, it was demonstrated that the MIF/CXCR axis is critical for MIF-dependent monocyte recruitment processes in atherosclerotic arteries [75]. MIF's critical role within the immune system is further underscored by the finding that MIF is induced by low concentrations of glucocorticoids and has the unique ability to override the anti-inflammatory and immunosuppressive effects of glucocorticoids [77, 81, 84]. Mouse modeling and human clinical studies have implicated MIF in the pathogenesis of various acute and chronic inflammatory diseases, including septic shock [79], asthma [165], rheumatoid arthritis [166], atherosclerosis [167], inflammatory bowel disease [168], and cancer [76].

The actions of MIF in sepsis pathophysiology have been studied extensively. The administration of recombinant MIF protein increases mortality following LPS administration [74]. Conversely, several studies showed that the neutralization of MIF reduced proinflammatory cytokine production, decreased organ injury, and increased the survival rate of mice in different animal models of sepsis, such as endotoxic shock, *Escherichia coli* injection or CLP [79, 169–172]. Recently, MIF was established as an important mediator of LPS-induced myocardial dysfunction [173, 174]. Serum MIF concentration of patients suffering from sepsis are significantly higher compared to healthy individuals [175] and correlate with the outcome [176]. Thus, MIF was suggested as an early predictor for survival in septic patients [177]. In the largest genetic study of sepsis performed to date, *MIF* alone among 20 candidate polymorphic loci within immune response genes was associated with clinical outcome from septic shock [178]. Notably, the role for *MIF* gene variants in this study of community-acquired pneumonia progressing to sepsis was found to be one of protection, with a 50% survival benefit observed in individuals with high expression *MIF* alleles at 30, 60, and 90 days of followup. Thus, despite prior suggestions that sepsis pathology results from an excessive or overreactive systemic inflammatory response, high MIF expression was protective, presumably

because of its high upstream role in eliminating invasive microbial infections or because of its ability to counteract the immunoparalytic state. A strong role for MIF also has been reported for clinical outcome from meningococcemia [179], invasive streptococcal infection [180], and severe malaria [181].

Very recently, the protein D-dopachrome tautomerase (D-DT), which is the only known MIF homolog in the human genome, was identified as a cytokine [82, 175]. While the precise biologic functions of D-DT (a.k.a. MIF-2) are yet to be clarified, it was demonstrated that D-DT is released in response to LPS and that its immunoneutralization protects mice from lethal endotoxic shock. This protective action of anti-D-DT was associated with a reduction in the circulating levels of TNF-$\alpha$, IFN-$\gamma$, IL-12, and IL-1 and increases in the serum concentration of IL-10. D-DT serum levels have been determined to be higher in septic patients compared to healthy controls and to correlate with MIF and with disease severity.

## 7. Anti-Inflammatory Cytokines

*7.1. IL-10.* IL-10 is a 35-kDa homodimeric cytokine that is produced by many types of immune cells, such as monocytes, macrophages, B and T lymphocytes, and NK cells [87]. Functional studies widely revealed anti-inflammatory functions of IL-10. *In vitro*, IL-10 suppresses the production of proinflammatory mediators, such as TNF-$\alpha$, IL-1, IL-6, IFN-$\gamma$, and GM-CSF, in immune cells [85, 86]. In contrast, it was reported that IL-10 has no effect on the constitutive expression of TGF-$\beta$, a cytokine with anti-inflammatory properties. Additionally, IL-10 stimulates the production of IL-1Ra and sTNFRs, thereby neutralizing the proinflammatory actions of IL-1 and TNF [88]. These results were supported *in vivo*. In an experimental murine model, the administration of recombinant IL-10 protein protected mice from lethal endotoxemia, even when IL-10 was injected 30 minutes after the LPS administration [182]. In contrast, the immunoneutralization of IL-10 led to elevated levels of circulating TNF-$\alpha$ and IL-6 in mice [183] and reversed the ability of IL-10 to protect mice from lethal endotoxemia [182]. Despite these clearly protective effects of IL-10 in LPS-induced pathologies, the actions of IL-10 were not always beneficial in the CLP model of polymicrobial sepsis. In fact, the inhibition of IL-10 12 hours after CLP markedly improved survival [184]. However, the administration of neutralizing IL-10 antibodies at the time of CLP partially exacerbated mortality [185]. These findings indicate that the time of anti-IL-10 antibody application is crucial for the outcome, and that IL-10 can exhibit protective or harmful effects in the course of sepsis. More recently, Latifi et al. reported that IL-10-deficient mice showed an earlier onset of lethality following CLP and showed a reduced response to rescue surgery (the removal of the necrotic cecum) compared with wildtype mice [87]. However, the administration of recombinant IL-10 protein to WT or IL-10 deficient mice increased survival and lengthened the therapeutic window for the rescue surgery. These results suggest that IL-10 might regulate the transition from early reversible sepsis to late irreversible septic shock. Recently, it was investigated whether polymorphisms in the IL-10 gene promotor affect sepsis susceptibility. Zeng et al. showed that the –1082A allele was associated with a lower IL-10 production following LPS stimulation and with the development of sepsis after major trauma [186].

*7.2. TGF-$\beta$.* TGF-$\beta$ is a member of a family of dimeric polypeptide growth factors and is an important anti-inflammatory cytokine. A role for TGF-$\beta$ was demonstrated in tissue repair und fibrosis [93], as well as in sepsis-induced immunosuppression [89]. *In vitro*, TGF-$\beta$ suppresses the release of proinflammatory mediators, such as IL-1, TNF-$\alpha$, and HMGB1, from monocytes and macrophages [90, 92], and stimulates the production of immunosuppressive factors such as sTNFRs and IL-1Ra [94]. TGF-$\beta$ also inhibits T lymphocyte functions, such as IL-2 secretion and T cell proliferation [187], and it promotes the development of T regulatory cells [188]. Moreover, studies demonstrated a role of TGF-$\beta$, as well as IL-10, in the tolerance of monocytes and macrophages to LPS, which is characterized by a downregulated cytokine response following a second LPS challenge [189].

In alignment with the *in vitro* studies, experiments in animal models of sepsis and clinical studies in humans supported the anti-inflammatory actions of TGF-$\beta$. Parrella et al. reported that treatment with TGF-$\beta$ blocked endotoxin-induced hypotension, potentially by inhibiting the hypotensive effects of NO and improved survival in a rat model of *Salmonella typhosa* endotoxin-induced septic shock [190]. Similar results were reported in a rat model using the endotoxin of *Salmonella enteritidis* [191] and in the murine endotoxic shock model [192]. Moreover, patients with sepsis had elevated levels of TGF-$\beta$ compared to healthy controls [91]. TGF-$\beta$ levels were shown to peak early in disease progression and not to correlate strongly with disease severity or prognosis [193]. Recent data demonstrated that TGF-$\beta$ reverses the depression of cardiac myocyte contraction, which is induced by proinflammatory cytokines, such as TNF-$\alpha$ and IL-1, and by serum from patients with septic shock [194]. This suggests that TGF-$\beta$ might have cardioprotective effects in sepsis-induced cardiac injury.

*7.3. IL-4.* IL-4 is a cytokine with many immunoregulatory functions, which was shown to participate in the regulation of proliferation, differentiation, and apoptosis of multiple cell types [195–197]. An important action of IL-4 is its critical role in the regulation of T lymphocyte differentiation, in which it promotes $T_H2$ cell differentiation while inhibiting $T_H1$ cell differentiation [96]. IL-4 is the principal cytokine produced by $T_H2$ lymphocytes, causes an enhanced release of further IL-4 and other anti-inflammatory cytokines, and suppresses the secretion of monocyte-derived proinflammatory cytokines [95].

Animal-based studies revealed that IL-4 increases survival of mice exposed to lethal doses of LPS [198]. However, protective as well as detrimental effects of IL-4 were described in *Staphylococcus aureus*-triggered murine sepsis, which appeared to depend on the host's genetic background [199].

In humans, it was reported that the mRNA expression of IL-4 was associated with survival of patients with severe sepsis, but that the plasma IL-4 levels in septic patients on the day of admission to the hospital did not differ between survivors and nonsurvivors [200]. Recently, it was suggested that *IL-4* promoter polymorphisms might affect the balance between the $T_H1$ and $T_H2$ immune response, and thereby predispose trauma patients to the development of sepsis [201]. While all these studies indicate that IL-4 plays an important role in the pathogenesis of sepsis, its precise role during the course of disease remains unknown.

## 8. Immunomodulating Treatment Strategies for Sepsis

Basic research and clinical studies performed over the past several years have led to a significant amount of data on immunoregulatory and modulating mechanisms in sepsis. Cytokines have proved to function as important regulators of the immune response, while various other agents, including growth factors or activated protein C (APC), have shown immunomodulating effects. Therefore, it would appear to be highly promising and beneficial to therapeutically target these mediators in order to decrease the unfavorable effects of sepsis-related host responses, and to improve the overall outcome. A number of potential therapeutic targets have been identified to date, and their clinical use has subsequently been assessed in sepsis, both in animal models and in clinical trials. The following paragraphs will give an overview of recent important therapeutic strategies for the treatment of sepsis with special respect to anticytokine approaches.

*8.1. Anti-TNF-α and Anti-IL-1.* In one of the first approaches of treating sepsis, therapies were directed against TNF-$\alpha$ and IL-1. These therapies included monoclonal antibodies against TNF-$\alpha$ [202], sTNFRs [45], IL-1Ra, and soluble IL-1 receptors [203]. While positive results were obtained in experimental models of sepsis, these agents failed to decrease the overall mortality of septic patients in clinical trials [204, 205]. Theses clinical results were unexpected, as the powerful cytokines TNF-$\alpha$ and IL-1 had been shown to initiate the excessive inflammatory immune response in sepsis, which was believed to cause the deleterious effects on the host organism. Subsequent studies were conducted to explain the lack of success of TNF-$\alpha$ and IL-1 blocking agents in clinical trials. Among many potential reasons, it was reported that the circulating levels of "early" cytokines like TNF-$\alpha$ and IL-1 return to almost baseline levels within the first few hours during the progression of disease [206]. Thus, the specific inhibition of "early" cytokines may provide only a narrow window for clinical intervention. Moreover, the elevation of their circulating levels may be downregulated even before the diagnosis of sepsis is made [207], indicating that the early diagnosis of sepsis is crucial for the outcome. It was proposed that inhibiting cytokines like MIF, whose immunoneutralization protected mice from lethal peritonitis even when the antibodies were administered after the onset of disease [79], or HMGB1, which may be involved in later stages of sepsis, might be beneficial in reducing sepsis mortality.

*8.2. Anti-MIF.* Given the complex role of MIF in various pathologies, such as sepsis, MIF is under investigation as a target for the development of novel pharmacological agents. Crystallographic studies of human MIF have identified a tautomerase enzymatic activity site that is important for MIF's cytokine activities [169, 208]. This offers the unique possibility to target a cytokine by a small molecule approach. In fact, small molecules like ISO-1 [(S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester] were found to inhibit this catalytic site and to block MIF interaction with its receptor [209] and its downstream effects. ISO-1 suppresses the MIF-induced activation of NF-$\kappa$B (although NF-$\kappa$B has so far not emerged as the predominant pathway induced by MIF) and the MIF-induced production of TNF-$\alpha$ from macrophages *in vitro* [169]. *In vivo*, the administration of ISO-1 dose-dependently improves survival in a murine model of lethal endotoxemia and rescues mice from polymicrobial sepsis, even when the ISO-1 treatment is started 24 hours after the CLP surgery [169]. ISO-1 or anti-MIF monoclonal antibody administration also was beneficial in a model of lethal flavivirus infection [210]. The positive results obtained from animal models have helped to prompt the clinical development of specific MIF blocking agents. Currently, a human anti-MIF antibody is in clinical development [211], and small molecule MIF inhibitors such as potent benzoxazol-2-ones are advancing towards clinical application [209, 212]. MIF-derived peptide sequences targeting MIF/receptor interfaces also have been considered as potential strategies [213].

*8.3. IFN-γ- and GM-CSF-Directed Strategies.* In light of recent research indicating that an immunosuppressive state may contribute to sepsis pathophysiology, it may be advantageous to apply IFN-$\gamma$ or growth factors, such as GM-CSF, in order to restore the host immune functions. Clinical studies showed that GM-CSF improved the gas exchange in patients with severe sepsis associated with respiratory dysfunction [214] and resulted in a more effective anti-infectious defense [215]. However, in neither study did treatment with GM-CSF improve mortality. Also, IFN-$\gamma$ given intravenously to severely injured patients was not successful in decreasing infection rates or improving survival [216].

*8.4. APC-Directed Strategies.* Numerous studies have revealed functional interactions between inflammation and coagulation that contribute significantly to sepsis pathophysiology [20, 217]. Inflammation mediates the coagulation cascade, leading in the extreme case to the development of disseminated intravascular coagulation, and clotting factors in return reciprocally modulate the local or the systemic inflammatory response. Therefore, therapeutic intervention in the coagulation pathway might not only counteract the deleterious effects attributed to a dysregulated coagulation system but also affect the dysregulated inflammatory and immune response in a beneficial manner.

Recombinant human activated protein C (rhAPC) was the first biological drug for the treatment of sepsis that was approved by the Food and Drug Administration in the United States. Protein C is produced by the liver as an acute phase zymogen and is subsequently activated by thrombin [218]. Upon its activation, APC proteolytically inactivates factors Va and VIIIa of the coagulation cascade, resulting in a decrease in thrombin production. Low thrombin levels ultimately lead to the inhibition of the thrombin-induced platelet activation. These anticoagulant actions of APC were considered initially to be responsible for its beneficial effect in sepsis. However, more recent studies have suggested an additional anti-inflammatory action of APC. By preventing the excessive generation of thrombin, APC reduces thrombin's strong proinflammatory actions [219], which include the release of chemokines and cytokines (such as MIF) and the expression of adhesion molecules on platelets and endothelium [220]. Moreover, APC was shown to inhibit chemotaxis and IL-6 release by human neutrophils [221] and to prevent the production of proinflammatory cytokines, such as TNF-$\alpha$, IL-1$\beta$, IL-6, MIF, and IL-8 by LPS-stimulated monocytes [222, 223]. Antiapoptotic functions have also been attributed to APC. Bilbault et al. showed that circulating mononuclear cells from septic shock patients treated with rhAPC had decreased Bax/Bcl-2 protein ratios compared to healthy controls [224]. Low Bax/Bcl-2 protein ratios are found in antiapoptotic states, which might be beneficial in the recovery from sepsis as high apoptotic rates of immune cells were shown to contribute to the immunoparalytic state of sepsis [225].

Following preclinical investigations of septic shock showing that the administration of APC improved survival [226], the first reports describing the impact of rhAPC administration in humans with severe sepsis were published in 2001. Bernard et al. reported that a 96-hour continuous infusion of rhAPC, also referred to as drotrecogin alfa (activated) (DrotAA), markedly reduced the circulating levels of D-dimers (fibrin degradation products) and IL-6 in patients with severe sepsis [227]. The PROWESS phase 3 clinical trial subsequently showed that the treatment of severe sepsis with rhAPC reduced the relative and absolute death risk by 19.4 and 6.1%, respectively [228]. However, an increased incidence of serious bleeding events was observed in the rhAPC-treated group compared to the placebo group. Nonetheless, on the strength of the survival results, rhAPC was approved for clinical use. Because subgroup analysis in the PROWESS study showed that reduced mortality in the rhAPC-treated group was limited to patients with high disease severity, for instance, those with at least two sepsis-induced dysfunctional organs or those with a high acute physiology and chronic health evaluation (APACHE) II score, the international guidelines for the management of severe sepsis, and septic shock released in 2008 recommended that rhAPC only be used for patients at high risk of death [229]. Unfortunately, more recently published results from the follow-up PROWESS-SHOCK trial indicated that rhAPC did not significantly reduce mortality of patients with septic shock. In fact, at both 28 and 90 days after the initiation of treatment, there was no significant difference in the mortality rate between septic patients treated with rhAPC and those given a placebo (26.4 versus 24.2% and 34.1 versus 32.7%, resp.) [230]. These results have now led to the withdrawal of rhAPC from the market. Future studies will be required to clarify whether rhAPC ultimately finds clinical utility, perhaps in a carefully defined subset of subjects with sepsis. Current treatment modalities for sepsis remain largely supportive rather than directly immunomodulating.

## 9. Conclusion

Sepsis remains a major challenge both for clinicians and researchers. Despite many years of intensive research and numerous clinical studies, its pathophysiology is still incompletely understood, and specific anticytokine treatments have not been successful in clinical trials. This is mainly due to the fact that sepsis can be characterized as a complex and dynamic disease process that involves excessive and suppressed inflammatory and immune responses. Moreover, it affects heterogeneous patient populations with diverse disease etiologies and comorbidities, further aggravating our difficulties in understanding and therapeutically intervening in this complex syndrome. Nonetheless, research studies have elucidated many different pathophysiologic processes involved in sepsis and have revealed an important regulatory role of pro- and anti-inflammatory cytokines in disease progression. These findings have led to the development of promising anticytokine and immunomodulating treatment strategies. We anticipate that ongoing research will expand our knowledge of currently described disease mechanisms and lead to the identification of new pathophysiologic features of sepsis. Also, we expect that novel antisepsis strategies will continue to be clinically assessed and potentially exploited for the more effective future treatment of sepsis.

## Acknowledgments

The authors were supported by the Deutsche Forschungsgemeinschaft (DFG) Grant SCHU2851/1-1 (to Wibke Schulte), DFG Grants BE1977/4-2 and SFB-TRR57/P07 (to Jürgen Bernhagen), and the National Institutes of Health Grant NIH RO1AI042310 (to Richard Bucala).

## 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 Medicine," *Chest*, vol. 101, no. 6, 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] 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.

[4] 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.

[5] R. Anel and A. Kumar, "Human endotoxemia and human sepsis: limits to the model," *Critical Care*, vol. 9, no. 2, pp. 151–152, 2005.

[6] T. van der Poll and S. M. Opal, "Host-pathogen interactions in sepsis," *The Lancet Infectious Diseases*, vol. 8, no. 1, pp. 32–43, 2008.

[7] A. N. Chalupka and D. Talmor, "The economics of sepsis," *Critical Care Clinics*, vol. 28, no. 1, pp. 57–76, 2012.

[8] H. Burchardi and H. Schneider, "Economic aspects of severe sepsis: a review of intensive care unit costs, cost of illness and cost effectiveness of therapy," *PharmacoEconomics*, vol. 22, no. 12, pp. 793–813, 2004.

[9] S. Geroulanos and E. T. Douka, "Historical perspective of the word 'sepsis'," *Intensive Care Medicine*, vol. 32, no. 12, p. 2077, 2006.

[10] 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.

[11] M. M. Levy, M. P. Fink, J. C. Marshall et al., "2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference," *Critical Care Medicine*, vol. 31, no. 4, pp. 1250–1256, 2003.

[12] C. Alberti, C. Brun-Buisson, S. V. Goodman et al., "Influence of systemic inflammatory response syndrome and sepsis on outcome of critically III infected patients," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 1, pp. 77–84, 2003.

[13] C. Alberti, C. Brun-Buisson, S. Chevret et al., "Systemic inflammatory response and progression to severe sepsis in critically ill infected patients," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 5, pp. 461–468, 2005.

[14] D. Pittet, S. Rangel-Frausto, N. Li et al., "Systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock: incidence, morbidities and outcomes in surgical ICU patients," *Intensive Care Medicine*, vol. 21, no. 4, pp. 302–309, 1995.

[15] R. P. Dellinger, J. M. Carlet, H. Masur et al., "Surviving sepsis campaign guidelines for management of severe sepsis and septic shock," *Critical Care Medicine*, vol. 32, no. 3, pp. 858–873, 2004.

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

[17] J. D. Hansen, L. N. Vojtech, and K. J. Laing, "Sensing disease and danger: a survey of vertebrate PRRs and their origins," *Developmental and Comparative Immunology*, vol. 35, no. 9, pp. 886–897, 2011.

[18] 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.

[19] S. Denk, M. Perl, and M. Huber-Lang, "Damage- and pathogen-associated molecular patterns and alarmins: keys to sepsis?" *European Surgical Research*, vol. 48, no. 4, pp. 171–179, 2012.

[20] M. Levi and T. van der Poll, "Inflammation and coagulation," *Critical Care Medicine*, vol. 38, supplement 2, pp. S26–S34, 2010.

[21] T. Atsumi, Y. Cho, L. Leng et al., "The proinflammatory cytokine macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation," *The Journal of Immunology*, vol. 179, no. 8, pp. 5399–5406, 2007.

[22] B. Mlinar and J. Marc, "New insights into adipose tissue dysfunction in insulin resistance," *Clinical Chemistry and Laboratory Medicine*, vol. 49, no. 12, pp. 1925–1935, 2011.

[23] M. Emonts, F. C. G. J. Sweep, N. Grebenchtchikov et al., "Association between high levels of blood macrophage migration inhibitory factor, inappropriate adrenal response, and early death in patients with severe sepsis," *Clinical Infectious Diseases*, vol. 44, no. 10, pp. 1321–1328, 2007.

[24] L. Capuron and A. H. Miller, "Immune system to brain signaling: neuropsychopharmacological implications," *Pharmacology and Therapeutics*, vol. 130, no. 2, pp. 226–238, 2011.

[25] K. M. Hook and C. S. Abrams, "The loss of homeostasis in hemostasis: new approaches in treating and understanding acute disseminated intravascular coagulation in critically ill patients," *Clinical and Translational Science*, vol. 5, no. 1, pp. 85–92, 2012.

[26] R. S. Hotchkiss and D. W. Nicholson, "Apoptosis and caspases regulate death and inflammation in sepsis," *Nature Reviews Immunology*, vol. 6, no. 11, pp. 813–822, 2006.

[27] H. Volk, P. Reinke, and W. Döcke, "Clinical aspects: from systemic inflammation to 'immunoparalysis'," *Chemical Immunology*, vol. 74, pp. 162–177, 2000.

[28] R. N. Germain, "Maintaining system homeostasis: the third law of Newtonian immunology," *Nature Immunology*, vol. 13, no. 10, pp. 902–906, 2012.

[29] R. S. Hotchkiss and S. Opal, "Immunotherapy for sepsis—a new approach against an ancient foe," *The New England Journal of Medicine*, vol. 363, no. 1, pp. 87–89, 2010.

[30] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

[31] K. J. Ishii, S. Koyama, A. Nakagawa, C. Coban, and S. Akira, "Host innate immune receptors and beyond: making sense of microbial infections," *Cell Host and Microbe*, vol. 3, no. 6, pp. 352–363, 2008.

[32] S. Akira and K. Takeda, "Toll-like receptor signalling," *Nature Reviews Immunology*, vol. 4, no. 7, pp. 499–511, 2004.

[33] S. A. Lakhani and C. W. Bogue, "Toll-like receptor signaling in sepsis," *Current Opinion in Pediatrics*, vol. 15, no. 3, pp. 278–282, 2003.

[34] L. A. J. O'Neill, "A critical role for citrate metabolism in LPS signalling," *Biochemical Journal*, vol. 438, no. 3, pp. e5–e6, 2011.

[35] J. Jawien, "New insights into immunological aspects of atherosclerosis," *Polskie Archiwum Medycyny Wewnetrznej*, vol. 118, no. 3, pp. 127–131, 2008.

[36] E. Choy, "Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis," *Rheumatology*, vol. 51, supplement 5, pp. v3–v11, 2012.

[37] N. Parameswaran and S. Patial, "Tumor necrosis factor-a signaling in macrophages," *Critical Reviews in Eukaryotic Gene Expression*, vol. 20, no. 2, pp. 87–103, 2010.

[38] J. M. Rubio-Perez and J. M. Morillas-Ruiz, "A review: inflammatory process in Alzheimer's disease, role of cytokines," *The Scientific World Journal*, vol. 2012, Article ID 756357, 15 pages, 2012.

[39] C. A. Dinarello, "Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed," *Journal of Endotoxin Research*, vol. 10, no. 4, pp. 201–222, 2004.

[40] M. Feldmann, F. M. Brennan, M. Elliott, P. Katsikis, and R. N. Maini, "TNF$\alpha$ as a therapeutic target in rheumatoid arthritis," *Circulatory Shock*, vol. 43, no. 4, pp. 179–184, 1994.

[41] B. Modzelewski, "The role of soluble TNF p55 and p75 receptors in the development of septic syndrome," *Polski Merkuriusz Lekarski*, vol. 14, no. 79, pp. 69–72, 2003.

[42] M. Schouten, W. J. Wiersinga, M. Levi, and T. van der Poll, "Inflammation, endothelium, and coagulation in sepsis," *Journal of Leukocyte Biology*, vol. 83, no. 3, pp. 536–545, 2008.

[43] A. L. Witsell and L. B. Schook, "Tumor necrosis factor alpha is an autocrine growth regulator during macrophage differentiation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 10, pp. 4754–4758, 1992.

[44] 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.

[45] C. J. Fisher Jr., J. M. Agosti, S. M. Opal et al., "Treatment of septic shock with the tumor necrosis factor receptor: Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group," *The New England Journal of Medicine*, vol. 334, no. 26, pp. 1697–1702, 1996.

[46] Y. Fong, K. J. Tracey, L. L. Moldawer et al., "Antibodies to cachectin/tumor necrosis factor reduce interleukin 1$\beta$ and interleukin 6 appearance during lethal bacteremia," *Journal of Experimental Medicine*, vol. 170, no. 5, pp. 1627–1633, 1989.

[47] L. A. J. O'Neill, "The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress," *Immunological Reviews*, vol. 226, no. 1, pp. 10–18, 2008.

[48] K. Ohlsson, P. Bjork, M. Bergenfeldt, R. Hageman, and R. C. Thompson, "Interleukin-1 receptor antagonist reduces mortality from endotoxin shock," *Nature*, vol. 348, no. 6301, pp. 550–552, 1990.

[49] E. C. Borden and P. Chin, "Interleukin-6: a cytokine with potential diagnostic and therapeutic roles," *Journal of Laboratory and Clinical Medicine*, vol. 123, no. 6, pp. 824–829, 1994.

[50] Z. Chai, S. Gatti, C. Toniatti, V. Poli, and T. Bartfai, "Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1$\beta$: a study on IL- 6-deficient mice," *Journal of Experimental Medicine*, vol. 183, no. 1, pp. 311–316, 1996.

[51] M. Kopf, H. Baumann, G. Freer et al., "Impaired immune and acute-phase responses in interleukin-6-deficient mice," *Nature*, vol. 368, no. 6469, pp. 339–342, 1994.

[52] M. W. N. Nijsten, C. E. Hack, M. Helle, H. J. Ten Duis, H. J. Klasen, and L. A. Aarden, "Interleukin-6 and its relation to the humoral immune response and clinical parameters in burned patients," *Surgery*, vol. 109, no. 6, pp. 761–767, 1991.

[53] D. A. Papanicolaou, R. L. Wilder, S. C. Manolagas, and G. P. Chrousos, "The pathophysiologic roles of interleukin-6 in human disease," *Annals of Internal Medicine*, vol. 128, no. 2, pp. 127–137, 1998.

[54] J. Y. Park and M. H. Pillinger, "Interleukin-6 in the pathogenesis of rheumatoid arthritis," *Bulletin of the NYU hospital for joint diseases*, vol. 65, supplement 1, pp. S4–S10, 2007.

[55] A. M. W. Petersen and B. K. Pedersen, "The anti-inflammatory effect of exercise," *Journal of Applied Physiology*, vol. 98, no. 4, pp. 1154–1162, 2005.

[56] J. Scheller and S. Rose-John, "Interleukin-6 and its receptor: from bench to bedside," *Medical Microbiology and Immunology*, vol. 195, no. 4, pp. 173–183, 2006.

[57] R. Schindler, J. Mancilla, S. Endres, R. Ghorbani, S. C. Clark, and C. A. Dinarello, "Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF," *Blood*, vol. 75, no. 1, pp. 40–47, 1990.

[58] A. Steensberg, C. P. Fischer, C. Keller, K. Møller, and B. K. Pedersen, "IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans," *American Journal of Physiology*, vol. 285, no. 2, pp. E433–E437, 2003.

[59] H. Tilg, C. A. Dinarello, and J. W. Mier, "IL-6 and APPs: anti-inflammatory and immunosuppressive mediators," *Immunology Today*, vol. 18, no. 9, pp. 428–432, 1997.

[60] H. Tilg, E. Trehu, M. B. Atkins, C. A. Dinarello, and J. W. Mier, "Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55," *Blood*, vol. 83, no. 1, pp. 113–118, 1994.

[61] T. van der Poll, M. Levi, C. E. Hack et al., "Elimination of interleukin 6 attenuates coagulation activation in experimental endotoxemia in chimpanzees," *Journal of Experimental Medicine*, vol. 179, no. 4, pp. 1253–1259, 1994.

[62] L. Z. Xiao, N. Topley, T. Ito, and A. Phillips, "Interleukin-6 regulation of transforming growth factor (TGF)-$\beta$ receptor compartmentalization and turnover enhances TGF-$\beta$1 signaling," *The Journal of Biological Chemistry*, vol. 280, no. 13, pp. 12239–12245, 2005.

[63] M. Rincon, "Interleukin-6: from an inflammatory marker to a target for inflammatory diseases," *Trends in Immunology*, vol. 33, no. 11, pp. 571–577, 2012.

[64] R. Bonecchi, G. Bianchi, P. P. Bordignon et al., "Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s," *Journal of Experimental Medicine*, vol. 187, no. 1, pp. 129–134, 1998.

[65] J. Estaquier, T. Idziorek, W. Zou et al., "T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD4+ T cells from human immunodeficiency virus-infected persons," *Journal of Experimental Medicine*, vol. 182, no. 6, pp. 1759–1767, 1995.

[66] P. Puccetti, M. L. Belladonna, and U. Grohmann, "Effects of IL-12 and IL-23 on antigen-presenting cells at the interface between innate and adaptive immunity," *Critical Reviews in Immunology*, vol. 22, no. 5-6, pp. 373–390, 2002.

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

[68] U. Boehm, T. Klamp, M. Groot, and J. C. Howard, "Cellular responses to interferon-gamma," *Annual Review of Immunology*, vol. 15, pp. 749–795, 1997.

[69] T. Calandra, J.-D. Baumgartner, G. E. Grau et al., "Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-$\alpha$, and interferon-$\gamma$ in the serum of patients with septic shock," *Journal of Infectious Diseases*, vol. 161, no. 5, pp. 982–987, 1990.

[70] S. B. Flohé, H. Agrawal, S. Flohé, M. Rani, J. M. Bangen, and F. U. Schade, "Diversity of interferon $\gamma$ and granulocyte-macrophage colony-stimulating factor in restoring immune dysfunction of dendritic cells and macrophages during polymicrobial sepsis," *Molecular Medicine*, vol. 14, no. 5-6, pp. 247–256, 2008.

[71] J. Leentjens, M. Kox, R. M. Koch et al., "Reversal of immunoparalysis in humans in vivo: a double-blind, placebo-controlled, randomized pilot study," *American Journal of Respiratory and Critical Care Medicine*, vol. 186, no. 9, pp. 838–845, 2012.

[72] H. Okamura, S. Kashiwamura, H. Tsutsui et al., "Regulation of interferon-gamma production by IL-12 and IL-18," *Current Opinion in Immunology*, vol. 10, no. 3, pp. 259–264, 1998.

[73] E. F. Wheelock, "Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin," *Science*, vol. 149, no. 3681, pp. 310–311, 1965.

[74] J. Bernhagen, T. Calandra, R. A. Mitchell et al., "MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia," *Nature*, vol. 365, no. 6448, pp. 756–759, 1993.

[75] J. Bernhagen, R. Krohn, H. Lue et al., "MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment," *Nature Medicine*, vol. 13, no. 5, pp. 587–596, 2007.

[76] R. Bucala and S. C. Donnelly, "Macrophage migration inhibitory factor: a probable link between inflammation and cancer," *Immunity*, vol. 26, no. 3, pp. 281–285, 2007.

[77] T. Calandra, J. Bernhagen, C. N. Metz et al., "MIF as a glucocorticoid-induced modulator of cytokine production," *Nature*, vol. 377, no. 6544, pp. 68–71, 1995.

[78] T. Calandra, J. Bernhagen, R. A. Mitchell, and R. Bucala, "The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor," *Journal of Experimental Medicine*, vol. 179, no. 6, pp. 1895–1902, 1994.

[79] T. Calandra, B. Echtenacher, D. Le Roy et al., "Protection from septic shock by neutralization of macrophage migration inhibitory factor," *Nature Medicine*, vol. 6, no. 2, pp. 164–170, 2000.

[80] T. Calandra and T. Roger, "Macrophage migration inhibitory factor: a regulator of innate immunity," *Nature Reviews Immunology*, vol. 3, no. 10, pp. 791–800, 2003.

[81] L. Leng, W. Wang, T. Roger et al., "Glucocorticoid-induced MIF expression by human CEM T cells," *Cytokine*, vol. 48, no. 3, pp. 177–185, 2009.

[82] M. Merk, R. A. Mitchell, S. Endres, and R. Bucala, "D-dopachrome tautomerase (D-DT or MIF-2): doubling the MIF cytokine family," *Cytokine*, vol. 59, no. 1, pp. 10–17, 2012.

[83] R. A. Mitchell, H. Liao, J. Chesney et al., "Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 345–350, 2002.

[84] T. Roger, A. Chanson, M. Knaup-Reymond, and T. Calandra, "Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1," *European Journal of Immunology*, vol. 35, no. 12, pp. 3405–3413, 2005.

[85] R. D. W. Malefyt, J. Abrams, B. Bennett, C. G. Figdor, and J. E. de Vries, "Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes," *Journal of Experimental Medicine*, vol. 174, no. 5, pp. 1209–1220, 1991.

[86] D. F. Fiorentino, A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra, "IL-10 inhibits cytokine production by activated macrophages," *The Journal of Immunology*, vol. 147, no. 11, pp. 3815–3822, 1991.

[87] S. Q. Latifi, M. A. O'Riordan, and A. D. Levine, "Interleukin-10 controls the onset of irreversible septic shock," *Infection and Immunity*, vol. 70, no. 8, pp. 4441–4446, 2002.

[88] M. Seitz, P. Loetscher, B. Dewald, H. Towbin, H. Gallati, and M. Baggiolini, "Interleukin-10 differentially regulates cytokine inhibitor and chemokine release from blood mononuclear cells and fibroblasts," *European Journal of Immunology*, vol. 25, no. 4, pp. 1129–1132, 1995.

[89] G. C. Blobe, W. P. Schiemann, and H. F. Lodish, "Role of transforming growth factor $\beta$ in human disease," *The New England Journal of Medicine*, vol. 342, no. 18, pp. 1350–1358, 2000.

[90] C. Bogdan and C. Nathan, "Modulation of macrophage function by transforming growth factor $\beta$, interleukin-4, and interleukin-10," *Annals of the New York Academy of Sciences*, vol. 685, pp. 713–739, 1993.

[91] C. Marie, J. M. Cavaillon, and M. R. Losser, "Elevated levels of circulating transforming growth factor-beta 1 in patients with the sepsis syndrome," *Annals of internal medicine*, vol. 125, no. 6, pp. 520–521, 1996.

[92] A. Pellacani, P. Wiesel, S. Razavi et al., "Down-regulation of high mobility group-I(Y) protein contributes to the inhibition of nitric-oxide synthase 2 by transforming growth factor-$\beta$1," *The Journal of Biological Chemistry*, vol. 276, no. 2, pp. 1653–1659, 2001.

[93] M. B. Sporn and A. B. Roberts, "Transforming growth factor-$\beta$: multiple actions and potential clinical applications," *Journal of the American Medical Association*, vol. 262, no. 7, pp. 938–941, 1989.

[94] M. Turner, D. Chantry, P. Katsikis, A. Berger, F. M. Brennan, and M. Feldmann, "Induction of the interleukin 1 receptor antagonist protein by transforming growth factor-$\beta$," *European Journal of Immunology*, vol. 21, no. 7, pp. 1635–1639, 1991.

[95] S. M. Opal and V. A. DePalo, "Anti-inflammatory cytokines," *Chest*, vol. 117, no. 4, pp. 1162–1172, 2000.

[96] R. A. Seder, W. E. Paul, M. M. Davis, and B. F. de st. Groth, "The presence of interleukin 4 during in vitro priming determines the lymphokine-producing potential of CD4+ T cells from T cell receptor transgenic mice," *Journal of Experimental Medicine*, vol. 176, no. 4, pp. 1091–1098, 1992.

[97] J.-M. Cavaillon, C. Munoz, C. Fitting, B. Misset, and J. Carlet, "Circulating cytokines: the tip of the iceberg?" *Circulatory Shock*, vol. 38, no. 2, pp. 145–152, 1992.

[98] T. S. Blackwell and J. W. Christman, "Sepsis and cytokines: current status," *British Journal of Anaesthesia*, vol. 77, no. 1, pp. 110–117, 1996.

[99] N. Aikawa, "Cytokine storm in the pathogenesis of multiple organ dysfunction syndrome associated with surgical insults," *Nippon Geka Gakkai Zasshi*, vol. 97, no. 9, pp. 771–777, 1996.

[100] W. G. Junger, D. B. Hoyt, F. C. Liu, W. H. Loomis, and R. Coimbra, "Immunosuppression after endotoxin shock: the result of multiple anti-inflammatory factors," *Journal of Trauma*, vol. 40, no. 5, pp. 702–709, 1996.

[101] B. M. Tang, S. J. Huang, and A. S. McLean, "Genome-wide transcription profiling of human sepsis: a systematic review," *Critical Care*, vol. 14, no. 6, article R237, 2010.

[102] C. E. Hack, L. A. Aarden, and L. G. Thijs, "Role of cytokines in sepsis," *Advances in Immunology*, vol. 66, pp. 101–195, 1997.

[103] T. van der Poll and S. J. H. van Deventer, "Cytokines and anticytokines in the pathogenesis of sepsis," *Infectious Disease Clinics of North America*, vol. 13, no. 2, pp. 413–426, 1999.

[104] C. Dinarello, W. Arend, J. Sims et al., "IL-1 family nomenclature," *Nature Immunology*, vol. 11, no. 11, article 973, 2010.

[105] M. Kapoor, J. Martel-Pelletier, D. Lajeunesse, J. Pelletier, and H. Fahmi, "Role of proinflammatory cytokines in the pathophysiology of osteoarthritis," *Nature Reviews Rheumatology*, vol. 7, no. 1, pp. 33–42, 2011.

[106] M. Lewis, L. A. Tartaglia, A. Lee et al., "Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species specific," *Proceedings of*

*the National Academy of Sciences of the United States of America*, vol. 88, no. 7, pp. 2830–2834, 1991.

[107] M. Bhatia and S. Moochhala, "Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome," *Journal of Pathology*, vol. 202, no. 2, pp. 145–156, 2004.

[108] P. Selby, S. Hobbs, C. Viner et al., "Tumour necrosis factor in man: clinical and biological observations," *British Journal of Cancer*, vol. 56, no. 6, pp. 803–808, 1987.

[109] M. L. Sherman, D. R. Spriggs, K. A. Arthur, K. Imamura, E. Frei III, and D. W. Kufe, "Recombinant human tumor necrosis factor administered as a five-day continuous infusion in cancer patients: phase I toxicity and effects on lipid metabolism," *Journal of Clinical Oncology*, vol. 6, no. 2, pp. 344–350, 1988.

[110] T. van der Poll, H. R. Buller, H. ten Cate et al., "Activation of coagulation after administration of tumor necrosis factor to normal subjects," *The New England Journal of Medicine*, vol. 322, no. 23, pp. 1622–1627, 1990.

[111] A. Tewari, W. C. Buhles Jr., and H. F. Starnes Jr., "Preliminary report: effects of interleukin-1 on platelet counts," *The Lancet*, vol. 336, no. 8717, pp. 712–714, 1990.

[112] C. A. Dinarello, "Interleukin-1 and interleukin-1 antagonism," *Blood*, vol. 77, no. 8, pp. 1627–1652, 1991.

[113] 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.

[114] J. G. Cannon, R. G. Tompkins, J. A. Gelfand et al., "Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever," *Journal of Infectious Diseases*, vol. 161, no. 1, pp. 79–84, 1990.

[115] D. G. Hesse, K. J. Tracey, Y. Fong et al., "Cytokine appearance in human endotoxemia and primate bacteremia," *Surgery Gynecology and Obstetrics*, vol. 166, no. 2, pp. 147–153, 1988.

[116] H. R. Michie, K. R. Manogue, D. R. Spriggs et al., "Detection of circulating tumor necrosis factor after endotoxin administration," *The New England Journal of Medicine*, vol. 318, no. 23, pp. 1481–1486, 1988.

[117] A. F. Suffredini, R. E. Fromm, M. M. Parker et al., "The cardiovascular response of normal humans to the administration of endotoxin," *The New England Journal of Medicine*, vol. 321, no. 5, pp. 280–287, 1989.

[118] C. Fahlman, F. W. Jacobsen, O. P. Veiby, I. K. McNiece, H. K. Blomhoff, and S. E. W. Jacobsen, "Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) potently enhances in vitro macrophage production from primitive murine hematopoietic progenitor cells in combination with stem cell factor and interleukin-7: novel stimulatory role of p55 TNF receptors," *Blood*, vol. 84, no. 5, pp. 1528–1533, 1994.

[119] D. Conte, M. Holcik, C. A. Lefebvre et al., "Inhibitor of apoptosis protein cIAP2 is essential for lipopolysaccharide-induced macrophage survival," *Molecular and Cellular Biology*, vol. 26, no. 2, pp. 699–708, 2006.

[120] H. Nakae, S. Endo, K. Inada, T. Takakuwa, and T. Kasai, "Changes in adhesion molecule levels in sepsis," *Research Communications in Molecular Pathology and Pharmacology*, vol. 91, no. 3, pp. 329–338, 1996.

[121] M. Shimaoka and E. J. Park, "Advances in understanding sepsis," *European Journal of Anaesthesiology*, vol. 25, no. 42, pp. 146–153, 2008.

[122] G. A. Spinas, U. Keller, and M. Brockhaus, "Release of soluble receptors for tumor necrosis factor (TNF) in relation to circulating TNF during experimental endotoxinemia," *The Journal of Clinical Investigation*, vol. 90, no. 2, pp. 533–536, 1992.

[123] D. B. Kuhns, W. G. Alvord, and J. I. Gallin, "Increased circulating cytokines, cytokine antagonists, and E-selectin after intravenous administration of endotoxin in humans," *Journal of Infectious Diseases*, vol. 171, no. 1, pp. 145–152, 1995.

[124] W. Ertel, F. A. Scholl, H. Gallati et al., "Increased release of soluble tumor necrosis factor receptors into blood during clinical sepsis," *Archives of Surgery*, vol. 129, no. 12, pp. 1330–1337, 1994.

[125] B. Gardlund, J. Sjolin, A. Nilsson, M. Roll, C.-J. Wickerts, and B. Wretlind, "Plasma levels of cytokines in primary septic shock in humans: correlation with disease severity," *Journal of Infectious Diseases*, vol. 172, no. 1, pp. 296–301, 1995.

[126] A. S. Goldie, K. C. H. Fearon, J. A. Ross et al., "Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome," *Journal of the American Medical Association*, vol. 274, no. 2, pp. 172–177, 1995.

[127] E. Fischer, M. A. Marano, K. J. Van Zee et al., "Interleukin-1 receptor blockade improves survival and hemodynamic performance in *Escherichia coli* septic shock, but fails to alter host responses to sublethal endotoxemia," *The Journal of Clinical Investigation*, vol. 89, no. 5, pp. 1551–1557, 1992.

[128] A. A. Creasey, P. Stevens, J. Kenney et al., "Endotoxin and cytokine profile in plasma of baboons challenged with lethal and sublethal *Escherichia coli*," *Circulatory Shock*, vol. 33, no. 2, pp. 84–91, 1991.

[129] P. Damas, D. Ledoux, M. Nys et al., "Cytokine serum level during severe sepsis in human IL-6 as a marker of severity," *Annals of Surgery*, vol. 215, no. 4, pp. 356–362, 1992.

[130] A. M. Cruickshank, W. D. Fraser, H. J. G. Burns, J. van Damme, and A. Shenkin, "Response of serum interleukin-6 in patients undergoing elective surgery of varying severity," *Clinical Science*, vol. 79, no. 2, pp. 161–165, 1990.

[131] R. C. Hoch, R. Rodriguez, T. Manning et al., "Effects of accidental trauma on cytokine and endotoxin production," *Critical Care Medicine*, vol. 21, no. 6, pp. 839–845, 1993.

[132] C. E. Hack, E. R. De Groot, R. J. F. Felt-Bersma et al., "Increased plasma levels of interleukin-6 in sepsis," *Blood*, vol. 74, no. 5, pp. 1704–1710, 1989.

[133] E. Borrelli, P. Roux-Lombard, G. E. Grau et al., "Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk," *Critical Care Medicine*, vol. 24, no. 3, pp. 392–397, 1996.

[134] A. Waage, P. Brandtzaeg, A. Halstensen, P. Kierulf, and T. Espevik, "The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome," *Journal of Experimental Medicine*, vol. 169, no. 1, pp. 333–338, 1989.

[135] J. Preiser, D. Schmartz, P. van der Linden et al., "Interleukin-6 administration has no acute hemodynamic or hematologic effect in the dog," *Cytokine*, vol. 3, no. 1, pp. 1–4, 1991.

[136] I. Kushner and D. L. Rzewnicki, "The acute phase response: general aspects," *Bailliere's Clinical Rheumatology*, vol. 8, no. 3, pp. 513–530, 1994.

[137] S. Cuzzocrea, L. Sautebin, G. De Sarro et al., "Role of IL-6 in the pleurisy and lung injury caused by carrageenan," *The Journal of Immunology*, vol. 163, no. 9, pp. 5094–5104, 1999.

[138] S. Cuzzocrea, G. De Sarro, G. Costantino et al., "Role of interleukin-6 in a non-septic shock model induced by zymosan," *European Cytokine Network*, vol. 10, no. 2, pp. 191–203, 1999.

[139] N. Pathan, C. A. Hemingway, A. A. Alizadeh et al., "Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock," *The Lancet*, vol. 363, no. 9404, pp. 203–209, 2004.

[140] K. Yoshizawa, M. Naruto, and N. Ida, "Injection time of interleukin-6 determines fatal outcome in experimental endotoxin shock," *Journal of Interferon and Cytokine Research*, vol. 16, no. 12, pp. 995–1000, 1996.

[141] Z. Xing, J. Gauldie, G. Cox et al., "IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses," *The Journal of Clinical Investigation*, vol. 101, no. 2, pp. 311–320, 1998.

[142] D. G. Remick, G. Bolgos, S. Copeland, and J. Siddiqui, "Role of interleukin-6 in mortality from and physiologic response to sepsis," *Infection and Immunity*, vol. 73, no. 5, pp. 2751–2757, 2005.

[143] L. L. Jones and D. A. A. Vignali, "Molecular interactions within the IL-6/IL-12 cytokine/receptor superfamily," *Immunologic Research*, vol. 51, no. 1, pp. 5–14, 2011.

[144] M. L. Steinhauser, C. M. Hogaboam, N. W. Lukacs, R. M. Strieter, and S. L. Kunkel, "Multiple roles for IL-12 in a model of acute septic peritonitis," *The Journal of Immunology*, vol. 162, no. 9, pp. 5437–5443, 1999.

[145] D. A. Zisman, S. L. Kunkel, R. M. Strieter et al., "Anti-interleukin-12 therapy protects mice in lethal endotoxemia but impairs bacterial clearance in murine *Escherichia coli* peritoneal sepsis," *Shock*, vol. 8, no. 5, pp. 349–356, 1997.

[146] P. M. Jansen, T. C. T. M. V. Kraan, I. W. de Jong et al., "Release of interleukin-12 in experimental *Escherichia coli* septic shock in baboons: relation to plasma levels of interleukin-10 and interferon-$\gamma$," *Blood*, vol. 87, no. 12, pp. 5144–5151, 1996.

[147] S. E. Moreno, J. C. Alves-Filho, T. M. Alfaya, J. S. da Silva, S. H. Ferreira, and F. Y. Liew, "IL-12, but not IL-18, is critical to neutrophil activation and resistance to polymicrobial sepsis induced by cecal ligation and puncture," *The Journal of Immunology*, vol. 177, no. 5, pp. 3218–3224, 2006.

[148] H. Weighardt, C. Heidecke, A. Westerholt et al., "Impaired monocyte IL-12 production before surgery as a predictive factor for the lethal outcome of postoperative sepsis," *Annals of Surgery*, vol. 235, no. 4, pp. 560–567, 2002.

[149] S. A. Stanilova, Z. T. Karakolev, G. S. Dimov et al., "High interleukin 12 and low interleukin 10 production after in vitro stimulation detected in sepsis survivors," *Intensive Care Medicine*, vol. 31, no. 3, pp. 401–407, 2005.

[150] H. Wu, C. Shih, C. Lin, C. Hua, and D. Chuang, "Serial increase of IL-12 response and human leukocyte antigen-DR expression in severe sepsis survivors," *Critical Care*, vol. 15, no. 5, article R224, 2011.

[151] Z. Wang, S. L. Reiner, S. Zheng, D. K. Dalton, and R. M. Locksley, "CD4+ effector cells default to the Th2 pathway in interferon $\gamma$-deficient mice infected with Leishmania major," *Journal of Experimental Medicine*, vol. 179, no. 4, pp. 1367–1371, 1994.

[152] S. Huang, W. Hendriks, A. Althage et al., "Immune response in mice that lack the interferon-$\gamma$ receptor," *Science*, vol. 259, no. 5102, pp. 1742–1745, 1993.

[153] A. M. Cooper, D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell, and I. M. Orme, "Disseminated tuberculosis in interferon $\gamma$ gene-disrupted mice," *Journal of Experimental Medicine*, vol. 178, no. 6, pp. 2243–2247, 1993.

[154] U. Müller, U. Steinhoff, L. F. L. Reis et al., "Functional role of type I and type II interferons in antiviral defense," *Science*, vol. 264, no. 5167, pp. 1918–1921, 1994.

[155] F. P. Heinzel, "The role of IFN-$\gamma$ in the pathology of experimental endotoxemia," *The Journal of Immunology*, vol. 145, no. 9, pp. 2920–2924, 1990.

[156] B. D. Car, V. M. Eng, B. Schnyder et al., "Interferon $\gamma$ receptor deficient mice are resistant to endotoxic shock," *Journal of Experimental Medicine*, vol. 179, no. 5, pp. 1437–1444, 1994.

[157] C. Munoz, J. Carlet, C. Fitting, B. Misset, J.-P. Bleriot, and J.-M. Cavaillon, "Dysregulation of in vitro cytokine production by monocytes during sepsis," *The Journal of Clinical Investigation*, vol. 88, no. 5, pp. 1747–1754, 1991.

[158] 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.

[159] A. R. Rich and M. R. Lewis, "The nature of allergy in tuberculosis as revealed by tissue culture studies," *Bulletin of the Johns Hopkins Hospital*, vol. 50, pp. 115–131, 1932.

[160] T. Calandra, L. A. Spiegel, C. N. Metz, and R. Bucala, "Macrophage migration inhibitory factor is a critical mediator of the activation of immune cells by exotoxins of Gram-positive bacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 19, pp. 11383–11388, 1998.

[161] M. Merk, J. Baugh, S. Zierow et al., "The Golgi-associated protein p115 mediates the secretion of macrophage migration inhibitory factor," *The Journal of Immunology*, vol. 182, no. 11, pp. 6896–6906, 2009.

[162] T. Roger, J. David, M. P. Glauser, and T. Calandra, "MIF regulates innate immune responses through modulation of Toll-like receptor 4," *Nature*, vol. 414, no. 6866, pp. 920–924, 2001.

[163] X. Shi, L. Leng, T. Wang et al., "CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex," *Immunity*, vol. 25, no. 4, pp. 595–606, 2006.

[164] L. Leng, C. N. Metz, Y. Fang et al., "MIF signal transduction initiated by binding to CD74," *Journal of Experimental Medicine*, vol. 197, no. 11, pp. 1467–1476, 2003.

[165] A. G. Rossi, C. Haslett, N. Hirani et al., "Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF): potential role in asthma," *The Journal of Clinical Investigation*, vol. 101, no. 12, pp. 2869–2874, 1998.

[166] A. Mikulowska, C. N. Metz, R. Bucala, and R. Holmdahl, "Macrophage migration inhibitory factor is involved in the pathogenesis of collagen type II-lnduced arthritis in mice," *The Journal of Immunology*, vol. 158, no. 11, pp. 5514–5517, 1997.

[167] S. Lin, X. Yu, Y. Chen et al., "De novo expression of macrophage migration inhibitory factor in atherogenesis in rabbits," *Circulation Research*, vol. 87, no. 12, pp. 1202–1208, 2000.

[168] Y. P. de Jong, A. C. Abadia-Molina, A. R. Satoskar et al., "Development of chronic colitis is dependent on the cytokine MIF," *Nature Immunology*, vol. 2, no. 11, pp. 1061–1066, 2001.

[169] Y. Al-Abed, D. Dabideen, B. Aljabari et al., "ISO-1 binding to the tautomerase active site of MIF inhibits its pro-inflammatory activity and increases survival in severe sepsis," *The Journal of Biological Chemistry*, vol. 280, no. 44, pp. 36541–36544, 2005.

[170] M. Bozza, A. R. Satoskar, G. Lin et al., "Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis," *Journal of Experimental Medicine*, vol. 189, no. 2, pp. 341–346, 1999.

[171] N. Matsuda, J. Nishihira, Y. Takahashi, O. Kemmotsu, and Y. Hattori, "Role of macrophage migration inhibitory factor in acute lung injury in mice with acute pancreatitis complicated by endotoxemia," *American Journal of Respiratory Cell and Molecular Biology*, vol. 35, no. 2, pp. 198–205, 2006.

[172] S. Kobayashi, J. Nishihira, S. Watanabe, and S. Todo, "Prevention of lethal acute hepatic failure by antimacrophage migration inhibitory factor antibody in mice treated with bacille Calmette-Guerin and lipopolysaccharide," *Hepatology*, vol. 29, no. 6, pp. 1752–1759, 1999.

[173] L. B. Garner, M. S. Willis, D. L. Carlson et al., "Macrophage migration inhibitory factor is a cardiac-derived myocardial depressant factor," *American Journal of Physiology*, vol. 285, no. 6, pp. H2500–H2509, 2003.

[174] E. J. Miller, J. Li, L. Leng et al., "Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart," *Nature*, vol. 451, no. 7178, pp. 578–582, 2008.

[175] M. Merk, S. Zierow, L. Leng et al., "The D-dopachrome tautomerase (DDT) gene product is a cytokine and functional homolog of macrophage migration inhibitory factor (MIF)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 34, pp. E577–E585, 2011.

[176] A. Beishuizen, L. G. Thijs, C. Haanen, and I. Vermes, "Macrophage migration inhibitory factor and hypothalamo-pituitary-adrenal function during critical illness," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 6, pp. 2811–2816, 2001.

[177] T. Brenner, S. Hofer, C. Rosenhagen et al., "Macrophage migration inhibitory factor (MIF) and manganese superoxide dismutase (MnSOD) as early predictors for survival in patients with severe sepsis or septic shock," *Journal of Surgical Research*, vol. 164, no. 1, pp. e163–e171, 2010.

[178] S. Yende, D. C. Angus, L. Kong et al., "The influence of macrophage migration inhibitory factor gene polymorphisms on outcome from community-acquired pneumonia," *The FASEB Journal*, vol. 23, no. 8, pp. 2403–2411, 2009.

[179] P. Renner, T. Roger, P. Bochud et al., "A functional microsatellite of the macrophage migration inhibitory factor gene associated with meningococcal disease," *The FASEB Journal*, vol. 26, no. 2, pp. 907–916, 2012.

[180] S. Doernberg, B. Schaaf, K. Dalhoff et al., "Association of macrophage migration inhibitory factor (MIF) polymorphisms with risk of meningitis from *Streptococcus pneumoniae*," *Cytokine*, vol. 53, no. 3, pp. 292–294, 2011.

[181] G. A. Awandare, J. J. Martinson, T. Were et al., "MIF (Macrophage Migration Inhibitory Factor) promoter polymorphisms and susceptibility to severe malarial anemia," *Journal of Infectious Diseases*, vol. 200, no. 4, pp. 629–637, 2009.

[182] M. Howard, T. Muchamuel, S. Andrade, and S. Menon, "Interleukin 10 protects mice from lethal endotoxemia," *Journal of Experimental Medicine*, vol. 177, no. 4, pp. 1205–1208, 1993.

[183] M. Howard and A. O'Garra, "Biological properties of interleukin 10," *Journal of Clinical Immunology*, vol. 12, no. 4, pp. 239–247, 1992.

[184] G. Y. Song, C. Chung, I. H. Chaudry, and A. Ayala, "What is the role of interleukin 10 in polymicrobial sepsis: anti-inflammatory agent or immunosuppressant?" *Surgery*, vol. 126, no. 2, pp. 378–383, 1999.

[185] T. van der Poll, A. Marchant, W. A. Buurman et al., "Endogenous IL-10 protects mice from death during septic peritonitis," *The Journal of Immunology*, vol. 155, no. 11, pp. 5397–5401, 1995.

[186] L. Zeng, W. Gu, K. Chen et al., "Clinical relevance of the interleukin 10 promoter polymorphisms in Chinese Han patients with major trauma: genetic association studies," *Critical Care*, vol. 13, no. 6, article R188, 2009.

[187] K. M. Gilbert, M. Thoman, K. Bauche, T. Pham, and W. O. Weigle, "Transforming growth factor-$\beta$1 induces antigen-specific unresponsiveness in naive T cells," *Immunological Investigations*, vol. 26, no. 4, pp. 459–472, 1997.

[188] Y. Y. Wan and R. A. Flavell, "TGF-$\beta$ and regulatory T cell in immunity and autoimmunity," *Journal of Clinical Immunology*, vol. 28, no. 6, pp. 647–659, 2008.

[189] F. Randow, U. Syrbe, C. Meisel et al., "Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor $\beta$," *Journal of Experimental Medicine*, vol. 181, no. 5, pp. 1887–1892, 1995.

[190] M. A. Perrella, C. M. Hsieh, W. S. Lee et al., "Arrest of endotoxin-induced hypotension by transforming growth factor beta1," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 5, pp. 2054–2059, 1996.

[191] B. S. Pender, "Transforming growth factor $\beta$1 alters rat peritoneal macrophage mediator production and improves survival during endotoxic shock," *European Cytokine Network*, vol. 7, no. 2, pp. 137–142, 1996.

[192] S. Zanotti, A. Kumar, and A. Kumar, "Cytokine modulation in sepsis and septic shock," *Expert Opinion on Investigational Drugs*, vol. 11, no. 8, pp. 1061–1075, 2002.

[193] S. Knapp, F. Thalhammer, G. J. Locker et al., "Prognostic value of MIP-1$\alpha$, TGF-$\beta$2, sELAM-1, and sVCAM-1 in patients with gram-positive sepsis," *Clinical Immunology and Immunopathology*, vol. 87, no. 2, pp. 139–144, 1998.

[194] A. Kumar, A. Kumar, B. Paladugu, J. Mensing, and J. E. Parrillo, "Transforming growth factor-$\beta$1 blocks in vitro cardiac myocyte depression induced by tumor necrosis factor-$\alpha$, interleukin-1$\beta$, and human septic shock serum," *Critical Care Medicine*, vol. 35, no. 2, pp. 358–364, 2007.

[195] J. Zamorano, H. Y. Wang, L. Wang, J. H. Pierce, and A. D. Keegan, "IL-4 protects cells from apoptosis via the insulin receptor substrate pathway and a second independent signaling pathway," *The Journal of Immunology*, vol. 157, no. 11, pp. 4926–4934, 1996.

[196] M. Yanagida, H. Fukamachi, K. Ohgami et al., "Effects of T-helper 2-type cytokines, interleukin-3 (IL-3), IL-4, IL-5, and IL-6 on the survival of cultured human mast cells," *Blood*, vol. 86, no. 10, pp. 3705–3714, 1995.

[197] M. B. Lutz, M. Schnare, M. Menges et al., "Differential functions of IL-4 receptor types I and II for dendritic cell maturation and IL-12 production and their dependency on GM-CSF," *The Journal of Immunology*, vol. 169, no. 7, pp. 3574–3580, 2002.

[198] J. M. Baumhofer, B. G. Beinhauer, J. E. Wang et al., "Gene transfer with IL-4 and IL-13 improves survival in lethal endotoxemia in the mouse and ameliorates peritoneal macrophages immune competence," *European Journal of Immunology*, vol. 28, no. 2, pp. 610–615, 1998.

[199] O. Hultgren, M. Kopf, and A. Tarkowski, "Outcome of *Staphylococcus aureus*-triggered sepsis and arthritis in IL-4-deficient mice depends on the genetic background of the host," *European Journal of Immunology*, vol. 29, no. 8, pp. 2400–2405, 1999.

[200] H. P. Wu, C. L. Wu, C. K. Chen et al., "The interleukin-4 expression in patients with severe sepsis," *Journal of Critical Care*, vol. 23, no. 4, pp. 519–524, 2008.

[201] W. Gu, L. Zeng, L. Zhang et al., "Association of interleukin 4-589T/C Polymorphism with TH1 and TH2 bias and sepsis in

chinese major trauma patients," *Journal of Trauma*, vol. 71, no. 6, pp. 1583–1587, 2011.

[202] C. J. Fisher Jr., S. M. Opal, J. F. Dhainaut et al., "Influence of an anti-tumor necrosis factor monoclonal antibody on cytokine levels in patients with sepsis. The CB0006 Sepsis Syndrome Study Group," *Critical Care Medicine*, vol. 21, no. 3, pp. 318–327, 1993.

[203] S. M. Opal, C. J. Fisher Jr., J. A. Dhainaut et al., "Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial," *Critical Care Medicine*, vol. 25, no. 7, pp. 1115–1124, 1997.

[204] F. Zeni, B. Freeman, and C. Natanson, "Anti-inflammatory therapies to treat sepsis and septic shock: a reassessment," *Critical Care Medicine*, vol. 25, no. 7, pp. 1095–1100, 1997.

[205] J. Cohen and J. Carlet, "INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-$\alpha$ in patients with sepsis," *Critical Care Medicine*, vol. 24, no. 9, pp. 1431–1440, 1996.

[206] W. R. Parrish, M. Gallowitsch-Puerta, C. J. Czura, and K. J. Tracey, "Experimental therapeutic strategies for severe sepsis: mediators and mechanisms," *Annals of the New York Academy of Sciences*, vol. 1144, pp. 210–236, 2008.

[207] L. Ulloa and K. J. Tracey, "The "cytokine profile": a code for sepsis," *Trends in Molecular Medicine*, vol. 11, no. 2, pp. 56–63, 2005.

[208] J. B. Lubetsky, A. Dios, J. Han et al., "The tautomerase active site of macrophage migration inhibitory factor is a potential target for discovery of novel anti-inflammatory agents," *The Journal of Biological Chemistry*, vol. 277, no. 28, pp. 24976–24982, 2002.

[209] L. Leng, L. Chen, J. Fan et al., "A small-molecule macrophage migration inhibitory factor antagonist protects against glomerulonephritis in Lupus-Prone NZB/NZW F1 and MRL/lpr mice," *The Journal of Immunology*, vol. 186, no. 1, pp. 527–538, 2011.

[210] A. Arjona, H. G. Foellmer, T. Town et al., "Abrogation of macrophage migration inhibitory factor decreases West Nile virus lethality by limiting viral neuroinvasion," *The Journal of Clinical Investigation*, vol. 117, no. 10, pp. 3059–3066, 2007.

[211] R. J. Kerschbaumer, M. Rieger, D. Völkel et al., "Neutralization of macrophage migration inhibitory factor (MIF) by fully human antibodies correlates with their specificity for the $\beta$-sheet structure of MIF," *The Journal of Biological Chemistry*, vol. 287, no. 10, pp. 7446–7455, 2012.

[212] A. A. Hare, L. Leng, S. Gandavadi et al., "Optimization of N-benzyl-benzoxazol-2-ones as receptor antagonists of macrophage migration inhibitory factor (MIF)," *Bioorganic and Medicinal Chemistry Letters*, vol. 20, no. 19, pp. 5811–5814, 2010.

[213] S. Kraemer, H. Lue, A. Zernecke et al., "MIF-chemokine receptor interactions in atherogenesis are dependent on an N-loop-based 2-site binding mechanism," *The FASEB Journal*, vol. 25, no. 3, pp. 894–906, 2011.

[214] J. J. Presneill, T. Harris, A. G. Stewart, J. F. Cade, and J. W. Wilson, "A randomized phase II trial of granulocyte-macrophage colony-stimulating factor therapy in severe sepsis with respiratory dysfunction," *American Journal of Respiratory and Critical Care Medicine*, vol. 166, no. 2, pp. 138–143, 2002.

[215] A. J. Rosenbloom, P. K. Linden, A. Dorrance, N. Penkosky, M. H. Cohen-Melamed, and M. R. Pinsky, "Effect of granulocyte-monocyte colony-stimulating factor therapy on leukocyte function and clearance of serious infection in nonneutropenic patients," *Chest*, vol. 127, no. 6, pp. 2139–2150, 2005.

[216] H. C. Polk Jr., W. G. Cheadle, D. H. Livingston et al., "A randomized prospective clinical trial to determine the efficacy of interferon-$\gamma$ in severely injured patients," *American Journal of Surgery*, vol. 163, no. 2, pp. 191–196, 1992.

[217] C. T. Esmon, "The interactions between inflammation and coagulation," *British Journal of Haematology*, vol. 131, no. 4, pp. 417–430, 2005.

[218] C. T. Esmon, "The protein C pathway," *Chest*, vol. 124, supplement 3, pp. 26S–32S, 2003.

[219] A. P. Neyrinck, K. D. Liu, J. P. Howard, and M. A. Matthay, "Protective mechanisms of activated protein C in severe inflammatory disorders," *British Journal of Pharmacology*, vol. 158, no. 4, pp. 1034–1047, 2009.

[220] P. L. Vera, T. E. Wolfe, A. E. Braley, and K. L. Meyer-Siegler, "Thrombin induces macrophage migration inhibitory factor release and upregulation in urothelium: a possible contribution to bladder inflammation," *PLoS ONE*, vol. 5, no. 12, Article ID e15904, 2010.

[221] H. F. Galley, N. E. El Sakka, N. R. Webster, D. A. Lowes, and B. H. Cuthbertson, "Activated protein C inhibits chemotaxis and interleukin-6 release by human neutrophils without affecting other neutrophil functions," *British Journal of Anaesthesia*, vol. 100, no. 6, pp. 815–819, 2008.

[222] K. Okajima, "Regulation of inflammatory responses by natural anticoagulants," *Immunological Reviews*, vol. 184, pp. 258–274, 2001.

[223] M. Schmidt-Supprian, C. Murphy, B. White et al., "Activated protein C inhibits tumor necrosis factor and macrophage migration inhibitory factor production in monocytes," *European Cytokine Network*, vol. 11, no. 3, pp. 407–413, 2000.

[224] P. Bilbault, T. Lavaux, A. Launoy et al., "Influence of drotrecogin alpha (activated) infusion on the variation of Bax/Bcl-2 and Bax/Bcl-xl ratios in circulating mononuclear cells: a cohort study in septic shock patients," *Critical Care Medicine*, vol. 35, no. 1, pp. 69–75, 2007.

[225] F. P. da Silva and V. Nizet, "Cell death during sepsis: integration of disintegration in the inflammatory response to overwhelming infection," *Apoptosis*, vol. 14, no. 4, pp. 509–521, 2009.

[226] F. B. Taylor Jr., A. Chang, and C. T. Esmon, "Protein C prevents the coagulopathic and lethal effects of *Escherichia coli* infusion in the baboon," *The Journal of Clinical Investigation*, vol. 79, no. 3, pp. 918–925, 1987.

[227] G. R. Bernard, E. W. Ely, T. J. Wright et al., "Safety and dose relationship of recombinant human activated protein C for coagulopathy in severe sepsis," *Critical Care Medicine*, vol. 29, no. 11, pp. 2051–2059, 2001.

[228] G. R. Bernard, J. Vincent, P. Laterre et al., "Efficacy and safety of recombinant human activated protein C for severe sepsis," *The New England Journal of Medicine*, vol. 344, no. 10, pp. 699–709, 2001.

[229] 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.

[230] 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.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 627831, 7 pages
http://dx.doi.org/10.1155/2013/627831

*Research Article*

# Dexmedetomidine Reduced Cytokine Release during Postpartum Bleeding-Induced Multiple Organ Dysfunction Syndrome in Rats

**Liu Xianbao,[1] Zhan Hong,[1] Zeng Xu,[2] Zhang Chunfang,[3] and Chen Dunjin[3]**

[1] *Department of Anesthesiology, Third Affiliated Hospital, Guangzhou Medical University, Guangzhou 510150, China*
[2] *Department of Pathology, Detroit Medical Center, Harper University Hospital, School of Medicine, Wayne State University, 3990 John R, Detroit, MI 48201, USA*
[3] *Department of Gynecology and Obstetrics, Third Affiliated Hospital, Guangzhou Medical University, Guangzhou 510150, China*

Correspondence should be addressed to Zhan Hong; 457163589@qq.com

Received 25 June 2012; Revised 7 March 2013; Accepted 22 May 2013

Academic Editor: Celeste C. Finnerty



Dexmedetomidine (DEX) is an $\alpha$2-adrenergic agonist. It decreases the levels of norepinephrine release, resulting in a reduction of postsynaptic adrenergic activity. In the present study, the effects of DEX on postpartum bleeding-induced multiple organ dysfunction syndrome (BMODS) were studied in rats in which BMODS was induced by the combination of hypotension and clamping of the superior mesenteric artery. We evaluated the role of dexmedetomidine (DEX) in cytokine release during postpartum BMODS in rats. In summary, the present study demonstrated that DEX administration reduced IFN-r and IL-4 release and decreased lung injury during postpartum BMODS. It is possible that DEX administration decreased inflammatory cytokine production in BMODS by inhibiting inflammation and free radical release by leukocytes independent of the DEX dose.

## 1. Introduction

The most common cause of multiple organ dysfunction syndrome (MODS) in obstetric patients is postpartum bleeding followed by gestational hypertension syndrome [1, 2]. Bleeding-induced multiple organ dysfunction syndrome (BMODS) is a rapidly progressive disease that commonly occurs in critically ill obstetric patients with a high mortality rate. It is also one of the major causes of death in the maternity intensive care unit (ICU) [2]. BMODS induces diffuse ischemia and functional impairment in multiple organ systems and, in severe cases, can progress to diffuse intravascular coagulation (DIC) [3]. For this reason, patients with a history of heavy blood loss during delivery should be monitored in the ICU. The management of BMODS is challenging. Beyond prevention, BMODS treatment options are limited, and they are a popular topic for future clinical explorations.

Dexmedetomidine (DEX) is an $\alpha$2-adrenergic agonist. It selectively binds to presynaptic $\alpha$2-adrenergic receptors ($\alpha$2AR) on the locus coeruleus and decreases the levels of norepinephrine release, resulting in a reduction of postsynaptic adrenergic activity [4]. In the periphery, $\alpha$2ARs are widely distributed in many organs, including the liver, kidney, pancreas, blood vessels, and platelets. The administration of DEX causes various effects in different organs [5, 6]. Currently, the primary clinical use of DEX is for its effects on the central nervous system, such as short-term sedation and antianxiety [7]. The pharmacological actions of DEX in other organs have not been fully evaluated in clinical patients. In animal studies, DEX has been shown to reduce mortality rates and inhibit inflammatory responses in endotoxemic rats without adverse effects on functions, such as respiration [8, 9]. However, the benefits of applying DEX to treat postpartum BMODS in maternity ICU patients have not been demonstrated due to the lack of a proper animal model [10]. In a pilot study, A. Sezer et al. found histopathological changes during sepsis-induced hypotension in rats [11]. In another study, liver and pancreatic dysfunctions were observed following the clamping of the superior mesenteric artery [12]. In the present study, the effects of DEX on postpartum BMODS were studied in

rats in which BMODS was induced by the combination of hypotension and clamping of the superior mesenteric artery. We demonstrated that DEX administration reduced cytokine release and lung injury during postpartum BMODS in rats.

## 2. Materials and Methods

*2.1. Reagents.* DEX was purchased from Hospira Inc. (Lake Forest, IL, USA). For reverse transcription polymerase chain reaction (RT-PCR) analysis, RNAiso Plus, and Taq DNA Polymerase were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The ReverTra Ace was purchased from Toyobo Biotech Co., Ltd. (Shanghai, China). Trizol reagent was purchased from Invitrogen (Carlsbad, CA, USA).

*2.2. Animals.* Sprague-Dawley (SD) rats (20 adult male and 40 adult female, 200–250 g) were purchased from the Animal Center of Guangdong Province. The rats were housed in the Guangzhou Animal Center in accordance with the specific pathogens animals standard with a 12 hr light/dark cycle. The room temperature was maintained at 22±1°C. The rats had *ad libitum* access to food and drinking water. The animal studies in the present experiment were approved by the Animal Use and Care Committee of Guangzhou Medical University. The rats were handled following the National Institutes of Health guidelines.

*2.3. Experimental Groups.* The male and female (1 : 2) rats were allowed to mate in the cage until the females were pregnant, as determined by detecting sperm in vaginal secretions using microscopy, at which point the females were separated from the males until delivery. Postpartum female rats were used within 24 hours after delivery. A total of 40 experimental female rats were randomly assigned into four groups, with 10 rats per group as follows.

The Sham (S) group: catheters were placed in the right femoral artery and the left femoral vein;

S+C group: bleeding-induced hypotension and clamping of the superior mesenteric artery were performed;

S+C+D2.5 group: administration of DEX (2.5 $\mu$g/kg/h) [13] to S+C rats;

S+C+D5.0 group: administration of DEX (5.0 $\mu$g/kg/h) [13] to S+C rats.

*2.4. Experiment.* For the S group, the rats were anesthetized by the intraperitoneal administration of 10% hydration chlorine aldehyde (0.4 mL/100 g). The rats were then placed supinely on an animal surgical table with a heating pad. The body temperature was maintained at 36–38°C throughout the experiment. To monitor blood pressure, a polyethylene catheter (PE-50) was inserted into the right femoral artery and connected to a blood pressure monitor. Another PE-50 was inserted into the left femoral vein for DEX administration. All of the catheters used in the present experiment were prepared with heparin to prevent blood clotting. For the S+C group, hypotension was induced by drawing blood from the right femoral artery into a glass syringe that was preloaded with heparin for temporary storage until the mean arterial pressure (MAP) reached the target level of 45–50 mmHg [14]. The MAP was constantly maintained at this low blood pressure level for 60 min by the withdrawal or reinfusion of storage blood via the right femoral artery. Thus, artificial bleeding and hypotension were established in the postpartum rats. Resuscitation was performed by re-infusing the full amount of blood that was previously taken from the rats to restore normal blood pressure. After a normal blood pressure was maintained for more than 30 min, the abdomen of the rat was opened under sterile conditions. The superior mesenteric artery was clamped for 60 min and then released. For the S+C+D2.5 and 5.0 groups, dexmedetomidine (2.5 $\mu$g/kg/h and 5.0 $\mu$g/kg/h, resp.) was administered via the left femoral vein for 4 hours.

After the previous experiment, the abdominal incision was sutured, and the catheters were removed. The rats were placed back in the cage and freely allowed to reach for food and drinking water. The rats were sacrificed 24 hours after the experiment. A 0.5-mL blood sample was taken from the abdominal aorta for immediate arterial blood gas level measurement. Another 0.7 mL of blood was taken for biochemical analysis. The left lung was harvested, immediately snap frozen in liquid nitrogen, and stored in the freezer for later histological and cytokine analyses.

The changes in pulmonary, liver, and kidney functions were evaluated in BMODS-induced rats by measuring arterial blood gas, aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine, and creatine phosphokinase (CPK). Morphological changes in the lung were evaluated using the index of quantitative assessment of histological lung injury (IQA) and alveolar interval thickness (AIT) [15, 16]. Histological sections from the lung were examined under the 200X power field. Lung injury was considered to be the presence of alveoli-containing erythrocytes or two or more neutrophils. The percentage of injured alveoli was counted from each field. The IQA was the mean percentage of injured alveoli from the 200X power field. The AIT from the lung was examined under the 200X power field using the IMS image analysis system (ShenTeng Information Technology Co., Ltd., Shanghai). Six fields were randomly selected from each power field and examined, making sure to avoid the bronchial and blood vessels. Six alveoli were randomly selected from each field for thickness measurements. The AIT was the mean thickness of the alveoli [15].

*2.5. Reverse Transcription Polymerase Chain Reaction (RT-PCR).* RT-PCR was performed on tissue from the left lung. Total RNA was purified using the TRIzol kit (Takara Co., Ltd.). Residual genomic DNA was removed by incubation with RNase-free DNase. For the first strand cDNA synthesis, RNA (2 $\mu$g) was converted to cDNA using superscript II reverse transcriptase (Invitrogen). The reaction mixture was inactivated by heating to 70°C for 15 min. One microliter of reaction mixture was amplified by Taq DNA polymerase (Takara Co., Ltd.) in a thermal cycler (GeneAmp, PCR system 2700, Applied Biosystems, Foster City, CA, USA). The first incubation was performed at 94°C for 3 min for

Table 1: Effects of DEX administration in liver, kidney functions during postpartum BMODS in rats.

| Group | ALT (IU/L) | TBIL (umol/L) | BUN (mmol/L) | Cr (umol/L) | AST (IU/L) | CPK (IU/L) |
|---|---|---|---|---|---|---|
| Sham | 46.1 ± 9.4 | 11.2 ± 1.9 | 2.6 ± 0.8 | 48.9 ± 4.7 | 168.3 ± 35.2 | 2103.4 ± 1045.6 |
| S+C | 383.7 ± 134.8$^{\Delta}$ | 56.6 ± 4.9$^{\Delta}$ | 5.5 ± 2.7$^{\Delta}$ | 196.6 ± 21.9$^{\Delta}$ | 611.3 ± 216.1$^{\Delta}$ | 9686.3 ± 1876.5$^{\Delta}$ |
| S+C+D2.5 | 378.6 ± 139.3$^{\Delta}$ | 52.1 ± 6.9$^{\Delta}$ | 5.2 ± 2.5$^{\Delta}$ | 197.6 ± 23.6$^{\Delta}$ | 580.1 ± 230.0$^{\Delta}$ | 9597.0 ± 1879.4$^{\Delta}$ |
| S+C+D5.0 | 376.1 ± 140.5$^{\Delta}$ | 53.5 ± 5.9$^{\Delta}$ | 5.5 ± 2.1$^{\Delta}$ | 195.9 ± 22.4$^{\Delta}$ | 601.4 ± 206.3$^{\Delta}$ | 9628.3 ± 1897.7$^{\Delta}$ |

$^{\Delta}$Compared to the Sham group $P < 0.05$; $^{\#}$compared to the S+C group $P < 0.05$.

initial denaturation, and the following steps were repeated 30 times: 30 s at 95°C (denaturation), 1 min (specific annealing temperature for each primer), and 1 min at 72°C (extension). The final incubation was at 72°C for 5 min (final extension). The sequences for the primers used in the present study are as follows: IL-4: sense 5′-TCCTTCACGGCAACAAGGAAC-3′ and antisense 5′-GTGAGTTCAGACCGCTGACA-3′ (predicted size: 168 bp, annealing temperature: 50°C); IFN-$\gamma$: sense 5′-GAACTGGCAAAAGGACGGTA-3′ and antisense 5′-GGATCTGTGGGTTGTTCACC-3′ (predicted size: 215 bp, annealing temperature: 49°C). The PCR products were size-fractioned by 1% agarose gel electrophoresis. After staining with ethidium bromide, the amplified DNA bands were analyzed with the image analysis software ScionImage (Scion Corp., Frederick, MD, USA).

*2.6. Statistical Analysis.* The statistical analysis was performed using Statistical Package from Social Sciences (SPSS) version 13.0 for Windows. All data were expressed as the mean ± SD. The Mann-Whitney $U$ and $\chi^2$ tests were used for the statistical analysis of the data among all the groups. $P < 0.05$ was considered to be statistically significant.

## 3. Results

*3.1. Effects of DEX on Liver and Kidney Dysfunctions.* Serum AST, ALT, BIL, BUN, Cr, and CPK were measured in the groups of 24 hours postpartum rats with BMODS (Table 1). There were significantly ($P < 0.05$) increased levels of AST, ALT, TBIL, BUN, Cr, and CPK in the S+C, S+C+D2.5, and S+C+D5.0 groups in comparison to the Sham group, indicating impaired liver and kidney functions in the postpartum rats with BMODS. There were no statistically significant differences ($P > 0.05$) between the S+C and S+C+D2.5 groups, the S+C and S+C+5.0 groups, and the S+C+D2.5 and S+C+5.0 groups, suggesting that DEX administration does not prevent liver and kidney damage in postpartum rats with BMODS.

*3.2. Arterial Blood Gas (ABS) Analysis.* ABS, including the potential of hydrogen (pH), partial pressure of carbon dioxide in arterial blood (PaCO2), partial pressure of oxygen in arterial blood (PaO2), and base excess (BE), was measured in postpartum rats with BMODS (Table 2). There were significant differences ($P < 0.05$) between the Sham, S+C, and S+C+D2.5/5.0 in the levels of PaCO2, PaO2, and BE, indicating impaired pulmonary function in postpartum rats with BMODS. There was no significant difference ($P > 0.05$) between S+C and S+C+D2.5 or between S+C and S+C+D5.0, suggesting that DEX administration does not improve ABS in postpartum rats with BMODS.

*3.3. Cytokine mRNA Expression.* The mRNA expression of IFN-$\gamma$ and IL-4 in the lung tissue of the S+C group was significantly ($P < 0.05$) higher than in that of the Sham group, indicating that an inflammatory response was elicited in postpartum rats with BMODS. The IFN-$\gamma$, IL-4, and IFN-$\gamma$/IL-4 mRNA ratios were significantly lower in S+C+D2.5 and S+C+D5.0 than in S+C, indicating reduced IFN-$\gamma$ and IL-4 release upon DEX administration during postpartum BMODS. There were no statistically significant differences ($P > 0.05$) in IFN-$\gamma$, IL-4, and IFN-$\gamma$/IL-4 mRNA expressions between S+C+D2.5 and S+C+D5.0 (Table 3).

*3.4. Morphological Evaluation of Lung Injury.* In comparison to the Sham group (Figure 1(a)), lung sections from the S+C group (Figure 1(b)) showed acute injury; the interstitium was expanded by edema and inflammatory infiltrates that were composed of neutrophils, lymphocytes, and histiocytes. The alveoli were congestive and hemorrhagic. Some of the alveoli were collapsed. The epithelium was edematous and displayed a loss of cilia. The IQA and AIT were significantly higher in the S+C group compared to the Sham group ($P < 0.05$). The S+C+D2.5 and S+C+D5.0 groups showed less severe injury as compared to the S+C group, with significantly lower IQA and AIT values ($P < 0.05$) (Figure 1(c)). No significant differences were detected between the S+C+D2.5 and S+C+D5.0 groups (Table 4).

## 4. Discussion

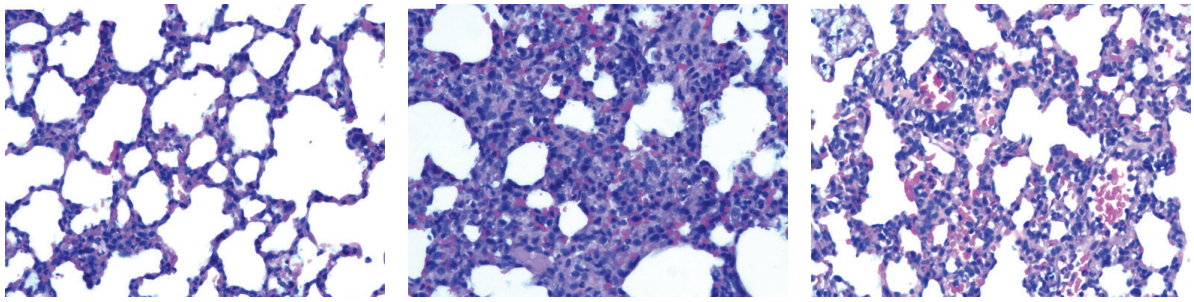
In addition to treatment for sedation and antianxiety [17], DEX has been administered to hypertensive patients during surgery [18], suggesting a relaxing effect on peripheral vessels. DEX exerts its effects via the selective activation of $\alpha$2AR. $\alpha$2AR is distributed not only in the central and peripheral nervous system but also in multiple organ systems, where it exerts its effects on different physiological and pathological processes. For example, the clinical use of DEX has become popular in neurosurgery and heart surgery under extracorporeal circulation anesthesia [19, 20]. However, the effects of DEX on BMODS have not yet been evaluated.

A small number of animal models are available for studying the pathogenesis of BMODS, but treatments have been largely unsuccessful, especially in patients with postpartum BMODS. It is difficult to study BMODS in human subjects

Table 2: Effects of DEX administration on arterial blood gas during postpartum BMODS in rats.

| Group | PH | PaCO2 (mmHg) | PaO2 (mmHg) | BE (mmol/L) |
|---|---|---|---|---|
| Sham | 7.40 ± 0.04 | 35.5 ± 4.2 | 92.2 ± 12.5 | −1.0 ± 0.5 |
| S+C | 7.35 ± 0.07 | 43.7 ± 5.3$^{\Delta}$ | 65.6 ± 13.8$^{\Delta}$ | −5.2 ± 0.4$^{\Delta}$ |
| S+C+D2.5 | 7.32 ± 0.08 | 44.3 ± 4.5$^{\Delta}$ | 64.5 ± 14.5$^{\Delta}$ | −4.8 ± 0.6$^{\Delta}$ |
| S+C+D5.0 | 7.31 ± 0.07 | 43.5 ± 4.8$^{\Delta}$ | 65.8 ± 14.8$^{\Delta}$ | −5.0 ± 0.7$^{\Delta}$ |

$^{\Delta}$Compared to the Sham group $P < 0.05$; $^{\#}$compared to the S+C group $P < 0.05$.



(a) Sham (200X) (b) S+C (200X) (c) S+C+D2.5 (200X)

Figure 1: Histological sections from the lung were examined under the 200X power field. The IQA was the mean percentage of injured alveoli from the 200X power field. The AIT was examined under the 200X power field using the IMS image analysis system (ShenTeng Information Technology Co., Ltd, Shanghai). The AIT was the mean thickness of the alveoli.

Table 3: Effects of DEX administration on IFN-$\gamma$, IL-4, and IFN-$\gamma$/IL-4 mRNA expression during postpartum BMODS in rats.

| Group | IFN-$\gamma$ | IL-4 | IFN-$\gamma$/IL-4 |
|---|---|---|---|
| Sham | 27.34 ± 1.56 | 23.38 ± 2.01 | 1.07 ± 0.04 |
| S+C | 34.51 ± 3.25$^{\Delta}$ | 32.35 ± 2.36$^{\Delta}$ | 1.06 ± 0.03$^{\Delta}$ |
| S+C+2.5 | 20.27 ± 1.68$^{\Delta\#}$ | 24.24 ± 2.02$^{\#}$ | 0.83 ± 0.02$^{\Delta\#}$ |
| S+C+5.0 | 19.06 ± 2.10$^{\Delta\#}$ | 22.36 ± 1.31$^{\#}$ | 0.85 ± 0.03$^{\Delta\#}$ |

$^{\Delta}$Compared to the Sham group $P < 0.05$; $^{\#}$compared to the S+C group $P < 0.05$.

Table 4: The index of quantitative assessment of lung (IQA) and alveolar interval thickness (AIT).

| Group | IQA (%) | AIT ($\mu$m) |
|---|---|---|
| Sham | 13.45 ± 3.84 | 6.9 ± 1.4 |
| S+C | 40.45 ± 4.24$^{\Delta}$ | 15.5 ±2.0$^{\Delta\#}$ |
| S+C+D2.5 | 30.67 ± 3.67$^{\Delta\#}$ | 10.2 ± 2.3$^{\Delta\#}$ |
| S+C+D5.0 | 28.85 ± 3.45$^{\Delta\#}$ | 9.8 ± 2.2$^{\Delta\#}$ |

$^{\Delta}$Compared to the Sham group $P < 0.05$; $^{\#}$compared to the S+C group $P < 0.05$.

because there are many factors involved in the pathogenesis and because there are a wide range of clinical presentations [21]. Therefore, the development of a successful animal model is a priority. In the present study, we established a BMODS model in postpartum rats. This animal model not only mimics postpartum bleeding-induced shock, but it also mimics reperfusion injury and endotoxicity. The majority of animal models for MODS are created by the external administration of endotoxin to induce hypotension [22]. Due to the wide range of individual sensitivities to endotoxin, many animals showed either a response too mild to induce hypotension or a response too strong, resulting in death of the animal. The level of hypotension and therefore the degree of injury are difficult to keep constant across groups. In the present study, hypotension was artificially controlled during the experiment. The time of clamping of the superior mesenteric artery was kept equal across the groups. Therefore, the level of damage was constant across the groups. The clamping of the superior mesenteric artery provided an ischemic and reperfusion process. This process induced endotoxin release rather than requiring external administration. Together with constant hypotension in rats, our animal model provides a reliable and constant level of damage across all groups for the study of BMODS. Our current BMODS model induced reperfusion injury. We observed inflammatory cytokine release in response to shock. Furthermore, clamping of the superior mesenteric artery induced multiple organ dysfunction syndrome, and it did not induce inflammatory cytokine release simply due to uncontrolled hemorrhagic shock; inflammatory cytokine release simply due to uncontrolled hemorrhagic shock differs from the response observed in patients undergoing postpartum hypotensive shock. Free radicals, calcium overload, and increased leukocytes play important roles in the process of ischemia-reperfusion injury. However, vasoactive peptides, regulatory peptides, inflammatory mediators, damaged cells, and metabolism also play important roles in the shock process. The results in present study showed that DEX administration decreased inflammatory cytokine release and damage in the lung in our current model of BMODS-induced reperfusion injury. The results showed that DEX may prevent organ damage through the inhibition of inflammation and free radical release from leukocytes. The specific mechanism

of action of DEX in the BMODS model needs further research. According to the diagnosis criteria of MODS, damage of two or more organs must occur at the same time or sequentially within 24 hours of induction by pathogenic factors. Therefore, we sacrificed all of the rats 24 hours after the experiment. Liver, kidney, and lung dysfunctions were evident in the S+C group in the present study (Tables 1 and 2). However, there were no statistically significant differences between the S+C and S+C+D2.5 groups, S+C and S+C+5.0 groups, and S+C+D2.5 and S+C+5.0 groups, suggesting that DEX administration did not prevent liver and kidney damage in postpartum rats with BMODS. Several variables may explain these results, including that the time of DEX administration was too short, the specimen collection was too early (24 hours), or the dose of DEX administration was not suitable for use. At later time points of BMODS, biochemical markers may be more obviously altered, and increases in inflammatory cytokine release may be more apparent. Therefore, DEX administration may more clearly reduce biochemical markers and inflammatory cytokines at later time points.

There have been several hypotheses regarding the mechanism of MODS (Figure 2), including ischemia reperfusion, inflammation, intestinal bacteria, toxin shifts, two strikes double preexcitation syndrome, and stress genes. However, none of these theories can fully explain the pathogenesis of MODS. The effects of DEX on the pathogenesis of MODS remain unclear. Regardless, the effect of DEX on inhibiting cytokine release was confirmed in this study. CD4+ T helper cells are divided into Th1 and Th2 subsets based on the cytokines they secrete [23]. Cytokines play an important role in lymphocyte development, maturation, differentiation, and activation. Th1 cells mainly secrete interleukin-2 (IL-2), IFN-$\gamma$, and tumor necrosis factor-$\alpha$ (TNF-$\alpha$). They mediate cellular immunity and participate in late onset allergic reactions and inflammation. Th2 cells secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 and are responsible for B lymphocyte proliferation, antibody production, immune tolerance, and humoral immunity [24, 25]. Therefore IFN-$\gamma$ and IL-4 are the best respective markers for Th1 and Th2 cells, and they were chosen to evaluate the changes in immune function in postpartum rats with BMODS in the present study. DEX has been shown to inhibit cytokine secretion in many studies. Some reports have shown that DEX decreases cytokine secretion after endotoxin injection [26]. Other reports have demonstrated that DEX inhibits cytokine release in rats following endotoxin administration [22]. In mice, DEX administration led to a decrease in the total number of lung inflammatory cells, a reduction in the concentration of macrophage inflammatory protein-2 (MIP-2) and interleukin-$\beta$ (IL-$\beta$), and a decrease in the ratio of dry/wet tissue in the lung [27]. To date, the mechanism underlying the role of DEX in the reduction of cytokine secretion is still a matter of debate. One group reported that DEX administration led to a reduced inflammatory response during the treatment of spinal cord injury, confirming the anti-inflammatory effects of DEX [28]. Another group reported that DEX protected nerve tissue from reperfusion-induced injury after -erm brain ischemia via reduced levels of TNF-$\alpha$ and decreased numbers of degenerative neurons in the hippocampus and dentate gyrus [29]. Our findings

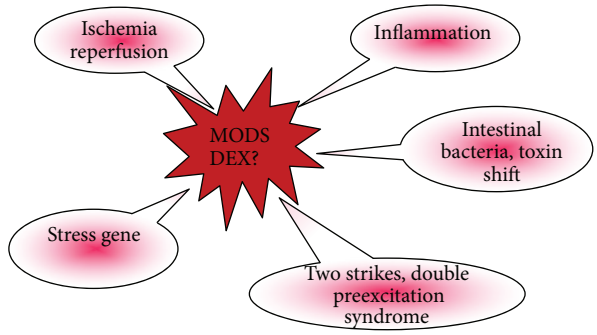



Figure 2: There have been several hypotheses regarding the mechanism of MODS (Figure 2), including ischemia reperfusion, inflammation, intestinal bacteria, toxin shifts, two strikes double preexcitation syndrome, and stress genes.

in the present study showed that DEX had strong anti-inflammatory effects on postpartum rats with BMODS. As shown in the S+C+D2.5 and S+C+D5.0 groups, DEX administration significantly reduced IFN-$\gamma$ and IL-4. Because both IFN-$\gamma$ and IL-4 represent humoral and cellular immunity, these findings suggested that both immune pathways were suppressed by DEX. Further, a decrease in the IFN-$\gamma$/IL-4 ratio suggested that DEX suppressed cellular immunity more than humoral immunity. There are few reports regarding a mechanism of DEX at the cellular level. Lower malondialdehyde (MDA) and nitric oxide (NO) levels and higher superoxide dismutase (SOD) and catalase (CAT) were observed in the hippocampus during reperfusion-induced injury in the ischemic rat brain after DEX administration [30]. DEX inhibited lipid peroxidation of the cell membrane in ischemia reperfusion models [31–33]. Further studies are required to address this issue in our model. Decreased immune function (both cellular and humoral) may induce septic shock. However, DEX administration did not lead to septic shock in our BMODS model, and this was confirmed by a report that DEX administration in early-stage sepsis patients decreased the mortality rate [27].

The decrease in cytokine release upon DEX treatment may protect organs from ischemic injuries. Clinically, DEX administration in combination with ketamine reduced mechanical ventilation-induced injury and inflammation in the lungs of endotoxemic rats [27]. In an animal model for spinal cord injury, the administration of DEX decreased edema and hemorrhage in gray matter without changing the number of neurons [34]. In the present study, DEX administration reduced inflammatory infiltration and edema in the lung, although no significant improvements were observed in ABS or liver and kidney function. This finding may provide a basis for additional management options for patients with BMODS. In current practice, BMODS patients are treated with mechanical approaches to prevent lung injury, including reducing the tidal volume of mechanical ventilation and positive pressure ventilation at the end of breath [35]. However, several studies have explored the combination of DEX and ketamine [36].

In summary, the present study demonstrated that DEX administration reduced IFN-$\gamma$ and IL-4 release and decreased

lung injury during postpartum BMODS. It is possible that DEX administration decreased inflammatory cytokine production in BMODS by inhibiting inflammation and free radical release by leukocytes independent of the DEX dose.

## Acknowledgments

The authors thank Liu Zhengzhuan for his assistance. This work was supported by the Youth Foundation of Guangzhou Medical University, China, Grant no. 2012A10 and the Science and Technology Foundation of Guangdong Province, China, Grant no. 2012B020700007.

## References

[1] A. Lalonde, B. A. Daviss, A. Acosta, and K. Herschderfer, "Postpartum hemorrhage today: ICM/FIGO initiative 2004–2006," *International Journal of Gynecology and Obstetrics*, vol. 94, no. 3, pp. 243–253, 2006.

[2] S. L. Clark, M. A. Belfort, G. A. Dildy, M. A. Herbst, J. A. Meyers, and G. D. Hankins, "Maternal death in the 21st century: causes, prevention, and relationship to cesarean delivery," *American Journal of Obstetrics and Gynecology*, vol. 199, no. 1, pp. 361–365, 2008.

[3] M. Levi, "Disseminated intravascular coagulation (DIC) in pregnancy and the peri-partum period," *Thrombosis Research*, vol. 123, pp. S63–S64, 2009.

[4] L. Quintin and M. Ghignone, "Risks associated with perioperative use of alpha-2 adrenoceptors agonists," in *Alpha-2 Adrenoceptor Agonists in Anaesthesia and Intensive Care*, pp. 347–368, Harcourt, London, UK, 2000.

[5] A. Aslan, M. Cemek, O. Eser et al., "Does dexmedetomidine reduce secondary damage after spinal cord injury? An experimental study," *European Spine Journal*, vol. 18, no. 3, pp. 336–344, 2009.

[6] C. A. Fairbanks, K. F. Kitto, H. O. Nguyen, L. S. Stone, and G. L. Wilcox, "Clonidine and dexmedetomidine produce antinociceptive synergy in mouse spinal cord," *Anesthesiology*, vol. 110, no. 3, pp. 638–647, 2009.

[7] D. B. Coursin, D. B. Coursin, and G. A. Maccioli, "Dexmedetomidine," *Current Opinion in Critical Care*, vol. 7, no. 4, pp. 221–226, 2001.

[8] T. Taniguchi, Y. Kidani, H. Kanakura, Y. Takemoto, and K. Yamamoto, "Effects of dexmedetomidine on mortality rate and inflammatory responses to endotoxin-induced shock in rats," *Critical Care Medicine*, vol. 32, no. 6, pp. 1322–1326, 2004.

[9] J. W. Suliburk, K. S. Helmer, E. A. Gonzalez, E. K. Robinson, and D. W. Mercer, "Ketamine attenuates liver injury attributed to endotoxemia: role of cyclooxygenase-2," *Surgery*, vol. 138, no. 2, pp. 134–140, 2005.

[10] M. J. Sheen and S. Ho, "Dexmedetomidine: more than a sedative and analgesic," *Acta Anaesthesiologica Taiwanica*, vol. 46, no. 4, pp. 149–150, 2008.

[11] A. Sezer, D. Memiş, U. Usta, and N. Süt, "The effect of dexmedetomidine on liver histopathology in a rat sepsis model: an experimental pilot study," *Ulusal Travma ve Acil Cerrahi Dergisi*, vol. 16, no. 2, pp. 108–112, 2010.

[12] D. G. Farmer, B. Ke, X. Shen et al., "Interleukin-13 protects mouse intestine from ischemia and reperfusion injury through regulation of innate and adaptive immunity," *Transplantation*, vol. 91, no. 7, pp. 737–743, 2011.

[13] C. L. Yang, P. S. Tsai, and C. J. Huang, "Effects of dexmedetomidine on regulating pulmonary inflammation in a rat model of ventilator-induced lung injury," *Acta Anaesthesiologica Taiwanica*, vol. 46, no. 4, pp. 151–159, 2008.

[14] S. Huang, P. Tsai, and C. Huang, "Dexmedetomidine-ketamine combination mitigates pulmonary type-2 cationic amino acid transporter isozymes upregulation in hemorrhagic shock rats," *Acta Anaesthesiologica Taiwanica*, vol. 48, no. 3, pp. 110–116, 2010.

[15] S.-Q. Chen, Y.-B. Zhang, C.-C. Li et al., "Antenatal exposure to different doses of dexamethasone leads to delayed alveolarization in newborn rats," *Chinese Journal of Pantophysiology*, vol. 27, no. 2, pp. 343–349, 2011.

[16] Y. Y. Dai, X. X. Qiu, Y. Wang et al., "Effect of hydroxysafflor yellow A on mitochondrial permeability transition in pneumonocytes after ischemia/reperfusion injure in rats," *Chinese Journal of Pathophysiology*, vol. 27, no. 6, pp. 1180–1186, 2011.

[17] J. R. Cormack, R. M. Orme, and T. G. Costello, "The role of $\alpha$2-agonists in neurosurgery," *Journal of Clinical Neuroscience*, vol. 12, no. 4, pp. 375–378, 2005.

[18] A. Koroglu, H. Teksan, O. Sagir, A. Yucel, H. I. Toprak, and O. M. Ersoy, "A comparison of the sedative, hemodynamic, and respiratory effects of dexmedetomidine and propofol in children undergoing magnetic resonance imaging," *Anesthesia and Analgesia*, vol. 103, no. 1, pp. 63–67, 2006.

[19] S. Mimuro, T. Katoh, A. Suzuki et al., "Deterioration of myocardial injury due to dexmedetomidine administration after myocardial ischaemia," *Resuscitation*, vol. 81, no. 12, pp. 1714–1717, 2010.

[20] J. C. Drummond and M. K. Sturaitis, "Brain tissue oxygenation during dexmedetomidine administration in surgical patients with neurovascular injuries," *Journal of Neurosurgical Anesthesiology*, vol. 22, no. 4, pp. 336–341, 2010.

[21] S. E. Geller, M. G. Adams, P. J. Kelly, B. S. Kodkany, and R. J. Derman, "Postpartum hemorrhage in resource-poor settings," *International Journal of Gynecology and Obstetrics*, vol. 92, no. 3, pp. 202–211, 2006.

[22] T. Taniguchi, A. Kurita, K. Kobayashi, K. Yamamoto, and H. Inaba, "Dose- and time-related effects of dexmedetomidine on mortality and inflammatory responses to endotoxin-induced shock in rats," *Journal of Anesthesia*, vol. 22, no. 3, pp. 221–228, 2008.

[23] S. A. Rosenberg, N. P. Restifo, J. C. Yang, R. A. Morgan, and M. E. Dudley, "Adoptive cell transfer: a clinical path to effective cancer immunotherapy," *Nature Reviews Cancer*, vol. 8, no. 4, pp. 299–308, 2008.

[24] O. Akbari, P. Stock, E. Meyer et al., "Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity," *Nature Medicine*, vol. 9, no. 5, pp. 582–588, 2003.

[25] B. Spellberg and J. E. Edwards Jr., "Type 1/type 2 immunity in infectious diseases," *Clinical Infectious Diseases*, vol. 32, no. 1, pp. 76–102, 2001.

[26] J. Szelényi, J. P. Kiss, and E. S. Vizi, "Differential involvement of sympathetic nervous system and immune system in the modulation of TNF-$\alpha$ production by $\alpha$2- and $\beta$-adrenoceptors in mice," *Journal of Neuroimmunology*, vol. 103, no. 1, pp. 34–40, 2000.

[27] C. Yang, C. Chen, P. Tsai, T. Wang, and C. Huang, "Protective effects of dexmedetomidine-ketamine combination against ventilator-induced lung injury in endotoxemia rats," *Journal of Surgical Research*, vol. 167, no. 2, pp. e273–e281, 2011.

[28] M. Can, S. Gul, S. Bektas, V. Hanci, and S. Acikgoz, "Effects of dexmedetomidine or methylprednisolone on inflammatory responses in spinal cord injury," *Acta Anaesthesiologica Scandinavica*, vol. 53, no. 8, pp. 1068–1072, 2009.

[29] O. Eser, H. Fidan, O. Sahin et al., "The influence of dexmedetomidine on ischemic rat hippocampus," *Brain Research*, vol. 1218, pp. 250–256, 2008.

[30] R. D. Sanders, J. Xu, Y. Shu et al., "Dexmedetomidine attenuates isoflurane-induced neurocognitive impairment in neonatal rats," *Anesthesiology*, vol. 110, no. 5, pp. 1077–1085, 2009.

[31] H. Yagmurdur, N. Ozcan, F. Dokumaci, K. Kilinc, F. Yilmaz, and H. Basar, "Dexmedetomidine reduces the ischemia-reperfusion injury markers during upper extremity surgery with tourniquet," *Journal of Hand Surgery*, vol. 33, no. 6, pp. 941–947, 2008.

[32] H. Okada, T. Kurita, T. Mochizuki, K. Morita, and S. Sato, "The cardioprotective effect of dexmedetomidine on global ischaemia in isolated rat hearts," *Resuscitation*, vol. 74, no. 3, pp. 538–545, 2007.

[33] K. Engelhard, C. Werner, E. Eberspächer et al., "The effect of the $\alpha$2-agonist dexmedetomidine and the N-methyl-D-aspartate antagonist S(+)-ketamine on the expression of apoptosis-regulating proteins after incomplete cerebral ischemia and reperfusion in rats," *Anesthesia and Analgesia*, vol. 96, no. 2, pp. 524–531, 2003.

[34] S. Gul, V. Hanci, B. Bahadir et al., "The effectiveness of dexmedetomidine in experimental spinal cord injury compared to methylprednisolone in rats," *Journal of Clinical Neuroscience*, vol. 17, no. 4, pp. 490–494, 2010.

[35] J. Kuhmonen, J. Pokorný, R. Miettinen et al., "Neuroprotective effects of dexmedetomidine in the gerbil hippocampus after transient global ischemia," *Anesthesiology*, vol. 87, no. 2, pp. 371–377, 1997.

[36] N. R. MacIntyre, "Ventilator-associated pneumonia: the role of ventilator management strategies," *Respiratory Care*, vol. 50, no. 6, pp. 766–772, 2005.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 187940, 6 pages
http://dx.doi.org/10.1155/2013/187940

*Clinical Study*

# Relationships of Adiponectin with Markers of Systemic Inflammation and Insulin Resistance in Infants Undergoing Open Cardiac Surgery

**Yukun Cao,[1] Ting Yang,[2] Shiqiang Yu,[1] Guocheng Sun,[1] Chunhu Gu,[1] and Dinghua Yi[1]**

[1] *Department of Cardiovascular Surgery, Xijing Hospital, Fourth Military Medical University, No. 15, Changle West Road, Xi'an, Shaanxi 710032, China*
[2] *Department of Oral Anatomy and Physiology and TMD, School of Stomatology, Fourth Military Medical University, Xi'an, Shaanxi 710032, China*

Correspondence should be addressed to Chunhu Gu; guchunhutga@hotmail.com and Dinghua Yi; yidh@fmmu.edu.cn

Received 4 August 2012; Accepted 22 May 2013

Academic Editor: Celeste C. Finnerty



*Background*. Insulin resistance and systemic inflammation frequently occur in infants undergoing cardiac surgery with cardiopulmonary bypass, while adiponectin has been demonstrated to have insulin-sensitizing and anti-inflammatory properties in obesity and type 2 diabetes mellitus. In this prospective study, we aimed to investigate the association of adiponectin with insulin resistance and inflammatory mediators in infants undergoing cardiac surgery with cardiopulmonary bypass. *Methods and Results*. From sixty infants undergoing open cardiac surgery, blood samples were taken before anesthesia, at the initiation of cardiopulmonary bypass and at 0, 6, 12, 24, and 48 hours after the termination of cardiopulmonary bypass. Plasma interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-$\alpha$), and adiponectin levels were assessed in blood samples. Insulin resistance was measured by assessment of the insulin requirement to maintain euglycaemia and repeated measurements of an insulin glycaemic index. Insulin glycaemic index, IL-6, and TNF-$\alpha$ increased up to 3–8-fold 6 h after the operation. Adiponectin is negatively correlated with markers of systemic inflammation 6 h after CPB. *Conclusions*. Although the level of serum adiponectin decreased significantly, there was a significant inverse association of adiponectin with markers of systemic inflammation and insulin resistance in infants undergoing open cardiac surgery.

## 1. Introduction

Insulin resistance and systemic inflammation frequently occur in infants undergoing cardiac surgery with cardiopulmonary bypass (CPB). Insulin resistance presenting with increased blood glucose level (hyperglycemia) and decreased sensitivity to insulin increases morbidity and mortality in critically ill patients [1, 2]. Intensive insulin therapy aiming at euglycemia improves their clinical outcome [3–5]. In a recently published study involving patients undergoing cardiac surgery, intraoperative insulin resistance was associated with an increased risk of short-term adverse outcomes [6]. The inflammatory reaction and injury may contribute to the development of postoperative complications [7, 8]. The magnitude and duration of the systemic inflammatory response determine the development of tissue damage, multiorgan failure, or even death [9, 10]. Our previous studies have demonstrated that ameliorating insulin resistance attenuates the systemic inflammatory response in infants undergoing CPB [11].

Adiponectin, a hormone derived from the adipose tissue, has been demonstrated to have insulin-sensitizing and anti-inflammatory properties in obesity and type 2 diabetes mellitus [12]. Recently adiponectin has also been shown to have a reverse correlation with insulin resistance and inflammatory mediators [13]. Studies on the relationship of adiponectin with insulin resistance and inflammatory mediators in infants undergoing cardiac surgery with cardiopulmonary bypass are scarce. The present study was undertaken to investigate the association of adiponectin with the development of insulin

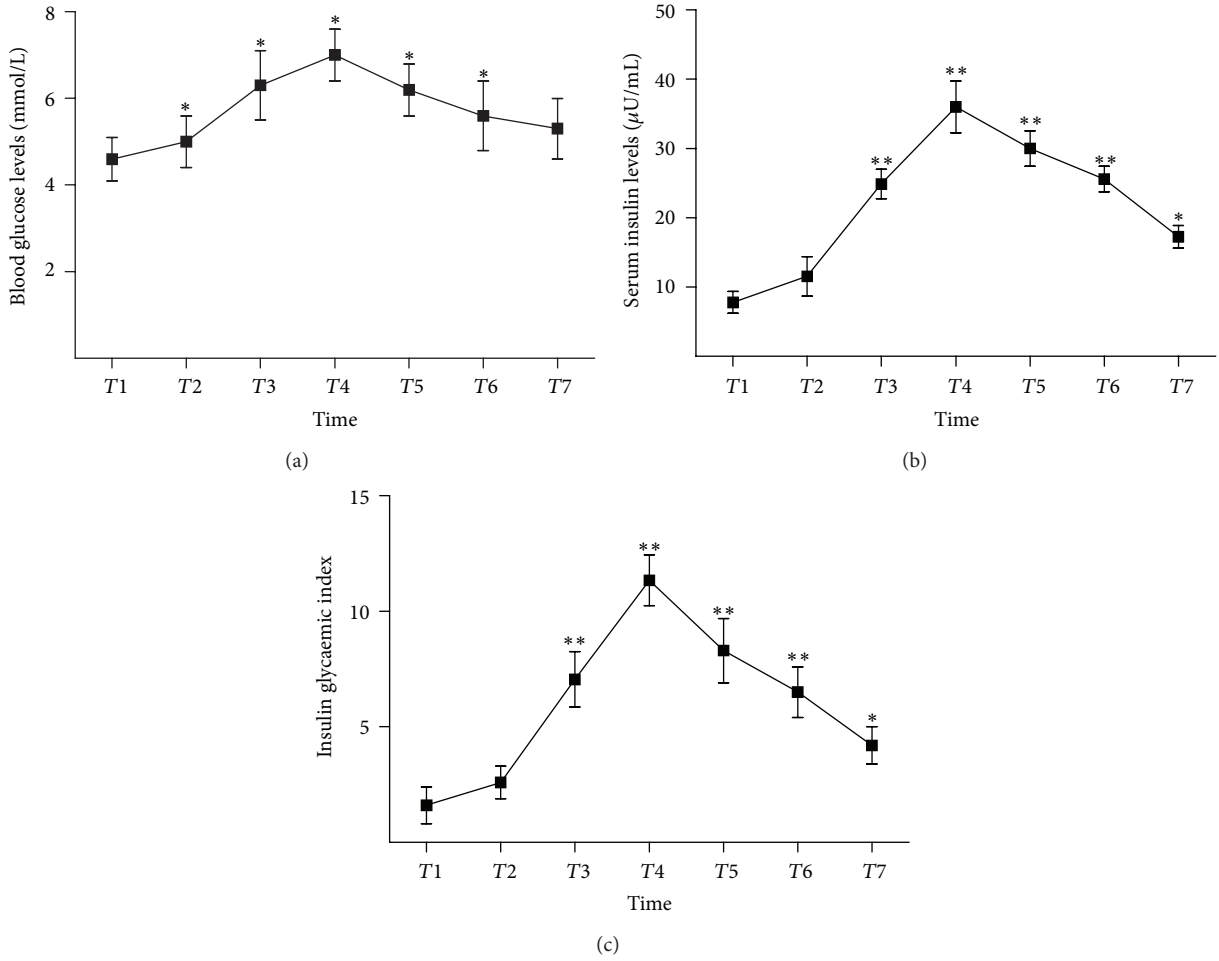



Figure 1: Changes in blood glucose levels, insulin levels, and insulin glycaemic index in the perioperative period. Reported significances ($^{*}P < 0.05$, $^{**}P < 0.01$ were calculated using pairwise comparisons with the preoperative level within a repeated measurement analysis of variance model for the respective parameter at different time points). The error bars designate the standard deviation (CPB, cardiopulmonary bypass; $T$: time; $T1$: before anesthesia; $T2$: initiation of CPB; $T3$: termination of CPB; $T4$: 6 h after CPB; $T5$: 12 h after CPB; $T6$: 24 h after CPB; $T7$: 48 h after CPB).

resistance and kinetic changes of inflammatory mediators in infants undergoing CPB.

## 2. Materials and Methods

The present study has been approved by the Ethics Committee of Xijing Hospital, The Fourth Military Medical University, and performed according to the World Medical Association Declaration of Helsinki.

*2.1. Patients.* Patient population: infants aged between 6 months and 3 years undergoing open cardiac surgery with CPB for congenital heart disease were enrolled for the study at our hospital from June 2010 to August 2011. Detailed information was given to the parents preoperatively and their written consent was obtained. None of the infants had a history of diabetes mellitus. Exclusive criteria included preoperative liver and kidney disease or dysfunction, preoperative coagulation disorder, palliative or second operation, and impaired blood glucose levels.

*2.2. Measurements of Insulin Resistance.* Overnight fasting was advised for all patients on the preoperative day. Insulin resistance was recorded by the individual insulin requirements to maintain euglycemia. Blood glucose was monitored on an hourly basis and insulin infusion rate was adjusted to maintain glucose levels between 4.4 and 8.3 mmol/L. The infusion of insulin is a standard of care and started when the glucose concentration became higher than 8.3 mmol/L. An insulin glycaemic index (insulin × glucose/22.5) was calculated at each time point.

*2.3. Determination of Insulin, Adiponectin, IL-6, and TNF-α Levels.* Blood samples were taken at 7 time points for each patient as follows: before anesthesia ($T1$), at the initiation of CPB ($T2$), at the termination of CPB ($T3$), 6 h after CPB ($T4$), 12 h after CPB ($T5$), 24 h after CPB ($T6$), and 48 h after CPB ($T7$). Serum level of adiponectin was determined with a commercial enzyme-linked immunosorbent assay (R&D, Wiesbaden, Germany). Serum insulin levels were measured with an insulin kit (R&D Systems, Abingdon, UK). Plasma

Table 1: Baseline characteristics and operative data of infants ($n$ = 60).

| Characteristics | Data |
|---|---|
| Male gender (%) | 36 (60%) |
| Age (year) | 1.5 ± 0.4 |
| Body weight (kg) | 5.9 ± 1.7 |
| Left ventricular ejection fraction (%) | 67.4 ± 8.6 |
| Cardiopulmonary bypass time (min) | 50.3 ± 7.9 |
| Cross-clamping time (min) | 35.4 ± 4.3 |
| Cardiopulmonary bypass flow (L/min/m$^2$) | 2.8 ± 0.4 |
| Ultrafiltration (mL/kg) | 337 ± 32 |
| Insulin ($\mu$U/mL) | 7.8 ± 1.6 |
| Blood glucose level (mmol/L) | 4.6 ± 0.5 |
| Tumor necrosis factor-$\alpha$ (pg/mL) | 32.7 ± 10.4 |
| Interleukin-6 (pg/mL) | 19.9 ± 15.7 |
| Adiponectin ($\mu$g/mL) | 9.5 ± 1.2 |

Data are presented as the number (%) of patients or mean values ± SD.

IL-6 and TNF-$\alpha$ levels were determined using commercially available ELISA kits (R&D Systems, Abingdon, UK) [14]. All enzyme-linked immunosorbent assay (ELISA) protocols were carried out according to kit guidelines.

*2.4. Statistical Analysis.* All data were expressed as mean with standard error of the mean. Pearson's correlation coefficient was estimated for associations between adiponectin and metabolic variables at different time points. Repeated measures analysis of variance (ANOVA) models (Figures 1, 2, and 3) were analysed using SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA).

## 3. Results

*3.1. Characteristics of the Study Group.* Baseline characteristics of the study participants are shown in Table 1. The cardiac surgery included repair of ventricular septal defects in 35 patients, atrial septal defects in 18 patients, and correction of tetralogy of Fallot in 7 patients.

*3.2. Kinetics of Insulin Resistance.* Blood glucose was monitored on an hourly basis throughout the observation period. All patients required insulin treatment to maintain euglycaemia. Figure 1(a) shows the stable blood glucose levels throughout the observation period. Serum insulin concentrations increased at the termination of CPB, following the course of exogenously applied insulin, and remained stable thereafter (Figure 1(b)). To create a more specific parameter of insulin resistance that combines serum glucose with serum insulin levels, we calculated an insulin glycaemic index (insulin × glucose/22.5) at each time point (Figure 1(c)). The insulin glycaemic index increased during the first 22 hours of the observation period and remained stable thereafter reflecting the kinetics of exogenously applied insulin.

Table 2: Correlations of adiponectin with metabolic variables.

| | Adiponectin with the insulin glycaemic index | Adiponectin with IL -6 | Adiponectin with TNF-$\alpha$ |
|---|---|---|---|
| $T1$ | $-0.415^{*}$ | $-0.397^{*}$ | $-0.419^{*}$ |
| $T2$ | $-0.408^{*}$ | $-0.384^{*}$ | $-0.379^{*}$ |
| $T3$ | −0.354 | −0.347 | −0.364 |
| $T4$ | $-0.465^{**}$ | $-0.427^{**}$ | $-0.447^{**}$ |
| $T5$ | −0.346 | −0.352 | −0.357 |
| $T6$ | −0.358 | −0.371 | −0.374 |
| $T7$ | −0.361 | −0.375 | −0.342 |

Pearson's correlation coefficient ($r$) and $P$ values of the corresponding significance test are both presented. ($T$: time; $T1$: before anesthesia; $T2$: initiation of CPB; $T3$: termination of CPB; $T4$: 6 h after CPB; $T5$: 12 h after CPB; $T6$: 24 h after CPB; $T7$: 48 h after CPB. $^{*}P < 0.05$ and $^{**}P < 0.001$.)

*3.3. Kinetics Inflammatory Cytokines.* During the observation period inflammatory cytokines rapidly increased with peak concentrations of TNF-$\alpha$ and IL-6 at the 6 h time point (Figures 2(a) and 2(b)). Adiponectin serum levels were repressed throughout the observation period reaching a minimum at the 6 h time point (Figure 3).

*3.4. Correlations of Adiponectin with Metabolic Variables at Different Time Points.* There was no association between the adiponectin at $T3$, $T5$, $T6$, and $T7$ time points and glycemic index, TNF-alpha and IL-6 (Table 2). At $T4$ (6 h after CPB) we found significant inverse correlations of adiponectin with insulin glycaemic index, IL-6, and TNF-$\alpha$ (Figure 4). Correlation of adiponectin with the insulin glycaemic index was $r = -0.465$ ($P < 0.001$) was adiponectin with IL-6, $r = -0.427$ ($P < 0.001$), and adiponectin with TNF-$\alpha$ was $r = -0.447$ ($P < 0.001$).

## 4. Discussion

Several studies have reported that adiponectin has a negative correlation with insulin resistance in chronic diseases such as metabolic syndrome and type 2 diabetes [15, 16]. However, the relationship of adiponectin with insulin resistance and inflammatory mediators in infants undergoing cardiac surgery with cardiopulmonary bypass has not been identified so far. The present study demonstrated the correlation of adiponectin with insulin resistance and the kinetic changes of inflammatory cytokines in infants undergoing CPB. CPB provokes a systemic inflammatory response. This inflammatory reaction may contribute to the development of postoperative complications. The marked increases in the amount of exogenous insulin requirement to maintain euglycemia as well as circulating insulin levels during CPB surgery suggest the development of insulin resistance. Our study showed significant increase in TNF-$\alpha$ and IL-6 levels after the initiation of CPB and their kinetics at various time points. At the same time, the need of an increased rate of insulin infusion to maintain euglycemia following the operation suggested the development of insulin resistance. Insulin resistance is

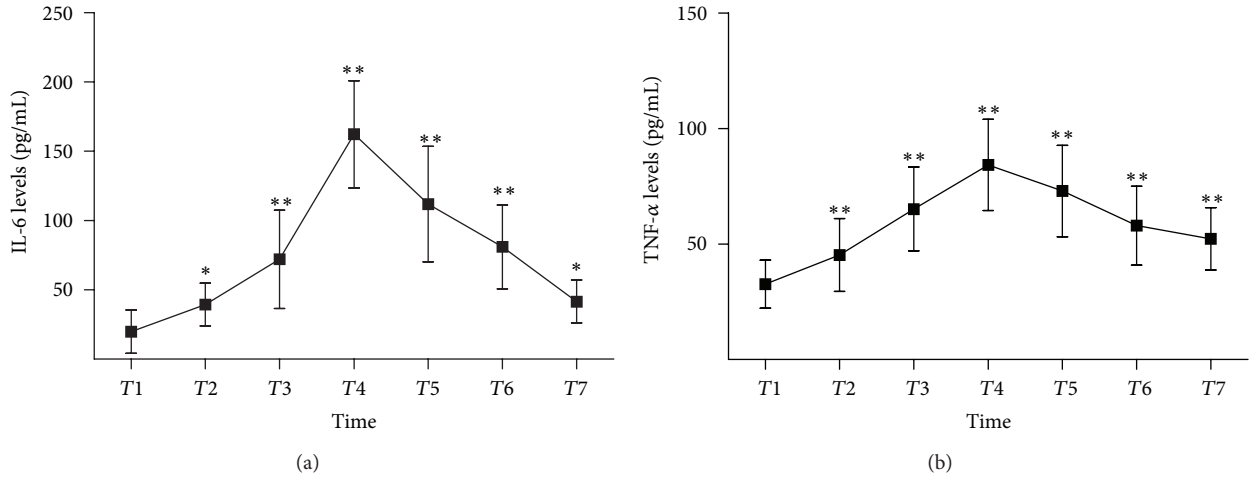



Figure 2: Pre- and postoperative TNF-$\alpha$ and IL-6 ($^{*}P < 0.05$, $^{**}P < 0.01$ compared with basal levels). The error bars designate standard deviation. IL-6 and TNF-$\alpha$ levels are higher than basal levels and did not normalize within the study period ((a) and (b)). (CPB: cardiopulmonary bypass; $T$: time; $T1$: before anesthesia; $T2$: initiation of CPB; $T3$: termination of CPB; $T4$: 6 h after CPB; $T5$: 12 h after CPB; $T6$: 24 h after CPB; $T7$: 48 h after CPB).

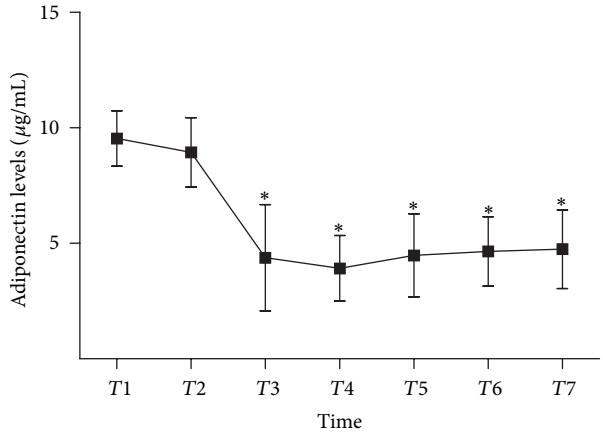



Figure 3: Changes in adiponectin levels in the perioperative period. Reported significances ($^{*}P < 0.05$ was calculated using pairwise comparisons with the preoperative level within a repeated measurement analysis of variance model for the respective parameter at different time points). The error bars designate the standard deviation (CPB: cardiopulmonary bypass; $T$: time; $T1$: before anesthesia; $T2$: initiation of CPB; $T3$: termination of CPB; $T4$: 6 h after CPB; $T5$: 12 h after CPB; $T6$: 24 h after CPB; $T7$: 48 h after CPB).

associated with the inflammatory response, but its molecular basis and physiological significance are not fully understood. Inflammatory mediators such as TNF-$\alpha$ and IL-6 either alone or through synergistic effect could lead to the development of insulin resistance by blocking the signal transduction of insulin, impairing insulin sensitivity, and increasing free fatty acids [17, 18]. Insulin resistance would be more intense as inflammatory mediator levels increase.

Adiponectin has been shown to directly or indirectly affect insulin sensitivity through modulation of insulin signaling and the molecules involved in glucose and lipid metabolism [12]. Adiponectin-deficient mice were shown to be prone to diet-induced obesity and insulin resistance and its reversal by adiponectin treatment [19]. In humans, low adiponectin was more closely associated with insulin resistance than adiposity [20]. In infants undergoing cardiac surgery, IL-6 and TNF-$\alpha$ levels were markedly increased while serum adiponectin levels were moderately decreased. This suggests the inverse relationship of circulating adiponectin levels to IL-6 and TNF-$\alpha$ and insulin resistance in critically ill patients. The repression of adiponectin serum levels in our model and its association with insulin resistance are in agreement with previous reports [13, 21]. Low adiponectin levels were associated with high inflammatory levels and intense insulin resistance. This indicates the role of adiponectin in regulation of glucose metabolism (insulin resistance) and inflammatory mediators.

## 5. Conclusions

In summary, we have demonstrated the significant inverse association of adiponectin with markers of systemic inflammation and insulin resistance in infants undergoing open cardiac surgery. The better understanding of the association of adiponectin with insulin resistance and systemic inflammation will be of high clinical value as it may have therapeutic implications.

## Authors' Contribution

Yukun Cao and Ting Yang contributed equally to this paper.

## Acknowledgment

This work was supported by the Grants from the Translational Medicine Foundation of Xijing Hospital (no. XJZT11Z08).

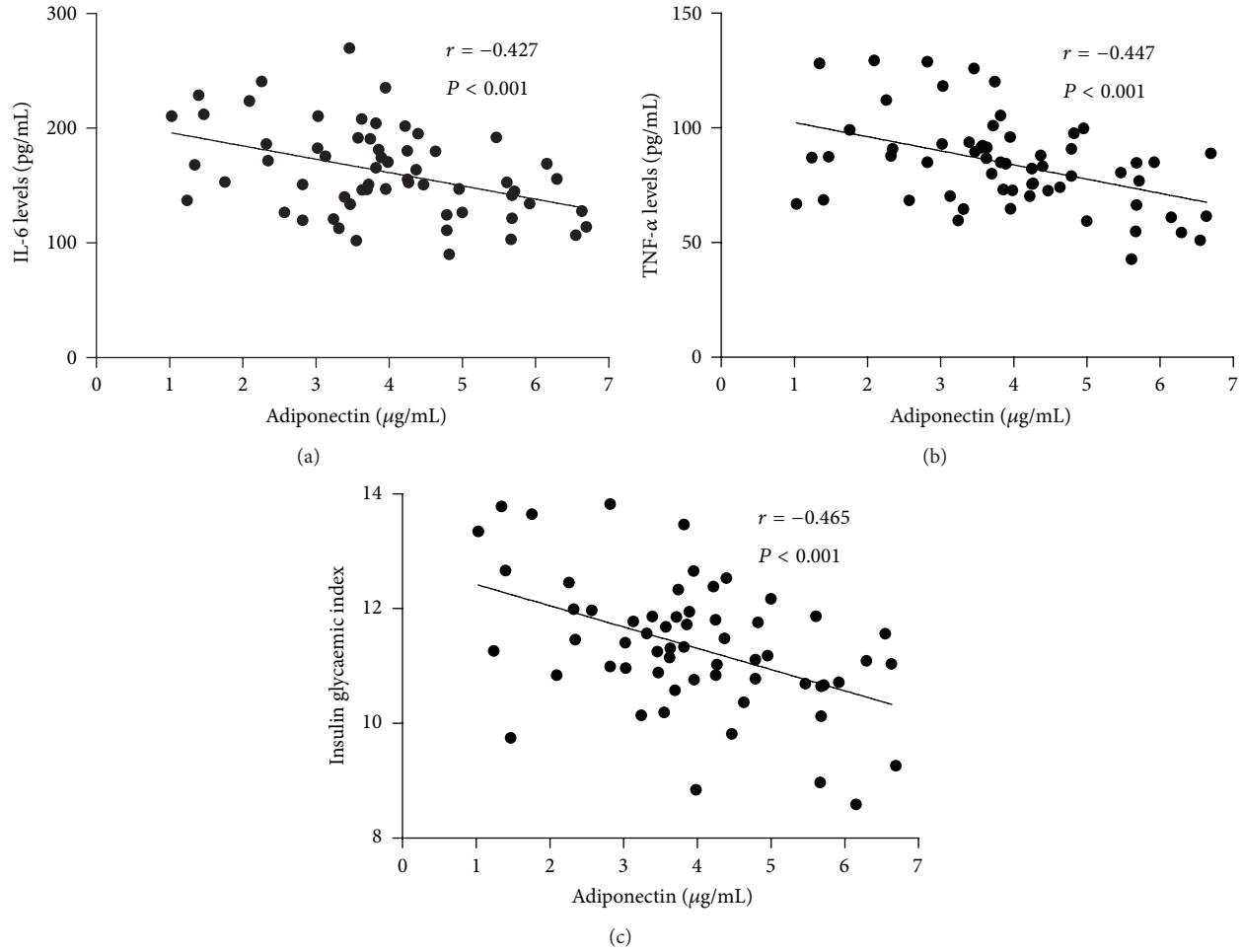



Figure 4: Correlations of adiponectin at $T4$ (6 h after CPB) with IL-6 (a), TNF-$\alpha$ (b), and insulin glycaemic index (c). Pearson's correlation coefficient ($r$) and $P$ values of the corresponding significance test are both presented.

## References


[1] S. O. Butler, I. F. Btaiche, and C. Alaniz, "Relationship between hyperglycemia and infection in critically ill patients," *Pharmacotherapy*, vol. 25, no. 7, pp. 963–976, 2005.

[2] I. Vanhorebeek, L. Langouche, and G. van den Berghe, "Glycemic and nonglycemic effects of insulin: how do they contribute to a better outcome of critical illness?" *Current Opinion in Critical Care*, vol. 11, no. 4, pp. 304–311, 2005.

[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] 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.

[5] R. Zheng, C. Gu, Y. Wang et al., "Impacts of intensive insulin therapy in patients undergoing heart valve replacement," *Heart Surgery Forum*, vol. 13, no. 5, pp. E292–E298, 2010.

[6] H. Sato, G. Carvalho, T. Sato, R. Lattermann, T. Matsukawa, and T. Schricker, "The association of preoperative glycemic control, intraoperative insulin sensitivity, and outcomes after cardiac surgery," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 9, pp. 4338–4344, 2010.

[7] H. Aebert, S. Kirchner, A. Keyser et al., "Endothelial apoptosis is induced by serum of patients after cardiopulmonary bypass," *The European Journal of Cardio-thoracic Surgery*, vol. 18, no. 5, pp. 589–593, 2000.

[8] T. Asahara, H. Masuda, T. Takahashi et al., "Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization," *Circulation Research*, vol. 85, no. 3, pp. 221–228, 1999.

[9] H. Wang, O. Bloom, M. Zhang et al., "HMG-1 as a late mediator of endotoxin lethality in mice," *Science*, vol. 285, no. 5425, pp. 248–251, 1999.

[10] K. J. Tracey, Y. Fong, D. G. Hesse et al., "Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia," *Nature*, vol. 330, no. 6149, pp. 662–664, 1987.

[11] C.-H. Gu, Q. Cui, Y.-Y. Wang et al., "Effects of insulin therapy on inflammatory mediators in infants undergoing cardiac surgery with cardiopulmonary bypass," *Cytokine*, vol. 44, no. 1, pp. 96–100, 2008.

[12] F. Ziemke and C. S. Mantzoros, "Adiponectin in insulin resistance: lessons from translational research," *The American Journal of Clinical Nutrition*, vol. 91, no. 1, pp. 258S–261S, 2010.

[13] M. Lehrke, U. C. Broedl, I. M. Biller-Friedmann et al., "Serum concentrations of cortisol, interleukin 6, leptin and adiponectin

predict stress induced insulin resistance in acute inflammatory reactions," *Critical Care*, vol. 12, no. 6, article R157, 2008.

[14] Y.-L. Liu, H. Bi, S.-M. Chi et al., "The effect of compound nutrients on stress-induced changes in serum IL-2, IL-6 and TNF-$\alpha$ levels in rats," *Cytokine*, vol. 37, no. 1, pp. 14–21, 2007.

[15] C. S. Mantzoros, T. Li, J. E. Manson, J. B. Meigs, and F. B. Hu, "Circulating adiponectin levels are associated with better glycemic control, more favorable lipid profile, and reduced inflammation in women with type 2 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 8, pp. 4542–4548, 2005.

[16] N. Soebijanto and S. Waspadji, "Adiponectin levels and its role in insulin resistance among adult women with metabolic syndrome," *Acta medica Indonesiana*, vol. 42, no. 4, pp. 187–191, 2010.

[17] G. S. Hotamisligil, "Inflammatory pathways and insulin action," *International Journal of Obesity*, vol. 27, supplement 3, pp. S53–S55, 2003.

[18] A. Shah, N. Mehta, and M. P. Reilly, "Adipose inflammation, insulin resistance, and cardiovascular disease," *Journal of Parenteral and Enteral Nutrition*, vol. 32, no. 6, pp. 638–644, 2008.

[19] T. Yamauchi, J. Kamon, H. Waki et al., "The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity," *Nature Medicine*, vol. 7, no. 8, pp. 941–946, 2001.

[20] C. Weyer, T. Funahashi, S. Tanaka et al., "Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia," *Journal of Clinical Endocrinology and Metabolism*, vol. 86, no. 5, pp. 1930–1935, 2001.

[21] J. Kremen, M. Dolinkova, J. Krajickova et al., "Increased subcutaneous and epicardial adipose tissue production of proinflammatory cytokines in cardiac surgery patients: possible role in postoperative insulin resistance," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 11, pp. 4620–4627, 2006.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 562154, 9 pages
http://dx.doi.org/10.1155/2013/562154

*Research Article*

# Dexmedetomidine Inhibits Inflammatory Reaction in Lung Tissues of Septic Rats by Suppressing TLR4/NF-$\kappa$B Pathway

**Yuqing Wu,[1,2] Yingchun Liu,[1] He Huang,[1] Yangzi Zhu,[1] Yong Zhang,[3] Fuzhao Lu,[1] Ce Zhou,[1] Li Huang,[4] Xin Li,[4] and Chenghua Zhou[4]**

[1] *Jiangsu Province Key Laboratory of Anesthesiology, Xuzhou Medical College, Tongshan Road 209, Xuzhou 221004, China*
[2] *Department of Anesthetic Pharmacology, Xuzhou Medical College, Tongshan Road 209, Xuzhou 221004, China*
[3] *The Sixth People's Hospital of Xuzhou City, Huaihai Road, Xuzhou 221002, China*
[4] *Department of Pharmacology, School of Pharmacy, Xuzhou Medical College, Tongshan Road 209, Xuzhou 221004, China*

Correspondence should be addressed to Chenghua Zhou; chzhou77@163.com

Received 14 June 2012; Revised 29 December 2012; Accepted 20 February 2013

Academic Editor: Celeste C. Finnerty



Dexmedetomidine has been reported to reduce mortality in septic rats. This study was designed to investigate the effects of dexmedetomidine on inflammatory reaction in lung tissues of septic rats induced by CLP. After induction of sepsis, the rats were treated with normal saline or dexmedetomidine (5, 10, or 20 $\mu$g/kg). The survival rate of septic rats in 24 h was recorded. The inflammation of lung tissues was evaluated by HE stain. The concentrations of IL-6 and TNF-$\alpha$ in BALF and plasma were measured by ELISA. The expressions of TLR4 and MyD88 were measured by western blotting. The activation of NF-$\kappa$B in rat lung tissues was assessed by western blotting and immunohistochemistry. It was found that the mortality rate and pulmonary inflammation were significantly increased in septic rats. IL-6 and TNF-$\alpha$ levels in BALF and plasma, NF-$\kappa$B activity, and TLR4/MyD88 expression in rat lung tissues were markedly enhanced after CLP. Dexmedetomidine (10 and 20 $\mu$g/kg) significantly decreased mortality and pulmonary inflammation of septic rats, as well as suppressed CLP-induced elevation of TNF-$\alpha$ and IL-6 and inhibited TLR4/MyD88 expression and NF-$\kappa$B activation. These results suggest that dexmedetomidine may decrease mortality and inhibit inflammatory reaction in lung tissues of septic rats by suppressing TLR4/MyD88/NF-$\kappa$B pathway.

## 1. Introduction

Sepsis is a main problem in the intensive care unit (ICU) and carries a very high mortality rate. It involves a network of proinflammatory cytokines such as TNF-$\alpha$ and IL-6 which are overexpressed after various noxious insults, especially bacterial infections [1–4]. Enhanced inflammatory reaction in lung tissues is believed to be an important component of the pathophysiology of sepsis [5]. Patients with endotoxemia are under the hyperstress state and often require drugs for sedation and analgesia to reduce anxiety and stress.

Dexmedetomidine, a selective agonist of $\alpha_2$-adrenergic receptor, is a potent sedative agent for critically ill patients and also provides effective analgesia [6]. The site of action for dexmedetomidine's sedative effects is thought to be the locus ceruleus in the midbrain, where it inhibits the release of neurotransmitters from synaptic terminals, thus inhibiting neuronal signaling and decreasing wakefulness. Dexmedetomidine's analgesic effects are mediated in the dorsal horn of spinal cord, where it is thought to decrease the firing of ascending nociceptive neurons. These actions result in effective sedation and analgesia without respiratory depression and drug-dependency issues. Along with its beneficial effects, dexmedetomidine was reported to exert potential anti-inflammatory effect during endotoxemia. Previous study revealed that dexmedetomidine, a new sedative agent, significantly reduced mortality and decreased the levels of inflammatory cytokines during endotoxemia in rats [7]. However, the detailed mechanisms by which dexmedetomidine regulates inflammatory responses during endotoxemia have not been clearly revealed.

Toll-like receptors (TLRs) are a family of transmembrane proteins and act as signal transduction molecules. Toll-like

receptor 4 (TLR4) has been considered the main sensor to recognize the pathogen-associated molecular patterns (PAMPs). TLR4 is a member of the IL-1R/TLR superfamily that is required for LPS responsiveness and is involved in the host defense against Gram-negative bacteria [8]. Stimulation of TLR4 can activate NF-kappaB protein through myeloid differentiation factor 88 (MyD88) dependent or independent pathway. After ligand binding, TLRs/IL-1Rs dimerize and undergo the conformational change required for the recruitment of downstream signaling molecules and stimulation of downstream kinases, including activation of the transcriptional factors NF-$\kappa$B [9]. The activation of NF-$\kappa$B leads to the induction of genes encoding proinflammatory cytokines such as IL-6 and TNF-$\alpha$ [10].

This study aims to investigate the effects of dexmedetomidine on inflammatory reaction in lung tissues of septic rats induced by cecal ligation and puncture (CLP) and relevant mechanisms. We hypothesized that dexmedetomidine could suppress inflammatory response in rat lung tissues during endotoxemia induced by CLP. Furthermore, we probed whether dexmedetomidine could attenuate CLP-induced pulmonary inflammation through inhibiting TLR4/MyD88 expression and NF-$\kappa$B activation, reducing proinflammatory cytokines production in septic rats.

## 2. Materials and Methods

*2.1. Animals.* Male Sprague-Dawley rats (250–300 g) were obtained from Shanghai Animal Center (Shanghai, China). The rats were housed at 23°C with 12 h of light and 12 h of darkness each day and allowed free access to food and water. All experiments were performed in accordance with the institutional criteria for the care and use of laboratory animals in research.

*2.2. Cecal Ligation and Puncture (CLP) Operation.* The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium 50 mg/kg. Polymicrobial sepsis was induced by CLP as previously described [11]. The procedure was performed under sterile conditions with the abdominal skin disinfected with 75% alcohol. Laparotomy was conducted through 2 cm lower-midline incision. The cecum was exposed and ligated immediately distal to the ileocecal valve to avoid intestinal obstruction and then punctured twice with an 18-gauge needle, squeezed gently to force out a small amount of feces, and then returned to the abdominal cavity. The abdomen is closed with 3–0 silk sutures in two layers.

*2.3. Experimental Protocol.* Rats were randomly divided into five groups: sham operation, CLP, small dose, medium dose, and large dose of dexmedetomidine. Following completion of CLP or sham operation dexmedetomidine or normal saline were administered. The rats in sham operation and CLP groups were administered by intraperitoneal injection normal saline, and the rats in small, medium, and large dose dexmedetomidine groups were administered by intraperitoneal injection 5, 10, and 20 $\mu$g/kg dexmedetomidine, respectively. Dexmedetomidine or normal saline was administered by intraperitoneal injection at 0, 2, 4, and 6 h after operation.

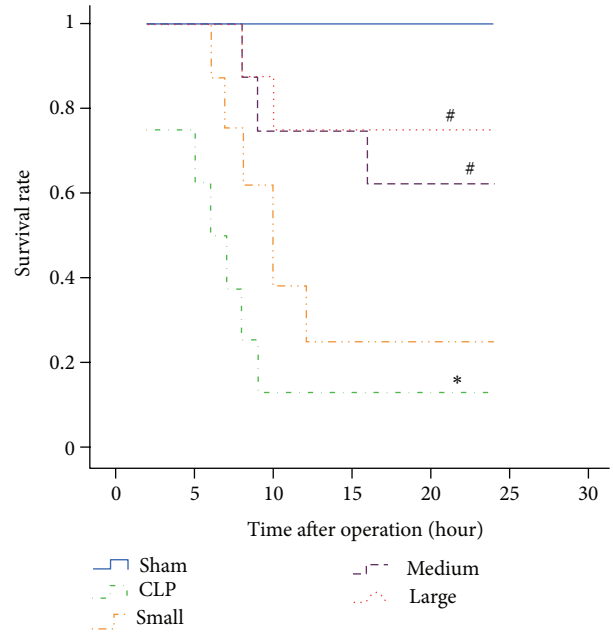



Figure 1: Effect of dexmedetomidine on survival curves of septic rats induced by CLP. Sham: sham operation group; CLP: cecal ligation and puncture operation group; Small: 5 $\mu$g/kg dexmedetomidine treatment group; Medium: 10 $\mu$g/kg dexmedetomidine treatment group; Large: 20 $\mu$g/kg dexmedetomidine treatment group. $n = 8$. The survival rate at 24 h after operation was analyzed. $^{*}P < 0.01$, versus sham group; $^{\#}P < 0.01$, versus CLP group.

*2.4. Mortality Rate.* Forty rats were randomly divided into five groups (8 per group) as aforementioned. The animals were performed by CLP or sham operation and administered as previously stated. All the animals were monitored after operation and administration. The time when animal died was recorded. The animals were observed up to 24 hours after operation and sacrificed by lethal sodium pentobarbital injection. The mortality rate within 24 hours was calculated.

*2.5. Plasma, Bronchoalveolar Lavage Fluid, and Lung Tissues Collection.* Venous blood samples were drawn at 0, 2, 4, and 6 hours after operation for the measurement of plasma cytokines. At 8 hours after operation or as soon as the animal died, the left main bronchus was tied and the left lung was removed. The upper lobe of the left lung tissues was snap-frozen in liquid nitrogen and stored at −80°C for subsequent protein detection by western blot, and the lower lobe of the left lung was used to HE stain and immunohistochemistry. The right lung from each group was lavaged with sterile normal saline through the tracheostomy tube as previous report [12], and then bronchoalveolar lavage fluid (BALF) was collected for measurement of cytokines by enzyme-linked immunosorbent assay (ELISA).

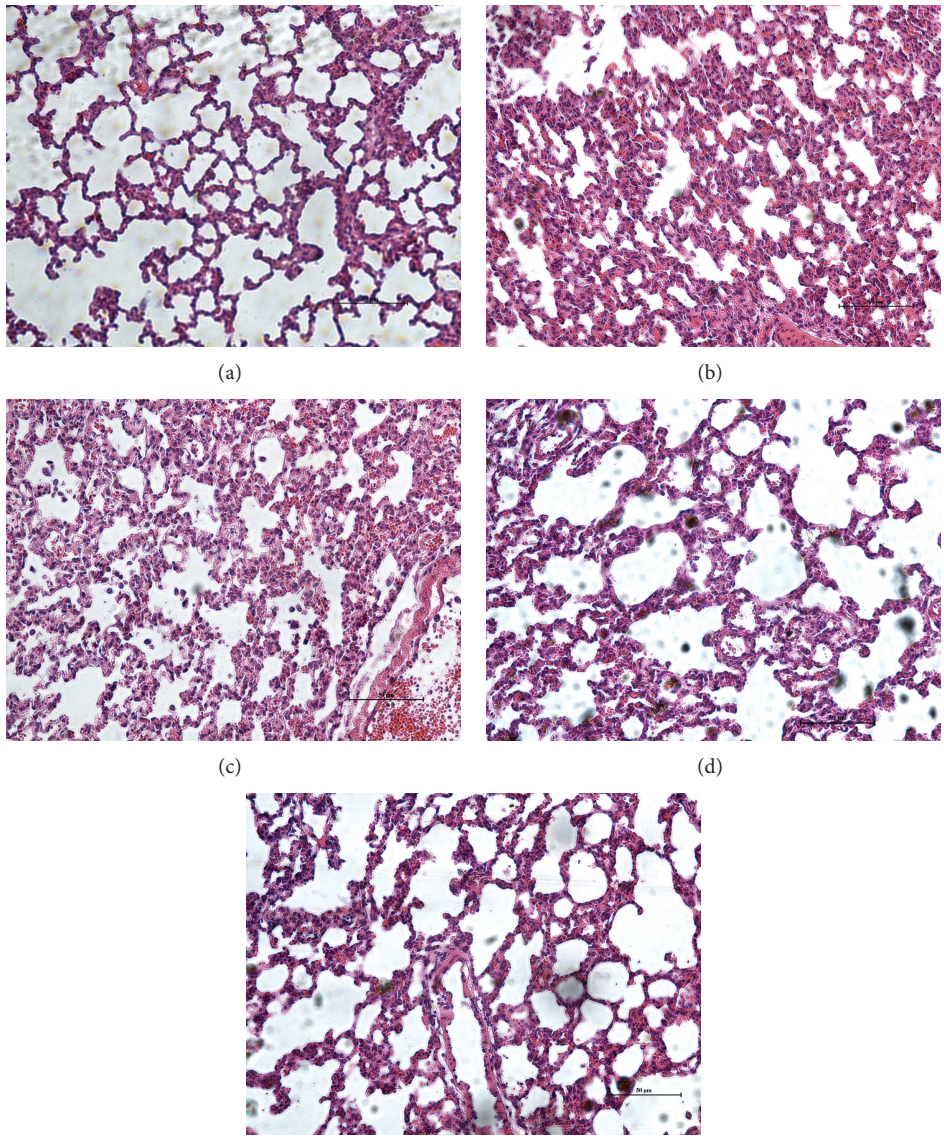


FIGURE 2: Microscopic findings of the lung tissues stained with hematoxylin and eosin. (a) Sham operation group. The lung tissues showed normal to minimal pulmonary inflammation. (b) CLP group. The lung tissues showed severe pulmonary inflammation. (c) Small dose (5 $\mu$g/kg) group. The lung tissues showed severe pulmonary inflammation. (d) Medium dose (10 $\mu$g/kg) group. The lung tissues showed moderate pulmonary inflammation. (e) Large dose (20 $\mu$g/kg) group. The lung tissues showed mild pulmonary inflammation. (magnification 200x).

*2.6. HE Stain for Lung Tissues.* Formaldehyde-fixed lower lobe of the left lung was embedded in paraffin wax, serial sectioned, and stained with hematoxylin and eosin. Histologic changes including alveolar wall edema, congestion, hemorrhage, and inflammatory cells infiltration were evaluated under a light microscope to assess pulmonary inflammation according to the previous report [12]. Each histologic characteristic was scored on a scale of 0 (normal) to 5 (severe) by a pathologist who was blind to this study. The overall pulmonary inflammation was categorized according to the sum of the score (0–5: normal to minimal inflammation; 6–10: mild inflammation; 11–15: moderate inflammation; 16–20: severe inflammation).

*2.7. ELISA for IL-6 and TNF-$\alpha$.* The levels of IL-6 and TNF-$\alpha$ in plasma and BALF were detected by ELISA. IL-6 and TNF-$\alpha$ were measured using commercially available ELISA kits according to the protocols of the kits. Briefly, cell free supernatant was added to each well of a monoclonal rabbit anti-rat IL-6 or TNF-$\alpha$ antibody coated microtitre plates (ELISA plates) for 12 h at 4°C. Unbound material was washed off and a biotinylated monoclonal rabbit anti-rat IL-6 or TNF-$\alpha$ antibody was used for 45 min. Bound antibody was detected by addition of avidin-peroxidase for 30 min followed by incubation of the ABTS substrate solution. Absorbance was measured 20 min after addition of substrate. A standard curve was constructed using various dilutions of IL-6 or TNF-$\alpha$

standard preparation. The levels of IL-6 and TNF-$\alpha$ were determined by extrapolation of absorbance to the standard curve.

*2.8. Western Blot for TLR4, MyD88, and NF-κB.* Frozen rat lung tissues were homogenized and the lysates were prepared in ice-cold lysis buffer. Nuclear extracts were collected and stored at −80°C for western blot analyses of nuclear translocation of NF-κB p65 protein. The extracts were normalized for equal amounts of total protein measured by the bicinchoninic acid (BCA) method. Seventy micrograms protein from each sample was separated on a sodium dodecyl sulfate-polyacrylamide gel and transferred to PVDF membranes. The membranes were blocked with 5% nonfat milk and incubated overnight with primary anti-NF-κB p-65 antibody (Cell Signaling Technology, USA), anti-TLR4 antibody (Santa Cruz), or anti-MyD88 antibody (Santa Cruz) at 4°C, followed by incubation with the suitable HRP-conjugated secondary antibody for 4 hours. Cellular GAPDH protein was immunodetected as the internal standard.

*2.9. Immunohistochemistry for NF-κB.* NF-κB p-65 protein activity was also observed by immunohistochemistry technique. Briefly, sections were microwaved for antigen retrieval and pretreated with 0.3% $H_2O_2$. Subsequently, the sections were blocked with goat serum and incubated in a primary antibody solution containing rabbit anti-NF-κB p-65 antibody overnight at 4°C. After washing, the samples were incubated in a suitable secondary antibody solution for 2 h at room temperature. Finally, the sections were incubated in HRP-streptavidin (1 : 100, Zhongshan Biotechnology) for 1 h at room temperature, and the color reaction was developed with diaminobenzidine (DAB). The sections were counterstained, dehydrated, coverslipped, and analyzed under light microscope. Five high-power fields per slide were observed. The degree of NF-κB activation was expressed as percentage of nuclear NF-κB p-65 positive cells to total alveolar epithelial cells.

*2.10. Statistical Analysis.* Data were presented as mean ± SD. SPSS-13.0 software was used for data analysis. Differences among groups were determined by one-way analysis of variance (ANOVA), followed by a post hoc test (Bonferroni's method). The mortality rates among groups were compared using the Kaplan Meier methods. Statistical significance was defined as $P < 0.05$.

## 3. Results

*3.1. Mortality Rate.* As shown in Figure 1, the mortality rates in 24 hours after operation were 0%, 87.5%, 75%, 37.5%, and 25%, respectively for sham, CLP, small, medium and large dose groups. Compared to sham group, the mortality rate in CLP group markedly increased. However, medium and large doses of dexmedetomidine significantly suppressed the elevated mortality rate induced by CLP. There is no significant difference between small dose dexmedetomidine group and CLP group for mortality rate.

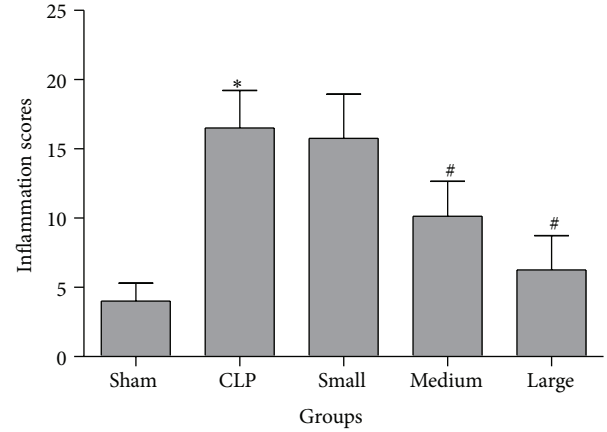



Figure 3: Pulmonary inflammation scores of rats from different groups. Sham: sham operation group; CLP: cecal ligation and puncture operation group; Small: 5 μg/kg dexmedetomidine treatment group; Medium: 10 μg/kg dexmedetomidine treatment group; Large: 20 μg/kg dexmedetomidine treatment group. Data are expressed as mean ± SD, $n = 8$. $^{*}P < 0.01$, versus sham group; $^{\#}P < 0.01$, versus CLP group.

*3.2. Histopathological Observation in Lung Tissues.* The representative micrographs in Figure 2 represent eight samples in each group. Observed under light microscope by hematoxylin and eosin staining, rat lung tissue sections in sham group showed normal alveolar architecture (Figure 2(a)). Rats in CLP group exhibited marked lung histopathologic abnormalities, characterized by alveolar wall edema, congestion, leakage of microvessel, and inflammatory cells infiltration (Figure 2(b)). Small dose of dexmedetomidine failed to improve the histopathologic abnormalities. However, after treatment with medium and large doses of dexmedetomidine, lung tissues appeared relatively better, with a prominent decrease in inflammation response (Figures 2(d) and 2(e)) compared to CLP group. Findings of the pulmonary inflammation score paralleled the findings of the histopathological observation (Figure 3).

*3.3. Levels of IL-6 and TNF-α in Plasma and BALF.* As shown in Figure 4, the baseline values of IL-6 and TNF-$\alpha$ in the five groups were similar. However, both IL-6 and TNF-$\alpha$ levels in plasma and BALF of rats were markedly increased at 2, 4, and 6 hours after CLP operation compared to those in sham group. Medium and large doses of dexmedetomidine significantly inhibited the production of IL-6 and TNF-$\alpha$ in plasma and BALF induced by CLP. While small dose of dexmedetomidine did not obviously affect the levels of these proinflammatory cytokines in plasma and BALF of CLP rats.

*3.4. Western Blot Analysis for TLR4/MyD88 Expression in Lung.* In order to clarify the mechanism through which dexmedetomidine inhibits pulmonary inflammation of septic rats induced by CLP, we further investigated the expression of TLR4/MyD88 in lung tissues. In this study, TLR4/MyD88 expression in lung tissues of rats from CLP

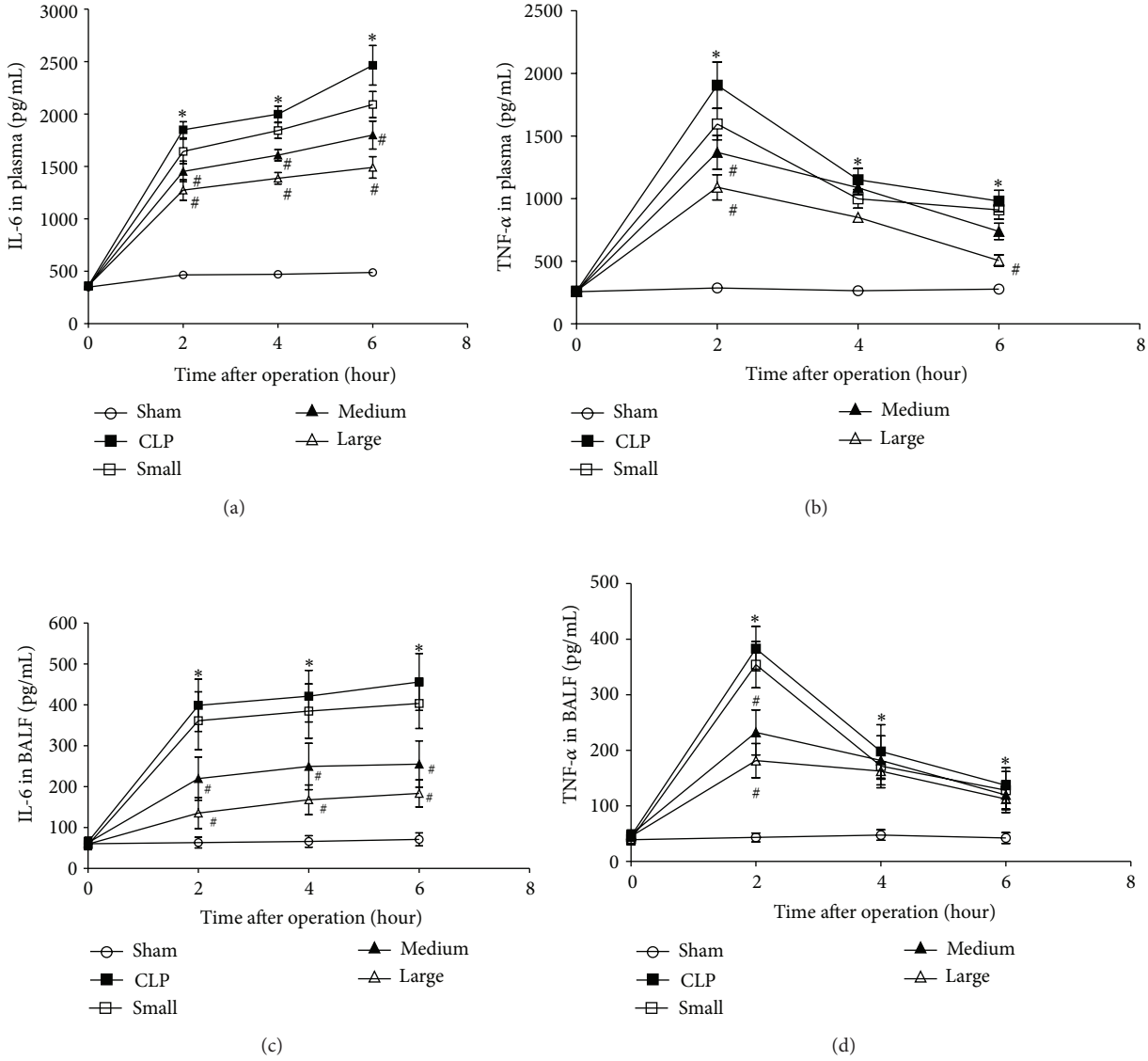



Figure 4: Levels of interleukin-6 (IL-6) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) in plasma ($n = 6$) and bronchoalveolar lavage fluid (BALF) ($n = 8$). (a) IL-6 in plasma. (b) TNF-$\alpha$ in plasma. (c) IL-6 in BALF. (d) TNF-$\alpha$ in BALF. Data are expressed as mean ± SD. $^{*}P < 0.01$, versus sham group; $^{\#}P < 0.01$, versus CLP group.

group was markedly upregulated compared to that in sham group. Medium and large doses of dexmedetomidine significantly suppressed TLR4/MyD88 expression in lung of septic rats. However, small dose of dexmedetomidine did not markedly reduce CLP induced overexpression of TLR4/MyD88 (Figure 5).

*3.5. Western Blot Analysis for NF-κB Activity.* As shown in Figure 6, the expression of NF-κB p65 in nuclear extracts was markedly upregulated after CLP operation compared to that in sham group. Dexmedetomidine at the medium and large doses significantly attenuated activation of NF-κB p65 in septic rat lung. However, small dose of dexmedetomidine did not obviously inhibit the activation of NF-κB p65 induced by CLP operation.

*3.6. Immunohistochemistry for NF-κB Activity.* The nuclear positive staining represents the activated form of NF-kappa B. In CLP group, about 54% of alveolar epithelial cells expressed nuclear positive staining, as compared to about 18.5% in sham group ($P < 0.01$). The percentage of cells showing nuclear positive staining significantly decreased after treatment with dexmedetomidine at medium and high doses compared to CLP group (23.1 ± 5.8%, $P < 0.01$; 20.9 ± 5.3%, $P < 0.01$; versus 54.9 ± 12.8%, Figures 7 and 8).

## 4. Discussion

In the present study, we investigated the effect of dexmedetomidine on mortality and inflammatory responses in lung tissues of septic rats induced by CLP. It was found

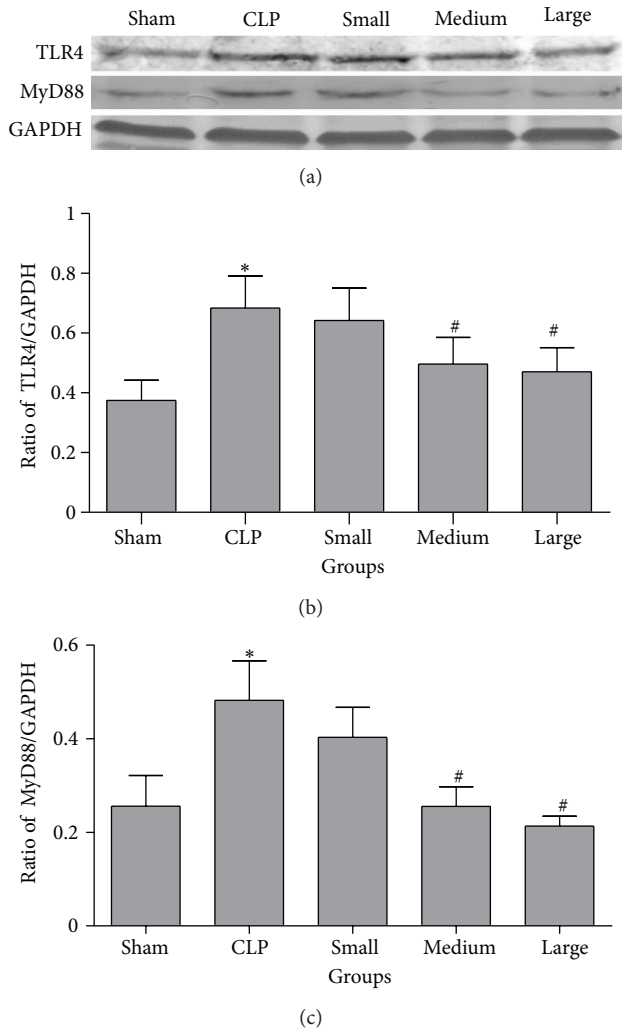



Figure 5: Effect of dexmedetomidine on TLR4 and MyD88 expression in lung tissues of septic rats by western blotting. Sham: sham operation group; CLP: cecal ligation and puncture operation group; Small: 5 $\mu$g/kg dexmedetomidine treatment group; Medium: 10 $\mu$g/kg dexmedetomidine treatment group; Large: 20 $\mu$g/kg dexmedetomidine treatment group. GAPDH: glyceraldehyde phosphate dehydrogenase. Data are expressed as mean ± SD, $n = 8$. $^{*}P < 0.01$, versus sham group; $^{\#}P < 0.01$, versus CLP group.

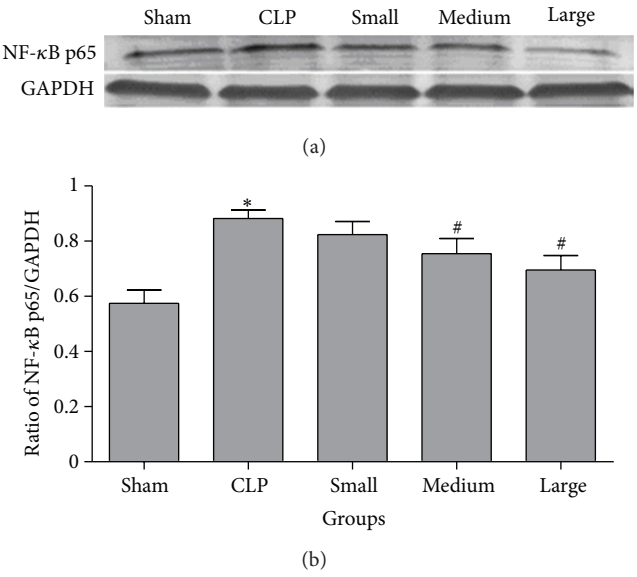



Figure 6: Effect of dexmedetomidine on NF-$\kappa$B p-65 activity in lung nuclear extracts of septic rats by western blotting. Sham: sham operation group; CLP: cecal ligation and puncture operation group; Small: 5 $\mu$g/kg dexmedetomidine treatment group; Medium: 10 $\mu$g/kg dexmedetomidine treatment group; Large: 20 $\mu$g/kg dexmedetomidine treatment group. GAPDH: glyceraldehyde phosphate dehydrogenase. Data are expressed as mean ± SD, $n = 8$. $^{*}P < 0.01$, versus sham group; $^{\#}P < 0.01$, versus CLP group.

that dexmedetomidine at the doses of 10 and 20 $\mu$g/kg decreased CLP-induced pulmonary inflammation and mortality, reduced production of IL-6 and TNF-$\alpha$ in plasma and BALF of septic rats, and suppressed TLR4/MyD88 expression and NF-$\kappa$B activation in lung of endotoxemia rats induced by CLP. Our results suggest that dexmedetomidine may modulate some of the inflammatory responses in lung of septic rats in vivo by suppressing TLR4/MyD88/NF-$\kappa$B pathway, which may contribute to decrease the mortality of septic rats induced by CLP.

Acute lung injury characterized by overinflammatory reaction is a serious consequence of sepsis [5]. Pulmonary inflammatory response, including upregulation of various proinflammatory factors and infiltration of many inflammatory cells, is crucial in endotoxemia and is also one of the important reasons leading to high mortality. Therefore, attenuating excessive pulmonary inflammation during endotoxemia is beneficial to decrease the mortality rate of septic patients. Previous study has demonstrated that dexmedetomidine reduced mortality rate and had an inhibitory effect on inflammatory response during endotoxemia induced by intravenous injection of Escherichia coli endotoxin [7]. Our results that dexmedetomidine markedly decreased CLP-induced mortality, reduced production of IL-6 and TNF-$\alpha$ in plasma, and attenuated inflammatory histopathological changes in lung tissues of septic rats induced by CLP were in concert with the previous report [7]. In addition, several studies [13–15] have demonstrated that dexmedetomidine could exert a potential protective effect by suppressing inflammatory responses on ventilator, lipopolysaccharide, or $\alpha$-naphthylthiourea-induced acute lung injury. Although the present and previous studies have shown the regulatory effects of dexmedetomidine on inflammatory reactions in acute lung injury, the exact mechanisms responsible for these actions are not well understood.

Toll-like receptor 4 (TLR4) is a transmembrane receptor protein with extracellular leucine-rich repeated domains and a cytoplasmic signaling domain. TLR4 is involved in immune responses, especially in the activation of innate immunity against foreign pathogens and microorganisms, but it also triggers adaptative immunity [16–18]. TLR4 is a CD-14 associated transmembrane signal transducer, which

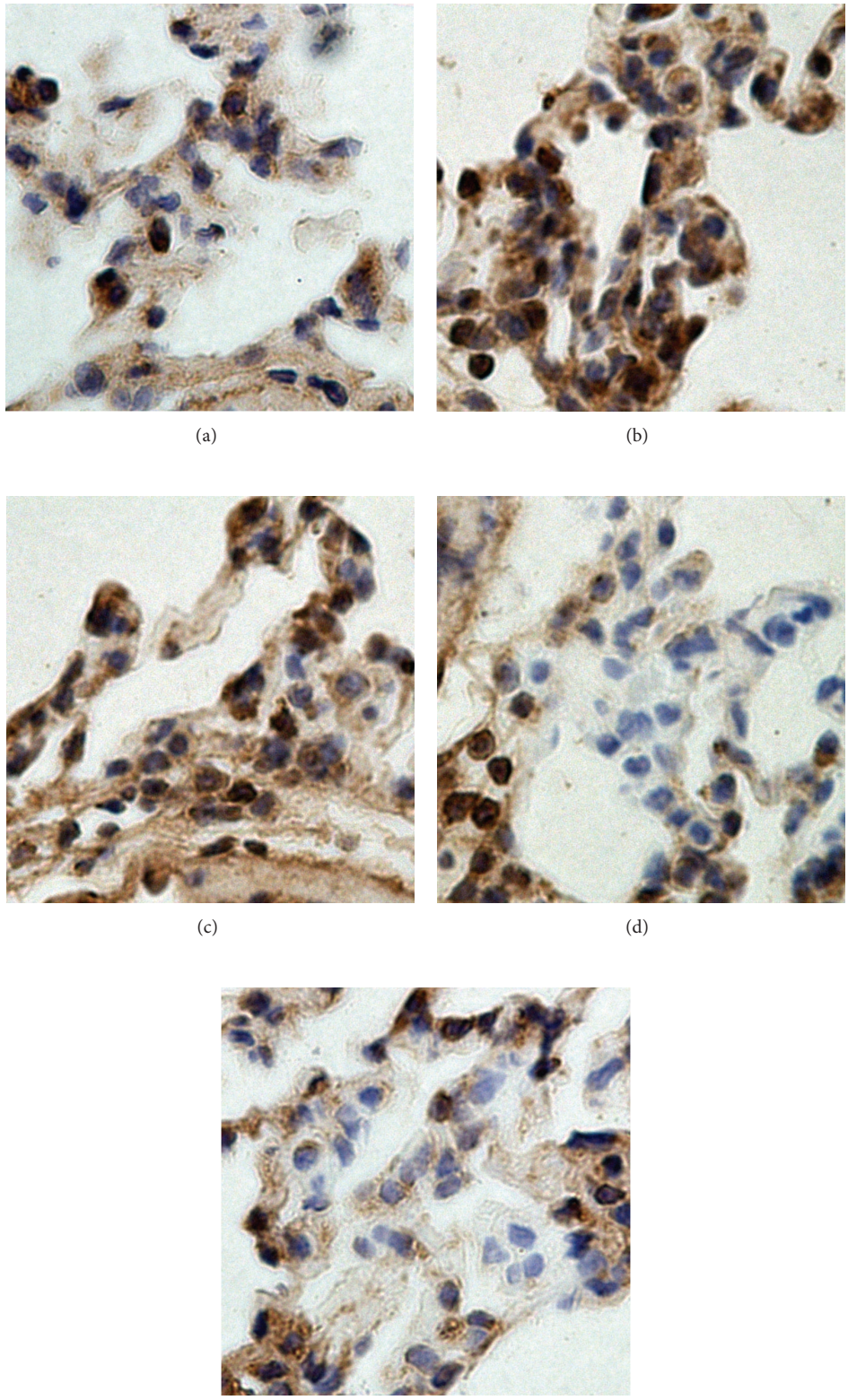



Figure 7: Effect of dexmedetomidine on NF-$\kappa$B p-65 activation in lung tissues of septic rats by immunohistochemistry staining. (a) Sham operation group; (b) cecal ligation and puncture operation group; (c) 5 $\mu$g/kg dexmedetomidine treatment group; (d) 10 $\mu$g/kg dexmedetomidine treatment group; (e) 20 $\mu$g/kg dexmedetomidine treatment group (magnification 400x).

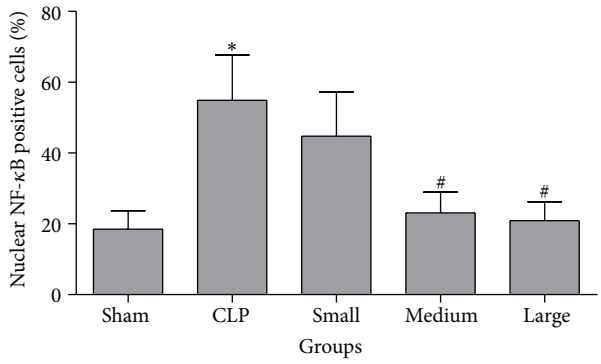



Figure 8: Effect of dexmedetomidine on NF-κB p-65 activation in lung tissues of septic rats by immunohistochemistry staining. Sham: sham operation group; CLP: cecal ligation and puncture operation group; Small: 5 μg/kg dexmedetomidine treatment group; Medium: 10 μg/kg dexmedetomidine treatment group; Large: 20 μg/kg dexmedetomidine treatment group. The degree of NF-κB activation was indicated as percentage of nuclear NF-κB p-65 positive cells to total alveolar epithelial cells. Data are expressed as mean ± SD, $n = 8$. $^{*}P < 0.01$, versus sham group; $^{\#}P < 0.01$, versus CLP group.

is necessary for the LPS-induced cellular response [19–21]. Recent studies have suggested that the association of TLR4 with myeloid differentiation factor 88 (MyD88) may induce the activation of IL-1R associated kinase and TNF receptor-associated factor [22], which triggers inflammatory cascade reactions.

Nuclear factor kappa B (NF-κB) is an important nuclear transcription factor. NF-κB heterodimer consists of p50 and p65 (Rel A) subunits. It plays a pivotal role in immune and inflammatory responses through the regulation of the expression of several proteins, including proinflammatory cytokines, chemokines, and adhesion molecules. Uncontrolled activation of the NF-κB pathway is involved in the pathogenesis of many acute and chronic inflammatory diseases. In its inactive state, the NF-kappaB dimer is present in the cytosol, where it is bound to an inhibitory protein, I-kappaB. Activation of NF-kappaB by several stimuli induces the release and degradation of the inhibitory protein I-kappaB from the dimeric complex [23], followed by phosphorylation of NF-kappaB p65 and translocation to the nucleus [24, 25].

TLR4-mediated signaling pathways mainly stimulate the activation of NF-κB. In the nucleus, NF-kappaB is bound to corresponding sites to regulate transcription of many proinflammatory genes such as IL-6 and TNF-α. In the present study, polymicrobial sepsis animal model was established by CLP. The results showed that both TLR4/MyD88 expression and NF-κB activation were significantly upregulated in lung tissues of septic rats. Here, we also showed that dexmedetomidine (10 and 20 μg/kg) treatment could reduce CLP-induced enhanced TLR4/MyD88 expression and NF-κB activation in rat lung. Since TLR4 is an essential upstream sensor for LPS from pathogens and microorganisms and may mediate the NF-κB activation through MyD88 dependent pathway, and NF-κB activation can increase the production of IL-6 and TNF-α, it is possible that dexmedetomidine reduces the production of these cytokines and attenuates pulmonary inflammation by suppressing TLR4/MyD88/NF-κB signaling pathway in lung of polymicrobial septic rats induced by CLP. Our results suggested that suppression of TLR4/MyD88/NF-κB signaling may be the probable mechanism through which dexmedetomidine attenuated inflammatory responses in lung of septic rats induced by CLP. In addition, small dose of dexmedetomidine did not affect the expression of TLR4/MyD88 and the activation of NF-κB in lung tissues of septic rats. These results were consistent with the effect that small dose of dexmedetomidine failed to inhibit pulmonary inflammation and decrease mortality rate of septic rats induced by CLP.

In conclusion, the present findings indicated that dexmedetomidine inhibited pulmonary inflammation and reduced the production of proinflammatory cytokines IL-6 and TNF-α by attenuation of TLR4/MyD88/NF-κB pathway activation in septic rats induced by CLP. These properties of dexmedetomidine may contribute to decrease the mortality rate of septic rats.

## Conflict of Interests

The authors declare that there is no conflict of interests that would prejudice their impartiality.

## Authors' Contribution

Y. Wu and Y. Liu contributed equally to this work.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (81171013, 81070889, and 81001431), Key Subject of Colleges and Universities, Natural Science Foundation of Jiangsu Province (10KJA320052), and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

## References

[1] G. Wakabayashi, J. A. Gelfand, W. K. Jung, R. J. Connolly, J. F. Burke, and C. A. Dinarello, "Staphylococcus epidermidis induces complement activation, *tumor necrosis factor* and *interleukin*-1, a shock-like state and tissue injury in rabbits without endotoxemia: comparison to Escherichia coli," *The Journal of Clinical Investigation*, vol. 87, no. 6, pp. 1925–1935, 1991.

[2] L. C. Casey, R. A. Balk, and R. C. Bone, "Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome," *Annals of Internal Medicine*, vol. 119, no. 8, pp. 771–778, 1993.

[3] C. Marty, B. Misset, F. Tamion, C. Fitting, J. Carlet, and J. M. Cavaillon, "Circulating *interleukin*-8 concentrations in patients with multiple organ failure of septic and nonseptic origin," *Critical Care Medicine*, vol. 22, no. 4, pp. 673–679, 1994.

[4] P. Damas, A. Reuter, P. Gysen, J. Demonty, M. Lamy, and P. Franchimont, "*Tumor necrosis factor* and *interleukin*-1 serum levels during severe sepsis in humans," *Critical Care Medicine*, vol. 17, no. 10, pp. 975–978, 1989.

[5] P. Delong, J. A. Murray, and C. K. Cook, "Mechanical ventilation in the management of acute respiratory distress syndrome," *Seminars in Dialysis*, vol. 19, no. 6, pp. 517–524, 2006.

[6] D. B. Coursin, D. B. Coursin, and G. A. Maccioli, "Dexmedetomidine," *Current Opinion in Critical Care*, vol. 7, no. 4, pp. 221–226, 2001.

[7] T. Taniguchi, Y. Kidani, H. Kanakura, Y. Takemoto, and K. Yamamoto, "Effects of dexmedetomidine on mortality rate and inflammatory responses to endotoxin-induced shock in rats," *Critical Care Medicine*, vol. 32, no. 6, pp. 1322–1326, 2004.

[8] L. O'Neill, "The Toll/*interleukin*-1 receptor domain: a molecular switch for inflammation and host defence," *Biochemical Society Transactions*, vol. 28, no. 5, pp. 557–563, 2000.

[9] S. Akira and K. Takeda, "Toll-like receptor signalling," *Nature Reviews Immunology*, vol. 4, no. 7, pp. 499–511, 2004.

[10] P. A. Baeuerle and T. Henkel, "Function and activation of NF-$\kappa$B in the immune system," *Annual Review of Immunology*, vol. 12, pp. 141–179, 1994.

[11] D. Rittirsch, M. S. Huber-Lang, M. A. Flierl, and P. A. Ward, "Immunodesign of experimental sepsis by cecal ligation and puncture," *Nature Protocols*, vol. 4, no. 1, pp. 31–36, 2009.

[12] C. H. Yang, P. S. Tsai, T. Y. Wang, and C. J. Huang, "Dexmedetomidine-ketamine combination mitigates acute lung injury in haemorrhagic shock rats," *Resuscitation*, vol. 80, no. 10, pp. 1204–1210, 2009.

[13] C. L. Yang, C. H. Chen, P. S. Tsai, T. Y. Wang, and C. J. Huang, "Protective effects of dexmedetomidine-ketamine combination against ventilator-induced lung injury in endotoxemia rats," *Journal of Surgical Research*, vol. 167, no. 2, pp. e273–e281, 2011.

[14] Q.-Q. Shi, H. Wang, and H. Fang, "Dose-response and mechanism of protective functions of selective alpha-2 agonist dexmedetomidine on acute lung injury in rats," *Saudi Medical Journal*, vol. 33, no. 4, pp. 375–381, 2012.

[15] V. Hanci, G. Yurdakan, S. Yurtlu, I. Ö. Turan, and E. Y. Sipahi, "Protective effect of dexmedetomidine in a rat model of $\alpha$-naphthylthiourea-induced acute lung injury," *Journal of Surgical Research*, vol. 178, no. 1, pp. 424–430, 2012.

[16] I. Bettoni, F. Comelli, C. Rossini et al., "Glial TLR4 receptor as new target to treat neuropathic pain: efficacy of a new receptor antagonist in a model of peripheral nerve injury in mice," *GLIA*, vol. 56, no. 12, pp. 1312–1319, 2008.

[17] O. Saito, C. I. Svensson, M. W. Buczynski et al., "Spinal glial TLR4-mediated nociception and production of prostaglandin $E_2$ and TNF," *British Journal of Pharmacology*, vol. 160, no. 7, pp. 1754–1764, 2010.

[18] G. J. Wu, T. L. Chen, Y. F. Ueng, and R. M. Chen, "Ketamine inhibits *tumor necrosis factor*-$\alpha$ and *interleukin*-6 gene expressions in lipopolysaccharide-stimulated macrophages through suppression of toll-like receptor 4-mediated c-Jun N-terminal kinase phosphorylation and activator protein-1 activation," *Toxicology and Applied Pharmacology*, vol. 228, no. 1, pp. 105–113, 2008.

[19] K. Hoshino, O. Takeuchi, T. Kawai et al., "Cutting edge: toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide evidence for TLR4 as the Lps gene product," *The Journal of Immunology*, vol. 162, no. 7, pp. 3749–3752, 1999.

[20] J. Da Silva Correia, K. Soldau, U. Christen, P. S. Tobias, and R. J. Ulevitch, "Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. Transfer from CD14 to TLR4 and MD-2," *The Journal of Biological Chemistry*, vol. 276, no. 24, pp. 21129–21135, 2001.

[21] E. Latz, A. Visintin, E. Lien et al., "Lipopolysaccharide rapidly traffics to and from the golgi apparatus with the toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction," *The Journal of Biological Chemistry*, vol. 277, no. 49, pp. 47834–47843, 2002.

[22] Y. Feng and G. D. Longmore, "The LIM protein Ajuba influences *interleukin*-1-induced NF-$\kappa$B activation by affecting the assembly and activity of the protein kinase C$\zeta$/p62/TRAF6 signaling complex," *Molecular and Cellular Biology*, vol. 25, no. 10, pp. 4010–4022, 2005.

[23] P. A. Baeuerle and D. Baltimore, "I$\kappa$B: a specific inhibitor of the NF-$\kappa$B transcription factor," *Science*, vol. 242, no. 4878, pp. 540–546, 1988.

[24] M. Grilli, J. J. S. Chiu, and M. J. Lenardo, "NF-$\kappa$B and Rel: participants in a multiform transcriptional regulatory system," *International Review of Cytology*, vol. 143, pp. 1–62, 1993.

[25] J. M. Muller, H. W. L. Ziegler-Heitbrock, and P. A. Baeuerle, "Nuclear factor kappa B, a mediator of lipopolysaccharide effects," *Immunobiology*, vol. 187, no. 3–5, pp. 233–256, 1993.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 793505, 12 pages
http://dx.doi.org/10.1155/2013/793505

*Review Article*

# The Involvement of Phospholipases $A_2$ in Asthma and Chronic Obstructive Pulmonary Disease

**Ewa Pniewska and Rafal Pawliczak**

*Department of Immunopathology, Faculty of Biomedical Sciences and Postgraduate Training, Medical University of Lodz, 7/9 Zeligowskiego Street, Building 2, Room 122, 90-752 Lodz, Poland*

Correspondence should be addressed to Rafal Pawliczak; rafal.pawliczak@umed.lodz.pl

Received 25 May 2012; Revised 2 January 2013; Accepted 27 February 2013

Academic Editor: Celeste C. Finnerty



The increased morbidity, mortality, and ineffective treatment associated with the pathogenesis of chronic inflammatory diseases such as asthma and chronic obstructive pulmonary disease (COPD) have generated much research interest. The key role is played by phospholipases from the $A_2$ superfamily: enzymes which are involved in inflammation through participation in pro- and anti-inflammatory mediators production and have an impact on many immunocompetent cells. The 30 members of the $A_2$ superfamily are divided into 7 groups. Their role in asthma and COPD has been studied *in vitro* and *in vivo* (animal models, cell cultures, and patients). This paper contains complete and updated information about the involvement of particular enzymes in the etiology and course of asthma and COPD.

## 1. Introduction

Both asthma and COPD are airway diseases characterized by impaired airflow in the respiratory tract, chronic airway inflammation, as well as symptoms such as coughing, dyspnea, and wheezing. Intensive studies focused on the pathogenesis of these conditions implicate, among others, the group of phospholipases $A_2$, which possess enzymatic and nonenzymatic properties. This paper presents general information about phospholipases and details the current knowledge about particular phospholipases $A_2$ involved in asthma and COPD in human and animal models. The data regarding interactions between members of this superfamily is summarized, as well as the role of these enzymes in exacerbations of inflammatory diseases.

## 2. Phospholipases

Phospholipases are enzymes that hydrolyze phospholipids. The main substrates for these enzymes are glycerophospholipids which contain glycerol with a saturated fatty acid in the *sn-1* position and an unsaturated fatty acid in the *sn-2* position. The phospholipases responsible for hydrolysis of glycerophospholipids are divided into two groups: acylhydrolases and phosphodiesterases. The first group comprises phospholipase $A_1$ ($PLA_1$) and $A_2$ ($PLA_2$), which hydrolyze the ester bond at the *sn-1* and *sn-2* positions, respectively. The second group comprises phospholipase C (PLC) which cleaves the glycerol-phosphate bond, and phospholipase D (PLD), which liberates phosphatidic acid and alcohol (Figure 1). Phospholipase B shares both the properties of $PLA_1$ and $PLA_2$.

The structure, function, and catalytic mechanism of the enzyme determine its place within the phospholipase $A_2$ superfamily, be it secretory $PLA_2$ ($sPLA_2$), cytosolic $PLA_2$ ($cPLA_2$), $Ca^{2+}$-independent phospholipase $A_2$ ($iPLA_2$), PAF acetylhydrolases (PAF-AH), or lysosomal $PLA_2$ ($LPLA_2$). The latest classification, based on genetic structure, divides these enzymes into groups from I to XVI (in each one, the enzyme is represented by a capital letter) [1]. The characteristic features of each group are presented in Table 1. Table 2 includes information about the mechanism of action and function of particular subgroups of $PLA_2$s concerning physiology and pathophysiology.

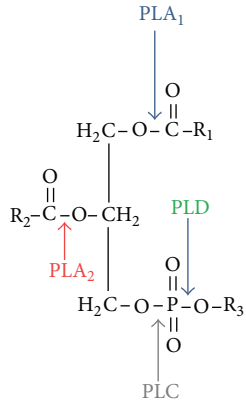



Figure 1: Phospholipases and their role in lipids metabolism.

## 3. Asthma and COPD

Currently about 300 million people worldwide suffer from asthma, and in 2025, this number is expected to grow by another 100 million. Annually, about 250 000 people die from asthma [2]. Asthma is defined according to the GINA (Global Initiative for Asthma) [3] as a chronic airway inflammatory disease in which many cells and cellular elements are involved. Chronic inflammation is a cause of bronchial hyperresponsiveness, leading to recurrent episodes of wheezing, dyspnea, chest tightness, and coughing, occurring particularly at night or dawn. This is usually accompanied by episodes of diffuse bronchial obstruction of varying severity, which often subside spontaneously or with treatment.

According to GOLD (The Global Initiative for Chronic Obstructive Lung Diseases) [6], COPD is characterized by a progressive and poorly reversible airflow limitation caused by both small airway diseases (airway inflammation and destruction) and parenchymal destruction (loss of alveolar attachment and decrease of elastic recall). Also, other extrapulmonary effects, such as weight loss, nutritional abnormalities, skeletal muscle dysfunction influence the severity of the disease. Apart from the genetic background (hereditary alpha-1 antitrypsin deficiency) [7] cigarette smoke is a crucial environmental factor in COPD development [8]; it is responsible for airway inflammation and further oxidant/antioxidant imbalance (oxidative stress) causing amplification of lung inflammation.

## 4. Analysis of Phospholipases $A_2$ Involvement in Asthma and COPD

An analysis of studies concerning the profile of $PLA_2$s expression in many experimental systems has revealed ambiguous results. Many different inductors used for cells stimulation cause expression of various types of enzymes in the same cells. Also, the presence of heterogeneous cells in experimental systems influences the expression of $PLA_2$s [9].

Mast cells, $Th_2$ lymphocytes, and eosinophils are the most important cellular components of asthma. It has been established that primary human lung mast cells constitutively express mRNA for the IB, IIA, IID, IIE, IIF, III, V, X, XIIA, and XIIB $sPLA_2$ groups and stimulation with anti-IgE antibodies can induce their secretion [10]. Hence $sPLA_2$ proteins are believed to belong to preformed mediators which are stored in mast cells granules. Cells stimulation by anti-IgE antibodies causes degranulation of mast cells, and $sPLA_2$ appears in the early phase of allergic reaction. Muñoz et al. have shown that $sPLA_2V$ is not expressed in eosinophils in detectable amounts. However exogenous $hPLA_2V$ can activate eosinophils, inducing the liberation of arachidonic acid (AA) and $LTC_4$ production [11]. Increased $cPLA_2\alpha$ phosphorylation and $cPLA_2\alpha$ activity was observed in eosinophils of asthmatics after allergen challenge [12].

Alveolar macrophages and neutrophils play a crucial role in the pathophysiology of COPD [13, 14]. Human macrophages express $cPLA_2$IVA, $iPLA_2$VIA, and several $sPLA_2$s (IIA, IID, IIE, IIF, V, X, and XIIA, but not group IB and III enzymes). Higher expression of $sPLA_2$IIA is observed after LPS treatment [15]. Neutrophils stimulated *in vitro* by the tripeptide formyl-Met-Leu-Phe (fMLP) demonstrate mRNA and protein expression of $sPLA_2V$ and $sPLA_2X$, where the $sPLA_2V$ protein is found in azurophilic and specific granules, and $sPLA_2X$ is found only in azurophilic granules. GIB, GIIA, GIID, GIIE, GIIF, GIII, and GXII $sPLA_2$s are undetectable. Cell activation by fMLP or zymosan results in the release of GV but not GX $sPLA_2$ [16].

The BALF of patients with COPD demonstrates a three- to fivefold higher activity of $PLA_2$s in comparison to a control BALF but the protein level shows no difference [17]. No differences in $sPLA_2$IIs serum levels exist between healthy smokers and nonsmokers. However, significantly greater levels of this enzyme are found in the BALF of smokers compared with nonsmokers [18]. Among $sPLA_2$s, $sPLA_2$IID is also considered as a molecule involved in the course of COPD. A change of Gly80Ser in the $sPLA_2$IID protein may be associated with body weight loss in patients suffering from COPD [19]. $sPLA_2$IID can be also involved in control of inflammation by inhibition of CD4+, CD8+ T cells proliferation and induction of regulatory T cell differentiation [20]. Cigarette smoke extract (CSE) can induce the production of cytosolic phospholipase $A_2$ in human pulmonary microvascular endothelial cells [21]. Moreover oxidative stress can increase the activity of $cPLA_2$ by promoting its phosphorylation [22]. $cPLA_2$ also participates in phosphodiesterase 4 signaling, whose inhibition attenuates neutrophilic inflammation in COPD [23]. The increased values of $PLA_2$VII in patients with longstanding pulmonary hypertension (severe complication in COPD) are related to severe endothelial dysfunction [24].

$sPLA_2V$ plays a different role in the activation of eosinophils and neutrophils. Hence, its involvement in the pathogenesis of asthma and COPD can vary. Exogenous $sPLA_2V$ can activate the production of AA and leukotrienes in both cell types. However, $LTB_4$ is preferentially produced in neutrophils, and $LTC_4$ in eosinophils [11]. The $sPLA_2V$-induced activation of neutrophils in contrast to eosinophils requires the presence and activation of $cPLA_2$ [25]. The inhibition of $cPLA_2$ may be more effective in diseases where neutrophils

Table 1: Characteristics of structure and localization of human phospholipase $A_2$ enzymes. Adapted and modified from [1, 4]. The Roman numeral indicates the group, and the capital letter after the number indicates the subgroup.

| Name | Members (human) | Molecular mass (kDa) | Relationship with $Ca^{2+}$ | Catalytic site | Localization |
|---|---|---|---|---|---|
| Secretory phospholipase $A_2$ (sPLA$_2$) | IB (sPLA$_2$IB) | 13–15 | Dependent | Histidine/Aspartic acid | Secreted |
| | IIA (sPLA$_2$IIA) | 13–15 | | | Secreted; membrane; secretory granules |
| | IID (sPLA$_2$IID) | 14-15 | | | Secreted |
| | IIE (sPLA$_2$IIE) | 14-15 | | | Secreted |
| | IIF (sPLA$_2$IIF) | 16-17 | | | Secreted |
| | III (sPLA$_2$III) | 55 | | | Secreted |
| | V (sPLA$_2$V) | 14 | | | Secreted; Golgi apparatus; nuclear envelope; plasma membrane |
| | X (sPLA$_2$X) | 14 | | | Secreted |
| | XIIA (sPLA$_2$XIIA) | 19 | | | Secreted; cytoplasm |
| | XIIB (sPLA$_2$XIIB, XIII) | 20 | | | Secreted |
| Cytosolic phospholipase $A_2$ (cPLA$_2$) | IVA (cPLA$_2\alpha$) | 85 | Dependent | Serine/Aspartic acid/Arginine | Nucleus; cytoplasmic vesicles |
| | IVB (cPLA$_2\beta$)-three splice variants | 114 | | | Cytosol |
| | IVC(cPLA$_2\gamma$) | 61 | Independent | | ER; Mitochondrium |
| | IVD (cPLA$_2\delta$) | 92-93 | | | Cytosol; Cytoplasmic vesicle membrane; peripheral membrane protein; cytoplasmic side |
| | IVE (cPLA$_2\varepsilon$) | 96 | Dependent | | Cytosol; lysosome membrane; peripheral membrane protein |
| | IVF (cPLA$_2\zeta$) | 95 | | | Cytosol; lysosome membrane; peripheral membrane protein; cytoplasmic side |
| $Ca^{2+}$-independent phospholipase $A_2$ (iPLA$_2$) | VIA-(iPLA$_2\beta$)-five splice variants | 84–90 | Independent | Serine | Cytosol |
| | VIB (iPLA$_2\gamma$)-four splice variants | 88–91 | | | ER; peroxisomal and mitochondrial membrane |
| | VIC (iPLA$_2\delta$, NTE) | 146 | | | ER; single-pass type I membrane protein; cytoplasmic side |
| | VID (iPLA$_2\varepsilon$, adiponutrin) | 53 | | | Membrane; single-pass type II membrane protein |
| | VIE (iPLA$_2\zeta$) | 57 | | | Lipid droplet membrane; single-pass type II membrane protein; cell membrane |
| | VIF (iPLA$_2\eta$) | 28 | | | Cytoplasm |
| Acidic $Ca^{2+}$-independent phospholipase $A_2$ | aiPLA$_2$ | 26 | Independent | Serine | Cytoplasm; Lysosome |
| Lysosomal phospholipase $A_2$ | XV (LPLA$_2$, LLPL, ACS) | 45 | Independent | Serine/Histidine/Aspartic acid | Secreted; Lysosome |

Table 1: Continued.

| Name | Members (human) | Molecular mass (kDa) | Relationship with $Ca^{2+}$ | Catalytic site | Localization |
|---|---|---|---|---|---|
| PAF acetylhydrolase (PAF-AH) or Lipoprotein-associated phospholipase $A_2$ | VIIA (Lp-$PLA_2$, Plasma PAF-AH) | 45 | Independent | Serine/Histidine/Aspartic acid | Secreted |
| | VIIB (PAF-AH II) | 40 | | | Cytoplasm |
| | VIIIA (PAF-AH Ib) $\alpha$1 subunit | 26 | | | Cytoplasm |
| | VIIIB (PAF-AH Ib) $\alpha$2 subunit | 26 | | | Cytoplasm |
| Adipose-specific phospholipase $A_2$ | XVI (H-Rev107) | 18 | Independent | Cystein/Histidine/Histidine | Cytoplasm, perinuclear region, Single-pass membrane protein |

ER: endoplasmic reticulum; NTE: neuropathy target esterase.

play a crucial role because they indirectly inhibit also the function of $sPLA_2$.

## 5. Role of $PLA_2$s in Asthma and COPD

The proposed mechanism of action of phospholipases $A_2$ ($PLA_2$s) in inflammatory diseases includes the liberation of arachidonic acid, generation of lysophospholipids, interaction between enzymes belonging to the $A_2$ superfamily, surfactant degradation, release of cytokines, and the impact on immunological and inflammatory cells (dendritic cells, T-cells, and leukocytes) [26].

*5.1. The Enzymatic Activity of $PLA_2$s.* The enzymatic properties of $PLA_2$s refer to their phospholipase, lysophospholipase, transacylase, adiponutrin-like, triglyceride lipase, peroxiredoxin 6, and acyl-ceramide synthase activities. Phospholipases $A_2$ play a pivotal role in eicosanoid production because they hydrolyze the ester bond at the *sn-2* position of the glycerophospholipid membrane, releasing arachidonic acid (AA) and lysophospholipids [27]. Arachidonic acid plays a dual role. It can act as a signaling molecule that regulates the activity of protein kinase C (PKC) and phospholipase C$\gamma$, influences $Ca^{2+}$ concentration, and acts as an endogenous ligand for PPAR$\gamma$ receptors [28, 29]. AA is also a precursor of lipid inflammatory mediators (eicosanoids). In cyclooxygenase (COX) pathways, it is transformed to prostaglandins and thromboxane while in lipoxygenase (ALOX) pathways, it is converted to leukotrienes. These molecules are responsible for bronchial constriction, increased vessel permeability, and inflammatory cell recruitment [30]. AA is also a substrate for resolvins and lipoxins (LXs) which have anti-inflammatory properties. Lipoxins can block granulocyte chemotaxis, migration, degranulation, oxidative burst, cytokine-mediated signaling in eosinophils, and secretion of cytokines from bronchial epithelial cells [31]. Several independent studies have reported that significantly lower levels of LXs are observed in severe asthmatics compared to patients with non-severe asthma [32, 33]. Resolvins demonstrate endogenous anti-inflammatory, proresolving, antifibrotic, antiangiogenic, anti-infective, and antihyperalgesic activity [31].

Among cytosolic phospholipases $A_2$, it has been well documented that $cPLA_2$IVA ($cPLA_2\alpha$) plays an important role in eicosanoid production. In patients with inherited $cPLA_2$ deficiency (loss-of-function mutations in both $cPLA_2$ alleles), a widespread decrease in eicosanoid concentrations has been observed [34]. S111P, R485H, and K651R mutations in *PLA2G4A* gene are thought to play a crucial role in this condition. The functional consequences of localized mutations concerning $cPLA_2$ catalytic activity, $Ca^{2+}$ recruitment, and affinity for the phospholipid membrane have been confirmed *in vitro* and in cell culture [35]. In patients with severe asthma, the microsatellite fragments $(T)_n$ and $(CA)_n$ in the promoter region of $cPLA_2\alpha$ gene (*PLA2G4A*) are shorter in comparison to healthy subjects [36]. In addition, asthmatic patients with shorter microsatellite sequences demonstrate greater expression of $cPLA_2\alpha$ mRNA, $cPLA_2\alpha$ protein, $PGE_2$ and 15-HETE, but not $LTC_4$ [37]. $cPLA_2$ participates in intracellular signaling, leading to allergen-induced production of inflammatory cytokines in the PBMC of asthmatics [38]. Hallstrand et al. [39] identified increased expression of three $cPLA_2$s, including $cPLA_2\alpha$, $cPLA_2\beta$, and $cPLA_2\gamma$ in induced sputum cells from subjects with asthma and exercise-induced bronchoconstriction. Both $cPLA_2\beta$ and $cPLA_2\gamma$ enzymes also participate in eicosanoids biosynthesis [40, 41]. Increased $cPLA_2$ expression and subsequent $PGE_2$ production are present in the asthma phenotype. The therapeutic decision to inhibit $cPLA_2$ in asthmatics may be unclear when considering the role of $PGE_2$ in airway inflammation. There is some evidence that $PGE_2$ can act as bronchodilator, as well as an inhibitor of both allergen-induced bronchoconstriction and inflammatory mediators production [42]. It should be noticed that $PGE_2$ acts through four different types of receptors ($EP_1$, $EP_2$, EP3, and $EP_4$). Changes in expression and combination of receptor subtypes actions may affect the action of $PGE_2$ giving it proinflammatory or bronchoprotective outcomes [43–45]. The pleiotropic properties of $PGE_2$ make it difficult to establish the direct impact of $PGE_2$ deficiency which appears as a consequence of $cPLA_2$ inhibition [46]. Moreover, although $cPLA_2$ is a major enzyme, it is not the only one providing substrates for eicosanoids synthesis; hence it cannot be excluded that other existing pathways can also perform this function.

Table 2: Mechanism of action and function of human phospholipase $A_2$ enzymes. Adapted and modified from [1, 4, 5].

| Name | Mechanism of action | Function | | Sources |
|---|---|---|---|---|
| | | Physiology | Pathophysiology | |
| Secretory phospholipases $A_2$ (sPLA$_2$s) | (i) Enzymatic (liberation of AA and lysophospholipids)<br>(ii) Autocrine and paracrine action by binding to N-type and M-type receptors or by binding to integrins | (i) Lipid remodeling for membrane homeostasis<br>(ii) Exocytosis<br>(iii) Phagocytosis<br>(iv) Anticoagulant activity<br>(v) Antibacterial activity (Gram-positive and Gram-negative bacteria)<br>(vi) Antifungal and antiadenoviral activity<br>(vii) Parturition<br>(viii) Spinal processing of nociception | (i) Inflammatory diseases (rheumatoid arthritis, adult respiratory distress syndrome, inflammatory bowel disease, and pancreatitis)<br>(ii) Sepsis<br>(iii) Atherosclerosis (foam cell formation)<br>(iv) Cancer<br>(v) Surfactant hydrolysis | Neutrophils, eosinophils, basophils, T-cells, monocytes, macrophages, platelets, mast cells, airway epithelial cells, alveolar type II epithelial cells, |
| Cytosolic phospholipases $A_2$ (cPLA$_2$s) | (i) enzymatic: lysophospholipase and transacylase activity | (i) AA releasing<br>(ii) Cellular signaling<br>(iii) Parturition<br>(iv) Nociception | (i) Inflammation<br>(ii) Intestinal ulceration<br>(iii) Psoriasis<br>(iv) Acute lung injury<br>(v) Polyposis<br>(vi) Brain injury<br>(vii) Anaphylaxis | Every tissue |
| $Ca^{2+}$-independent phospholipases $A_2$ (iPLA$_2$s) | VIA, VIB, VIC, VID, VIEVIF-phospholipase $A_2$ activity<br>VIC-lysophospholipase activity<br>VID-adiponutrin-like activity<br>VIE-triglyceride lipase activity<br>VIF-transacylase activity | (i) Remodeling of phospholipids<br>(ii) AA releasing<br>(iii) Protein expression<br>(iv) Acetylcholine-mediated endothelium-dependent relaxation of the vasculature<br>(v) Apoptosis<br>(vi) Insulin secretion<br>(vii) Bone formation<br>(viii) Sperm development<br>(ix) Cell proliferation<br>(x) Activation of $Ca^{2+}$ influx<br>(xi) Axon regeneration in nerve injury (VIA) | (i) Wallerian degeneration (VIA)<br>(ii) regulation of monocyte migration (VIB)<br>(iii) Oxidant-induced cell injury (VIC)<br>(iv) Ischemia-induced ventricular tachyarrhythmias | (i) Alveolar cells<br>(ii) Macrophages<br>(iii) Normal and cancer lung tissue<br>(iv) Neurons |
| | aiPLA$_2$-phospholipase $A_2$ and peroxiredoxin 6 activity | (i) Degradation and recycling of surfactant phospholipids (remodeling of phosphatidylcholine to dipalmitoyl-phosphatidylcholine (DPPC)<br>(ii) Antioxidative activity | (i) lung cancer, mesothelioma, sarcoidosis | (i) Alveolar macrophages<br>(ii) Type II epithelial cells<br>(iii) Clara cells |
| Lysosomal phospholipase $A_2$ | (i) Acyl-ceramide synthase<br>(ii) Transacylase activity<br>(iii) Lysophospholipase activity | (i) may be the crucial enzyme of pulmonary surfactant phospholipid degradation by alveolar macrophages | (i) Phospholipidosis<br>(ii) Complement activation<br>(iii) Induced lung injury | (i) Alveolar macrophages<br>(ii) Peripheral blood monocytes |
| PAF acetylhydrolases (PAF-AH) or Lipoprotein-associated phospholipases $A_2$ | (i) Phospholipase $A_2$ activity | (i) Anti-inflammatory properties by hydrolyzing platelet activating factor (PAF)<br>(ii) Protection against oxidative stress<br>(iii) Brain development | (i) Generation of lysophospholipids and fatty acid hydroperoxides (ROS)<br>(ii) Acute respiratory distress syndrome<br>(iii) Marker of coronary heart disease<br>(iv) Miller-Diker lissencephaly | (i) Alveolar macrophages<br>(ii) Epithelial type II cells |

Table 2: Continued.

| Name | Mechanism of action | Function | | Sources |
|---|---|---|---|---|
| | | Physiology | Pathophysiology | |
| Adipose-specific phospholipase $A_2$ | (i) Phospholipase $A_1$ and $A_2$ activity | (i) catalyzes the release of fatty acids from phospholipids in adipose tissue | (i) Obesity<br>(ii) Metabolic syndrome | Adipose tissue |

$sPLA_2$s and arachidonic acid accumulate in the BALF of asthmatics after allergen challenge [47, 48]. Despite being specific to the *sn-2* bond, $sPLA_2$s play more of a supporting role in AA liberation. Only $sPLA_2V$ and $sPLA_2X$ can efficiently interact and hydrolyze phospholipids from the outer surface of the cell membrane [9]. In acute and chronic animal asthma models, a deficit of $sPLA_2X$ diminishes the features of asthma (eosinophilia, airway hyperresponsiveness to methacholine, airway remodeling, eicosanoids, and Th2 cytokine production) [49].

Hallstrand et al. [50] showed that the expression of $sPLA_2X$ predominates in the airway epithelium, and both $sPLA_2X$ and $sPLA_2$IIA are the main phospholipases produced by BALF cells. The activity of the $sPLA_2V$ protein was found to be greatly lowered and undetectable. They have suggested that $sPLA_2X$ is most important among secretory phospholipases. Only $sPLA_2X$, not $sPLA_2$IIA, is correlated with asthma features such as lung function, recruitment of neutrophils in asthmatics [50]. $sPLA_2X$ is responsible for production of cysteinyl leukotrienes (cysLTs) which are proinflammatory in asthma and can be responsible for observable features of asthma. Moreover, the level of prostaglandin $E_2$ ($PGE_2$) is also connected with $sPLA_2X$, which can be explained by the fact that $sPLA_2X$ increases activity of $cPLA_2$IV which in turn leads to production of $PGE_2$. These results are consistent with earlier studies by the same authors in which gene expression of $sPLA_2X$ and $sPLA_2$ XII was demonstrated to be elevated in induced sputum cells of patients with asthma. The level of $sPLA_2X$ in induced sputum cells supernatant increased after exercise challenge among asthmatics with exercise-induced bronchoconstriction (EIB) [39]. Lai et al. [51] have confirmed the involvement of $sPLA_2X$. They demonstrated that recombinant $sPLA_2X$ caused AA release and rapid onset of cysLT synthesis in human eosinophils.

Limited information suggests a possible anti-inflammatory role of $sPLA_2X$. However in asthma, $sPLA_2X$ facilitates the polarization toward proasthmatic M2-macrophage phenotype [52]. It is possible that in a proinflammatory environment, that the $sPLA_2X$ propeptide is more rapidly converted to an active form that might influence the Th1/Th2 balance [53]. All these factors may suppress its anti-inflammatory action.

Other $sPLA_2$s (IIA, IID, IIE) contain a heparin-binding domain which allows these enzymes to be taken into the cells and further directed to compartments enriched in AA and enzymes responsible for eicosanoid production [54].

In spite of the fact that several studies have confirmed the participation of $iPLA_2\beta$ [55] and $iPLA_2\gamma$ [56] in AA release and eicosanoid production, there is no data indicating that these enzymes play a direct role in asthma. By the induction of $Ca^{2+}$ influx they can influence the translocation and activity of $Ca^{2+}$-dependent $PLA_2$s isoforms.

Group VII and VIII PAF-AH hydrolyze the short *sn-2* residue of PAF (platelet activating factor). As they lack activity against membrane phospholipids with long-chain *sn-2* residues, they are unable to release arachidonic acid from membrane phospholipids [57]. They exhibit pro- and anti-inflammatory properties. On the one hand, they inactivate PAF—the proinflammatory mediator—by hydrolyzing it to inactive acetate and lysolipid but on the other hand, they assist in the generation of lysophospholipids and fatty acid hydroperoxides [4]. Stafforini et al. [58] have established that asthmatics have a decreased level of PAF-AH, and that asthma incidence and severity correlate to PAF-AH deficiency in the Japanese population. Also some *PAF-AH* gene polymorphisms (Ile198Thr and Ala379Val variants) are known to be a risk factors for developing atopy and asthma [59]. Despite positive effects in animal models [60], administration of human recombinant PAF-AH (rPAF-AH) does not reduce both early and late phase of asthmatic response in mild asthmatics challenged with allergens [61].

The enzymatic activity of $PLA_2$s embraces also lysophospholipid generation. Lysophospholipids are biologically active molecules acting through specific receptors. They are a precursor of platelet activating factor (PAF) and lysophosphatidic acid (LPA). LPA is involved in cell adhesion, motility, and survival. In animal models, lysophospholipid receptors are required for proper development and function of the cardiovascular, immune, respiratory, and reproductive systems [62]. Lysophosphocholine and polyunsaturated fatty acids, including AA, can activate $cPLA_2$ and 5-lipoxygenase by increasing $Ca^{2+}$ and inducing $cPLA_2$ phosphorylation, which then leads to $LTB_4$ biosynthesis [25]. Lysophospholipid has nonspecific cytotoxic effect that depends on its concentration (critical micelle concentration). At concentration below their unspecific cytotoxic effect lysophospholipids can induce apoptosis by interrupting the synthesis of phosphatidylcholine [63].

Phospholipases $A_2$ activity is also connected with disturbed lipid homeostasis in the lung. Asthma and other inflammatory lung diseases are characterized by impaired surfactant function [64]. Secretory phospholipases degrade phosphatidylcholine (PC), the main component of the surfactant responsible for maintenance of small airway patency. The generation of lysophospholipids and free fatty acids by $sPLA_2$-mediated PC hydrolysis has been implicated in small airway closure in asthma. $sPLA_2$ action is enhanced by

eosinophilic lysophospholipases that use lysophospholipids as a substrate [65–68]. The presence of iPLA$_2$ proteins in alveolar macrophages suggests that they might play a role in surfactant degradation [69].

It should be mentioned that some PLA$_2$s are involved in antibacterial defense thanks to their ability to hydrolyze the lipids of the bacterial membrane. sPLA$_2$s IIA, V, X, and IB demonstrate bactericidal activity against gram-positive pathogens but the most effective is sPLA$_2$IIA. Group XII can directly kill *E.coli*, unlike the other sPLA$_2$s that require cofactors [70]. This property of phospholipases can be important in bacterial exacerbations of asthma and COPD.

*5.2. Nonenzymatic Activity of PLA$_2$s.* The secretory forms of many PLA$_2$s exert a range of actions in airway inflammation. Apart from their enzymatic activity, they can act as extracellular mediators involved in chemotaxis, cytokine production, and induction of cellular signaling pathways.

Mammalian N-type receptors have been identified for sPLA$_2$IB and IIA, X and M-type receptors for sPLA$_2$IB, IIA, IIE, IIF, V, and X [71]. N-type like receptors are present in lungs whereas M-type receptors have been identified in lung and myeloid cells [72]. The binding of sPLA$_2$s to their M-type receptor deactivates their enzymatic properties [73].

sPLA$_2$s are stored in intrinsic mast cell granulates and are released after cell activation by IgE and non-IgE stimuli [9]. After exocytosis, they can act in both autocrine and paracrine manners. By interacting with heparan sulphate proteoglycans and M-type receptors, they can induce PGD$_2$ and LTC$_4$ production and stimulate the subsequent degranulation of mast cells [74]. Granata et al. [17] delivered an evidence that sPLA$_2$s can act as proinflammatory connections between mast cells and macrophages in the airway. They suggest that the activation of macrophages by sPLA$_2$s leads to production of proinflammatory cytokines which sustain the inflammatory and immune response, chemokines responsible for recruitment of monocytes and neutrophils, as well as destructive lysosomal enzymes, NO, PGE$_2$, and metalloproteinases connected with airway remodeling [17]. The sPLA$_2$s induce $\beta$-glucuronidase release and production of IL-6 from human lung macrophages [75]. They influence the migration and adhesion of neutrophils as well as the release of elastase [76, 77]. In eosinophils, sPLA$_2$ IA and IIA stimulate $\beta$-glucuronidase release and cytokine production (IL-6, IL-8) by AA and lysophospholipid generation, by interaction with membrane peptidoglycans via their heparin-binding site, and through binding with specific M-type or N-type receptors [78]. The functions of sPLA$_2$s receptors require further studies because there are still some missing or unequivocal information [52].

*5.3. Crosstalk between PLA$_2$s.* The phospholipases can cooperate in mechanism leading to eicosanoid production. sPLA$_2$ and cPLA$_2$ interaction is quite well documented [79, 80]. The effect of group IIa and V PLA$_2$s on $H_2O_2$-induced AA release is dependent upon the presence of cPLA$_2$ and the activation of PKC and ERK1/2 in murine mesangial cells. Offer et al. [81] have described negative feedback between sPLA$_2$ and cPLA$_2$ in eicosanoid production. sPLA$_2$ activation induces production of bronchoconstrictor cysteinyl leukotrienes and suppresses cPLA$_2$ expression and the subsequent production of bronchodilator PGE$_2$. Recently it has been established that in human eosinophils, sPLA$_2$ initiates Ser(505) phosphorylation of cPLA$_2\alpha$ and stimulates leukotriene synthesis through involvement of p38 and JNK MAPK, cPLA$_2\alpha$, and 5-lipoxygenase activation, which may be an important process also in airways of asthmatics [51]. Also in bone-marrow-derived mast cells, sPLA$_2$ mediates the selective release of AA by binding M-type receptors and then inducing MAPK signaling pathways that lead to cPLA$_2$ activation [82].

*5.4. PLA$_2$s in the Exacerbation of Disease.* Another aspect of phospholipases and the asthma/COPD relationship is the participation of these enzymes in the pathogenetic mechanisms of disease exacerbation caused by bacterial factors. This role relates to increased expression of selective PLA$_2$s, modulation of their activity and involvement in cellular signaling. Elevated cPLA$_2\alpha$ expression was found in primary human lung macrophages after LPS treatment [15, 83]. LPS stimulates expression of cPLA$_2$ and COX-2 in macrophages, leading to increased production of AA and PGE$_2$ [83]. LPS treatment was also followed by rapid changes in cPLA$_2$ phosphorylation [84, 85]. This is one of the mechanisms of regulating enzyme activity [86]. The LPS-phosphorylated form of cPLA$_2$ is present in induction of iNOS and TNF-$\alpha$ expression [87, 88] and metalloproteinase production [89]. Selective sPLA$_2$ contributes to LPS-intracellular signaling in liver macrophages [84, 90, 91].

In mice with LPS-induced lung inflammation, the expression of sPLA$_2$X remains the same before and after treatment. In this study, increased expression of sPLA$_2$IID and sPLA$_2$V has been observed, as well as decreased sPLA$_2$IIE and sPLA2IIF levels in the lungs. In rats, sPLA$_2$IIA was seen to have the highest expression after LPS administration [92]. In msPLA$_2$X$^{-/-}$ mice with knock-in of human sPLA$_2$X (hsPLA$_2$X), allergen-induced inflammatory cell recruitment into airways (eosinophils) was restored, as well as hyperresponsiveness to methacholine. The application of specific hsPLA$_2$X inhibitor (RO 061606) significantly attenuates airway inflammation symptoms, mucous secretion, and hyperresponsiveness [93]. In sPLA$_2$V$^{-/-}$ knock-out mice, sPLA$_2$V has been proven to play a role in the development of lung injury and neutrophilic inflammation after bacterial stimulus (LPS) [94]. In addition, sPLA$_2$V was seen to be connected with regulation of cell migration and generation of airway hyperresponsiveness after ovalbumin challenge [95]. In a murine allergen-challenged asthma model, administration of rPAF-AH is effective in blocking late-phase pulmonary inflammation [60].

## 6. The Clinical Significance of Studying the Participation of PLA$_2$s in Airway Inflammatory Diseases

Taking into consideration the severe asthma phenotype, the difficulties related to obtain asthma control utilizing currently

available treatments and the progressive character of inflammation in patients with COPD that increases the morbidity, it seems reasonable to study the differences in pathogenesis of the diseases conditions, especially in relation to possible new therapies and drugs. The $PLA_2$s are an interesting object of study for several reasons. The superfamily of these enzymes contains approximately 30 members that have similar and isoform-specific properties. It has been confirmed that they are strictly connected with inflammation. The inhibitors of particular $PLA_2$s show the positive effect in treatment of inflammatory diseases [96] and they inhibit allergic reaction *in vitro* [38]. The $cPLA_2\alpha$ that evolved together with receptors for eicosanoids, present only in vertebrate, seems to play crucial role in course of inflammation. Its inhibitors such as efipladib [97] and ecopladib [98] successfully inhibit inflammation in rheumatoid arthritis and osteoporosis. The inhaled form of $cPLA_2\alpha$ inhibitor, the PLA-950, is considered as potential new treatment in asthmatic patients as well as other $PLA_2$s can influence the function of $cPLA_2\alpha$ or have similar effects. Recent studies report positive results of a preclinical evaluation of a $cPLA_2\alpha$ inhibitor [99]. The studies and analysis of protein involved in regulation of particular $sPLA_2$ involved in inflammatory diseases could result in finding new target for drugs.

Since 1980, it has been known that glucocorticoids (GCs) can inhibit the activity of $PLA_2$ [100]. The underlying mechanism concerns induction of mRNA and protein expression of lipocortin 1 (annexin 1) and the $PLA_2$ inhibitory protein [101–104]. The structure, function, and mechanism behind the anti-inflammatory action of annexin 1 have been well described elsewhere [105]. Glucocorticoids can also suppress the production of $sPLA_2$IIA by blocking mRNA synthesis and posttranslational expression in rats [106]. It is questionable whether therapeutic doses of glucocorticoids have sufficient power to satisfactorily inhibit the activity of $PLA_2$. Juergens et al. [107] demonstrated that topical GCs at therapeutically relevant concentration ($10^{-8}$ M) inhibit the spontaneous activity of $cPLA_2$ in the range of 8.6–17.3% depending on the type of GC. They suggest also that this effect may appear as a consequence of a decreased ability to binding the receptors by GCs present in airway in subtherapeutical doses. Although it has been established that treatment with GCs can indirectly inhibit $cPLA_2$ and AA-derivates production resistance to GCs in patients with asthma and COPD could also be problematic. Moreover the GCs have systemic effects and long-term application can cause the side effects. The approach to attack the inflammation process more precisely and downstream (inhibition the eicosanoids production) seems to be rationale.

Another aspect regarding annexin 1 and $PLA_2$s is their cell-specific manner of interactions [105]. Kwon et al. [108] demonstrated that cleavage of annexin 1 causes phosphorylation of $cPLA_2$ during mast-cell activation. Hence it is not clear whether GCs-induced expression of annexin always leads to inhibition of $cPLA_2$ activity. Posttranslational changes can dramatically influence the primary protein function. As previous studies indicate that GCs can stimulate expression of $cPLA_2$ in amnion fibroblast it cannot be excluded that in some specific circumstances GCs may directly induce $cPLA_2$ [109, 110].

## 7. Conclusions

Previous studies confirm the involvement of phospholipases $A_2$ in asthma and COPD although there are some gaps relating to the roles of specific enzymes. The participation of $PLA_2$ in asthma pathogenesis has been better investigated. The diagnostic problems concerning the overlap syndrome that shares the features of asthma and COPD demand further studies on the pathogenesis of these diseases. The phospholipases $A_2$ through their involvement in the course of inflammation seem to be important aspects of this investigation. As they demonstrate pro- and anti-inflammatory properties, a detailed analysis of their role should act as a focus for further studies intended to bring new insights into the pathogenesis of the diseases and identify targets for new drugs.

Data from studies focused on role of $PLA_2$s in inflammatory diseases facilitate the understanding of molecular aspects of inflammation. It can be observed that $cPLA_2$ plays a main role in eicosanoid production and other $PLA_2$s may influence their activity thanks to enzymatic properties or act as regulators of inflammation through their nonenzymatic activity. The pleiotropic properties of single phospholipase and their differential expression in many cells confirm that this is well-organized network of interaction, and further studies focused on this aspect may provide more useful knowledge. A comparison of how this network works in different inflammatory diseases, as well as in healthy subjects may indicate a key molecule, whose activity or presence will be a diagnostic parameter or whose activation or inhibition will have therapeutic value.

Asthma and COPD are heterogeneous diseases and current treatment gives only the possibility to obtain the phenotype of well-controlled diseases. Analysis of data regarding the involvement of $PLA_2$s in course of diseases arises the concept to use combined therapy rather than the treatment based on inhibition of one of them. The results from preclinical studies of $cPLA_2$ inhibitors are promising but clinical trials will give concrete knowledge about the effectiveness and possible side effects.

## Acknowledgments

This paper was partially funded by Polish National Science Centre Grant no. DEC-2012/05/N/NZ5/02630 and Medical University of Lodz Grants: 502-03/0-149-03/502-04-004 and 503/0-149-03/503-01 and was supported from science budget for years 2010-2013 as research project (N N402 516939).

## References

[1] R. H. Schaloske and E. A. Dennis, "The phospholipase $A_2$ superfamily and its group numbering system," *Biochimica et Biophysica Acta*, vol. 1761, no. 11, pp. 1246–1259, 2006.

[2] M. Masoli, D. Fabian, S. Holt, and R. Beasley, "The global burden of asthma: executive summary of the GINA Dissemination Committee Report," *Allergy*, vol. 59, no. 5, pp. 469–478, 2004.

[3] Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA), May 2012, http://www.ginasthma.org.

[4] E. Kitsiouli, G. Nakos, and M. E. Lekka, "Phospholipase $A_2$ subclasses in acute respiratory distress syndrome," *Biochimica et Biophysica Acta*, vol. 1792, no. 10, pp. 941–953, 2009.

[5] J. E. Burke and E. A. Dennis, "Phospholipase $A_2$ structure/function, mechanism, and signaling," *Journal of Lipid Research*, vol. 50, pp. S237–S242, 2009.

[6] The Global Initiative for Chronic Obstructive Lung Disease (GOLD), May 2012, http://www.goldcopd.com/.

[7] H. Nakamura, "Genetics of COPD," *Allergology International*, vol. 60, no. 3, pp. 253–258, 2011.

[8] E. K. Silverman and F. E. Speizer, "Risk factors for the development of chronic obstructive pulmonary disease," *Medical Clinics of North America*, vol. 80, no. 3, pp. 501–522, 1996.

[9] G. Lambeau and M. H. Gelb, "Biochemistry and physiology of mammalian secreted phospholipases $A_2$," *Annual Review of Biochemistry*, vol. 77, pp. 495–520, 2008.

[10] M. Triggiani, G. Giannattasio, C. Calabrese et al., "Lung mast cells are a source of secreted phospholipases $A_2$," *Journal of Allergy and Clinical Immunology*, vol. 124, no. 3, pp. 558.e3–565.e3, 2009.

[11] N. M. Muñoz, Y. J. Kim, A. Y. Meliton et al., "Human group V phospholipase $A_2$ induces group IVA phospholipase $A_2$-independent cysteinyl leukotriene synthesis in human eosinophils," *Journal of Biological Chemistry*, vol. 278, no. 40, pp. 38813–38820, 2003.

[12] M. C. Seeds, K. K. Peachman, D. L. Bowton, K. L. Sivertson, and F. H. Chilton, "Regulation of arachidonate remodeling enzymes impacts eosinophil survival during allergic asthma," *American Journal of Respiratory Cell and Molecular Biology*, vol. 41, no. 3, pp. 358–366, 2009.

[13] P. J. Barnes, "Alveolar macrophages as orchestrators of COPD," *Journal of Chronic Obstructive Pulmonary Disease*, vol. 1, no. 1, pp. 59–70, 2004.

[14] M. Ichinose, "Differences of inflammatory mechanisms in asthma and COPD," *Allergology International*, vol. 58, no. 3, pp. 307–313, 2009.

[15] G. Giannattasio, Y. Lai, F. Granata et al., "Expression of phospholipases $A_2$ in primary human lung macrophages. Role of cytosolic phospholipase $A_2$-$\alpha$ in arachidonic acid release and platelet activating factor synthesis," *Biochimica et Biophysica Acta*, vol. 1791, no. 2, pp. 92–102, 2009.

[16] N. Degousee, F. Ghomashchi, E. Stefanski et al., "Groups IV, V, and X phospholipases $A_2$s in human neutrophils. Role in eicosanoid production and gram-negative bacterial phospholipid hydrolysis," *Journal of Biological Chemistry*, vol. 277, no. 7, pp. 5061–5073, 2002.

[17] F. Granata, V. Nardicchi, S. Loffredo et al., "Secreted phospholipases $A_2$: a proinflammatory connection between macrophages and mast cells in the human lung," *Immunobiology*, vol. 214, no. 9-10, pp. 811–821, 2009.

[18] J. I. Yamashita, M. Ogawa, and T. Shirakusa, "Increased expression of membrane-associated phospholipase $A_2$ in the lower respiratory tract of asymptomatic cigarette smokers," *Respiratory Medicine*, vol. 90, no. 8, pp. 479–483, 1996.

[19] A. Igarashi, Y. Shibata, K. Yamauchi et al., "Gly80Ser polymorphism of phospholipase $A_2$-IID is associated with cytokine inducibility in A549 cells," *Respiration*, vol. 78, no. 3, pp. 312–321, 2009.

[20] C. E. Von Allmen, N. Schmitz, M. Bauer et al., "Secretory phospholipase $A_2$-IID is an effector molecule of $CD4^+$ $CD25^+$ regulatory T cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 28, pp. 11673–11678, 2009.

[21] S. P. Nana, J. D. Lee, S. Sotto-Santiago et al., "Prostacyclin prevents pulmonary endothelial cell apoptosis induced by cigarette smoke," *American Journal of Respiratory and Critical Care Medicine*, vol. 175, no. 7, pp. 676–685, 2007.

[22] R. Pawliczak, "The role of radical oxygen species in airway inflammation," *Polski Merkuriusz Lekarski*, vol. 14, no. 84, pp. 493–496, 2003.

[23] A. Y. Meliton, N. M. Muñoz, A. Lambertino et al., "Phosphodiesterase 4 inhibition of $\beta$2-integrin adhesion caused by leukotriene B4 and TNF-$\alpha$ in human neutrophils," *European Respiratory Journal*, vol. 28, no. 5, pp. 920–928, 2006.

[24] C. Tanaseanu, S. Tudor, I. Tamsulea, D. Marta, G. Manea, and E. Moldoveanu, "Vascular endothelial growth factor, lipoporotein-associated phospholipase $A_2$, sP-selectin and antiphospholipid antibodies, biological markers with prognostic value in pulmonary hypertension associated with chronic obstructive pulmonary disease and systemic lupus erithematosus," *European Journal of Medical Research*, vol. 12, no. 4, pp. 145–151, 2007.

[25] Y. J. Kim, K. P. Kim, S. K. Han et al., "Group V phospholipase $A_2$ induces leukotriene biosynthesis in human neutrophils through the activation of group IVA phospholipase $A_2$," *Journal of Biological Chemistry*, vol. 277, no. 39, pp. 36479–36488, 2002.

[26] D. L. Bowton, A. A. Dmitrienko, E. Israel, B. G. Zeiher, and G. D. Sides, "Impact of a soluble phospholipase $A_2$ inhibitor on inhaled allergen challenge in subjects with asthma," *Journal of Asthma*, vol. 42, no. 1, pp. 65–71, 2005.

[27] E. A. Dennis, "Phospholipase $A_2$ in eicosanoid generation," *American Journal of Respiratory and Critical Care Medicine*, vol. 161, no. 2, part 2, pp. S32–S35, 2000.

[28] T. J. Shuttleworth, "Arachidonic acid activates the noncapacitative entry of $Ca^{2+}$ during $[Ca^{2+}]_i$ oscillations," *Journal of Biological Chemistry*, vol. 271, no. 36, pp. 21720–21725, 1996.

[29] M. N. Graber, A. Alfonso, and D. L. Gill, "$Ca^{2+}$ pools and cell growth: arachidonic acid induces recovery of cells growth-arrested by $Ca^{2+}$ pool depletion," *Journal of Biological Chemistry*, vol. 271, no. 2, pp. 883–888, 1996.

[30] C. D. Funk, "Prostaglandins and leukotrienes: advances in eicosanoid biology," *Science*, vol. 294, no. 5548, pp. 1871–1875, 2001.

[31] M. Uddin and B. D. Levy, "Resolvins: natural agonists for resolution of pulmonary inflammation," *Progress in Lipid Research*, vol. 50, no. 1, pp. 75–88, 2011.

[32] A. Planagumà, S. Kazani, G. Marigowda et al., "Airway lipoxin $A_4$ generation and lipoxin $A_4$ receptor expression are decreased in severe asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 178, no. 6, pp. 574–582, 2008.

[33] B. D. Levy, C. Bonnans, E. S. Silverman, L. J. Palmer, C. Marigowda, and E. Israel, "Diminished lipoxin biosynthesis in severe asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 172, no. 7, pp. 824–830, 2005.

[34] D. H. Adler, J. D. Cogan, J. A. Phillips et al., "Inherited human $cPLA_2\alpha$ deficiency is associated with impaired eicosanoid biosynthesis, small intestinal ulceration, and platelet dysfunction," *Journal of Clinical Investigation*, vol. 118, no. 6, pp. 2121–2131, 2008.

[35] K. A. Reed, D. E. Tucker, A. Aloulou et al., "Functional characterization of mutations in inherited human cPLA$_2$ deficiency," *Biochemistry*, vol. 50, no. 10, pp. 1731–1738, 2011.

[36] M. Sokolowska, M. Borowiec, A. Ptasinska et al., "85-kDa cytosolic phospholipase A$_2$ group IV$\alpha$ gene promoter polymorphisms in patients with severe asthma: a gene expression and case-control study," *Clinical and Experimental Immunology*, vol. 150, no. 1, pp. 124–131, 2007.

[37] M. Sokolowska, J. Stefanska, K. Wodz-Naskiewicz, and R. Pawliczak, "Cytosolic phospholipase A$_2$ group IVA influence on GM-CSF expression in human lung cells: a pilot study," *Medical Science Monitor*, vol. 16, no. 9, pp. BR300–BR306, 2010.

[38] K. A. Whalen, H. Legault, C. Hang et al., "In vitro allergen challenge of peripheral blood induces differential gene expression in mononuclear cells of asthmatic patients: inhibition of cytosolic phospholipase A$_2\alpha$ overcomes the asthma-associated response," *Clinical and Experimental Allergy*, vol. 38, no. 10, pp. 1590–1605, 2008.

[39] T. S. Hallstrand, E. Y. Chi, A. G. Singer, M. H. Gelb, and W. R. Henderson, "Secreted phospholipase A$_2$ group X overexpression in asthma and bronchial hyperresponsiveness," *American Journal of Respiratory and Critical Care Medicine*, vol. 176, no. 11, pp. 1072–1078, 2007.

[40] K. Asai, T. Hirabayashi, T. Houjou, N. Uozumi, R. Taguchi, and T. Shimizu, "Human group IVC phospholipase A$_2$ (cPLA$_2\gamma$): roles in the membrane remodeling and activation induced by oxidative stress," *Journal of Biological Chemistry*, vol. 278, no. 10, pp. 8809–8814, 2003.

[41] D. J. Mancuso, D. R. Abendschein, C. M. Jenkins et al., "Cardiac ischemia activates calcium-independent phospholipase A$_2\beta$, precipitating ventricular tachyarrhythmias in transgenic mice: rescue of the lethal electrophysiologic phenotype by mechanism-based inhibition," *Journal of Biological Chemistry*, vol. 278, no. 25, pp. 22231–22236, 2003.

[42] G. M. Gauvreau, R. M. Watson, and P. M. O'Byrne, "Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation," *American Journal of Respiratory and Critical Care Medicine*, vol. 159, no. 1, pp. 31–36, 1999.

[43] K. F. Chung, "Evaluation of selective prostaglandin E$_2$ (PGE$_2$) receptor agonists as therapeutic agents for the treatment of asthma," *Science's STKE*, vol. 2005, no. 303, p. pe47, 2005.

[44] B. Sastre and V. del Pozo, "Role of PGE$_2$ in asthma and nonasthmatic eosinophilic bronchitis," *Mediators of Inflammation*, vol. 2012, Article ID 645383, 9 pages, 2012.

[45] R. P. Phipps, S. H. Stein, and R. L. Roper, "A new view of prostaglandin E regulation of the immune response," *Immunology Today*, vol. 12, no. 10, pp. 349–352, 1991.

[46] S. Yedgar, M. Krimsky, Y. Cohen, and R. J. Flower, "Treatment of inflammatory diseases by selective eicosanoid inhibition: a double-edged sword?" *Trends in Pharmacological Sciences*, vol. 28, no. 9, pp. 459–464, 2007.

[47] F. H. Chilton, F. J. Averill, W. C. Hubbard, A. N. Fonteh, M. Triggiani, and M. C. Liu, "Antigen-induced generation of lysophospholipids in human airways," *Journal of Experimental Medicine*, vol. 183, no. 5, pp. 2235–2245, 1996.

[48] D. L. Bowton, M. C. Seeds, M. B. Fasano, B. Goldsmith, and D. A. Bass, "Phospholipase A$_2$ and arachidonate increase in bronchoalveolar lavage fluid after inhaled antigen challenge in asthmatics," *American Journal of Respiratory and Critical Care Medicine*, vol. 155, no. 2, pp. 421–425, 1997.

[49] W. R. Henderson, E. Y. Chi, J. G. Bollinger et al., "Importance of group X-secreted phospholipase A$_2$ in allergen-induced airway inflammation and remodeling in a mouse asthma model," *Journal of Experimental Medicine*, vol. 204, no. 4, pp. 865–877, 2007.

[50] T. S. Hallstrand, Y. Lai, Z. Ni et al., "Relationship between levels of secreted phospholipase A$_2$ groups IIA and X in the airways and asthma severity," *Clinical and Experimental Allergy*, vol. 41, no. 6, pp. 801–810, 2011.

[51] Y. Lai, R. C. Oslund, J. G. Bollinger et al., "Eosinophil cysteinyl leukotriene synthesis mediated by exogenous secreted phospholipase A$_2$ group X," *Journal of Biological Chemistry*, vol. 285, no. 53, pp. 41491–41500, 2010.

[52] M. Murakami, Y. Taketomi, H. Sato, and K. Yamamoto, "Secreted phospholipase A$_2$ revisited," *Journal of Biochemistry*, vol. 150, no. 3, pp. 233–255, 2011.

[53] M. Murakami, Y. Taketomi, Y. Miki, H. Sato, T. Hirabayashi, and K. Yamamoto, "Recent progress in phospholipase A$_2$ research: from cells to animals to humans," *Progress in Lipid Research*, vol. 50, no. 2, pp. 152–192, 2011.

[54] I. Kudo and M. Murakami, "Phospholipase A$_2$ enzymes," *Prostaglandins and Other Lipid Mediators*, vol. 68-69, pp. 3–58, 2002.

[55] H. K. Tay and A. J. Melendez, "Fc$\gamma$RI-triggered generation of arachidonic acid and eicosanoids requires iPLA$_2$ but not cPLA$_2$ in human monocytic cells," *Journal of Biological Chemistry*, vol. 279, no. 21, pp. 22505–22513, 2004.

[56] M. Murakami, S. Masuda, K. Ueda-Semmyo et al., "Group VIB Ca$^{2+}$-independent phospholipase A$_2\gamma$ promotes cellular membrane hydrolysis and prostaglandin production in a manner distinct from other intracellular phospholipases A$_2$," *Journal of Biological Chemistry*, vol. 280, no. 14, pp. 14028–14041, 2005.

[57] T. M. McIntyre, S. M. Prescott, and D. M. Stafforini, "The emerging roles of PAF acetylhydrolase," *Journal of Lipid Research*, vol. 50, pp. S255–S259, 2009.

[58] D. M. Stafforini, T. Numao, A. Tsodikov et al., "Deficiency of platelet-activating factor acetylhydrolase is a severity factor for asthma," *Journal of Clinical Investigation*, vol. 103, no. 7, pp. 989–997, 1999.

[59] S. Kruse, X. Q. Mao, A. Heinzmann et al., "The Ile198Thr and Ala379Val variants of plasmatic Paf-acetylhydrolase impair catalytical activities and are associated with atopy and asthma," *American Journal of Human Genetics*, vol. 66, no. 5, pp. 1522–1530, 2000.

[60] W. R. Henderson, J. Lu, K. M. Poole, G. N. Dietsch, and E. Y. Chi, "Recombinant human platelet-activating factor-acetylhydrolase inhibits airway inflammation and hyperreactivity in mouse asthma model," *Journal of Immunology*, vol. 164, no. 6, pp. 3360–3367, 2000.

[61] N. R. Henig, M. L. Aitken, M. C. Liu, A. S. Yu, and W. R. Henderson, "Effect of recombinant human platelet-activating factor-acetylhydrolase on allergen-induced asthmatic responses," *American Journal of Respiratory and Critical Care Medicine*, vol. 162, no. 2, part 1, pp. 523–527, 2000.

[62] R. Rivera and J. Chun, "Biological effects of lysophospholipids," *Reviews of Physiology, Biochemistry and Pharmacology*, vol. 160, pp. 25–46, 2008.

[63] J. A. Urbina, "Mechanisms of action of lysophospholipid analogues against trypanosomatid parasites," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 100, supplement 1, pp. S9–S16, 2006.

[64] B. P. Hurley and B. A. McCormick, "Multiple roles of phospholipase A$_2$ during lung infection and inflammation," *Infection and Immunity*, vol. 76, no. 6, pp. 2259–2272, 2008.

[65] R. D. Hite, M. C. Seeds, R. B. Jacinto, B. L. Grier, B. M. Waite, and D. A. Bass, "Lysophospholipid and fatty acid inhibition of pulmonary surfactant: non-enzymatic models of phospholipase $A_2$ surfactant hydrolysis," *Biochimica et Biophysica Acta*, vol. 1720, no. 1-2, pp. 14–21, 2005.

[66] M. A. Kwatia, C. B. Doyle, W. Cho, G. Enhorning, and S. J. Ackerman, "Combined activities of secretory phospholipases and eosinophil lysophospholipases induce pulmonary surfactant dysfunction by phospholipid hydrolysis," *Journal of Allergy and Clinical Immunology*, vol. 119, no. 4, pp. 838–847, 2007.

[67] S. J. Ackerman, M. A. Kwatia, C. B. Doyle, and G. Enhorning, "Hydrolysis of surfactant phospholipids catalyzed by phospholipase $A_2$ and eosinophil lysophospholipases causes surfactant dysfunction: a mechanism for small airway closure in asthma," *Chest*, vol. 123, no. 3, p. 355S, 2003.

[68] G. Enhorning, "Surfactant in airway disease," *Chest*, vol. 133, no. 4, pp. 975–980, 2008.

[69] M. Hiraoka, A. Abe, Y. Lu et al., "Lysosomal phospholipase $A_2$ and phospholipidosis," *Molecular and Cellular Biology*, vol. 26, no. 16, pp. 6139–6148, 2006.

[70] R. S. Koduri, J. O. Grönroos, V. J. O. Laine et al., "Bactericidal properties of human and murine groups I, II, V, X, and XII secreted phospholipases $A_2$," *Journal of Biological Chemistry*, vol. 277, no. 8, pp. 5849–5857, 2002.

[71] M. Rouault, C. Le Calvez, E. Boilard et al., "Recombinant production and properties of binding of the full set of mouse secreted phospholipases $A_2$ to the mouse M-type receptor," *Biochemistry*, vol. 46, no. 6, pp. 1647–1662, 2007.

[72] M. Triggiani, F. Granata, A. Frattini, and G. Marone, "Activation of human inflammatory cells by secreted phospholipases $A_2$," *Biochimica et Biophysica Acta*, vol. 1761, no. 11, pp. 1289–1300, 2006.

[73] P. Ancian, G. Lambeau, and M. Lazdunski, "Multifunctional activity of the extracellular domain of the M-type (180 kDa) membrane receptor for secretory phospholipases $A_2$," *Biochemistry*, vol. 34, no. 40, pp. 13146–13151, 1995.

[74] F. Granata, R. I. Staiano, S. Loffredo et al., "The role of mast cell-derived secreted phospholipases $A_2$ in respiratory allergy," *Biochimie*, vol. 92, no. 6, pp. 588–593, 2010.

[75] M. Triggiani, F. Granata, A. Oriente et al., "Secretory phospholipases $A_2$ induce $\beta$-glucuronidase release and IL-6 production from human lung macrophages," *Journal of Immunology*, vol. 164, no. 9, pp. 4908–4915, 2000.

[76] C. C. Silliman, E. E. Moore, G. Zallen et al., "Presence of the M-type $sPLA_2$ receptor on neutrophils and its role in elastase release and adhesion," *American Journal of Physiology*, vol. 283, no. 4, pp. C1102–C1113, 2002.

[77] A. Gambero, E. C. T. Landucci, M. H. Toyama et al., "Human neutrophil migration in vitro induced by secretory phospholipases $A_2$: a role for cell surface glycosaminoglycans," *Biochemical Pharmacology*, vol. 63, no. 1, pp. 65–72, 2002.

[78] M. Triggiani, F. Granata, B. Balestrieri et al., "Secretory phospholipases $A_2$ activate selective functions in human eosinophils," *Journal of Immunology*, vol. 170, no. 6, pp. 3279–3288, 2003.

[79] Z. Ni, N. M. Okeley, B. P. Smart, and M. H. Gelb, "Intracellular actions of group IIA secreted phospholipase $A_2$ and group IVA cytosolic phospholipase $A_2$ contribute to arachidonic acid release and prostaglandin production in rat gastric mucosal cells and transfected human embryonic kidney cells," *Journal of Biological Chemistry*, vol. 281, no. 24, pp. 16245–16255, 2006.

[80] M. A. Balboa, R. Pérez, and J. Balsinde, "Amplification mechanisms of inflammation: paracrine stimulation of arachidonic acid mobilization by secreted phospholipase $A_2$ is regulated by cytosolic phospholipase $A_2$-derived hydroperoxyeicosatetraenoic acid," *Journal of Immunology*, vol. 171, no. 2, pp. 989–994, 2003.

[81] S. Offer, S. Yedgar, O. Schwob et al., "Negative feedback between secretory and cytosolic phospholipase $A_2$ and their opposing roles in ovalbumin-induced bronchoconstriction in rats," *American Journal of Physiology*, vol. 288, no. 3, pp. L523–L529, 2005.

[82] A. N. Fonteh, G. I. Atsumi, T. LaPorte, and F. H. Chilton, "Secretory phospholipase $A_2$ receptor-mediated activation of cytosolic phospholipase $A_2$ in murine bone marrow-derived mast cells," *Journal of Immunology*, vol. 165, no. 5, pp. 2773–2782, 2000.

[83] Y. J. Jiang, B. Lu, P. C. Choy, and G. M. Hatch, "Regulation of cytosolic phospholipase $A_2$, cyclooxygenase-1 and -2 expression by PMA, TNF$\alpha$, LPS and M-CSF in human monocytes and macrophages," *Molecular and Cellular Biochemistry*, vol. 246, no. 1-2, pp. 31–38, 2003.

[84] P. Dieter, A. Kolada, S. Kamionka, A. Schadow, and M. Kaszkin, "Lipopolysaccharide-induced release of arachidonic acid and prostaglandins in liver macrophages: regulation by Group IV cytosolic phospholipase $A_2$, but not by Group V and Group IIA secretory phospholipase $A_2$," *Cellular Signalling*, vol. 14, no. 3, pp. 199–204, 2002.

[85] H. Y. Qi and J. H. Shelhamer, "Toll-like receptor 4 signaling regulates cytosolic phospholipase $A_2$ activation and lipid generation in lipopolysaccharide-stimulated macrophages," *Journal of Biological Chemistry*, vol. 280, no. 47, pp. 38969–38975, 2005.

[86] W. Tian, G. T. Wijewickrama, J. H. Kim et al., "Mechanism of regulation of group IVA phospholipase $A_2$ activity by Ser727 phosphorylation," *Journal of Biological Chemistry*, vol. 283, no. 7, pp. 3960–3971, 2008.

[87] X. Zhou, W. Yang, and J. Li, "$Ca^{2+}$- and protein kinase C-dependent signaling pathway for nuclear factor-$\kappa$B activation, inducible nitric-oxide synthase expression, and tumor necrosis factor-$\alpha$ production in lipopolysaccharide-stimulated rat peritoneal macrophages," *Journal of Biological Chemistry*, vol. 281, no. 42, pp. 31337–31347, 2006.

[88] M. J. Coffey, S. M. Phare, and M. Peters-Golden, "Induction of inducible nitric oxide synthase by lipopolysaccharide/interferon gamma and sepsis down-regulates 5-lipoxygenase metabolism in murine alveolar macrophages," *Experimental Lung Research*, vol. 30, no. 7, pp. 615–633, 2004.

[89] U. T. Shankavaram, D. L. DeWitt, and L. M. Wahl, "Lipopolysaccharide induction of monocyte matrix metalloproteinases is regulated by the tyrosine phosphorylation of cytosolic phospholipase $A_2$," *Journal of Leukocyte Biology*, vol. 64, no. 2, pp. 221–227, 1998.

[90] U. A. Kessen, R. H. Schaloske, D. L. Stephens, K. K. Lucas, and E. A. Dennis, "PGE2 release is independent of upregulation of Group V phospholipase $A_2$ during long-term stimulation of P388D1 cells with LPS," *Journal of Lipid Research*, vol. 46, no. 11, pp. 2488–2496, 2005.

[91] P. Shridas, W. M. Bailey, K. R. Talbott, R. C. Oslund, M. H. Gelb, and N. R. Webb, "Group X secretory phospholipase $A_2$ enhances TLR4 signaling in macrophages," *Journal of Immunology*, vol. 187, no. 1, pp. 482–489, 2011.

[92] K. Hamaguchi, H. Kuwata, K. Yoshihara et al., "Induction of distinct sets of secretory phospholipase $A_2$ in rodents during

inflammation," *Biochimica et Biophysica Acta*, vol. 1635, no. 1, pp. 37–47, 2003.

[93] W. R. Hendersen, R. C. Oslund, J. G. Bollinger, X. Ye, Y. T. Tien, J. Xue et al., "Blockade of human group X secreted phospholipase $A_2$-induced airway inflammation and hyperresponsiveness in a mouse asthma model by a selective group X secreted phospholipase $A_2$ inhibitor," *The Journal of Biological Chemistry*. In press.

[94] N. M. Muñoz, A. Y. Meliton, L. N. Meliton, S. M. Dudek, and A. R. Leff, "Secretory group V phospholipase $A_2$ regulates acute lung injury and neutrophilic inflammation caused by LPS in mice," *American Journal of Physiology*, vol. 296, no. 6, pp. L879–L887, 2009.

[95] N. M. Muñoz, A. Y. Meliton, J. P. Arm, J. V. Bonventre, W. Cho, and A. R. Leff, "Deletion of secretory group V phospholipase $A_2$ attenuates cell migration and airway hyperresponsiveness in immunosensitized mice," *The Journal of Immunology*, vol. 179, no. 7, pp. 4800–4807, 2007.

[96] R. Malaviya, J. Ansell, L. Hall et al., "Targeting cytosolic phospholipase $A_2$ by arachidonyl trifluoromethyl ketone prevents chronic inflammation in mice," *European Journal of Pharmacology*, vol. 539, no. 3, pp. 195–204, 2006.

[97] J. C. McKew, K. L. Lee, M. W. H. Shen et al., "Indole cytosolic phospholipase $A_2\alpha$ inhibitors: discovery and in vitro and in vivo characterization of 4-3-[5-chloro-2-(2-[(3,4-dichlorobenzyl)sulfonyl]aminoethyl)-1-(diphenylmethyl)-1H-indol-3-yl]propyl benzoic acid, efipladib," *Journal of Medicinal Chemistry*, vol. 51, no. 12, pp. 3388–3413, 2008.

[98] K. L. Lee, M. A. Foley, L. Chen et al., "Discovery of ecopladib, an indole inhibitor of cytosolic phospholipase $A_2\alpha$," *Journal of Medicinal Chemistry*, vol. 50, no. 6, pp. 1380–1400, 2007.

[99] C. A. Hewson, S. Patel, L. Calzetta et al., "Preclinical evaluation of an inhibitorof cytosolic phospholipase $A_2\alpha$ for the treatment of asthma," *Journal of Pharmacology and Experimental Therapeutics*, vol. 340, no. 3, pp. 656–665, 2012.

[100] F. Hirata, E. Schiffmann, and K. Venkatasubramanian, "A phospholipase $A_2$ inhibitory protein in rabbit neutrophils induced by glucocorticoids," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 77, no. 5, pp. 2533–2536, 1980.

[101] R. De Caterina, R. Sicari, D. Giannessi et al., "Macrophage-specific eicosanoid synthesis inhibition and lipocortin-1 induction by glucocorticoids," *Journal of Applied Physiology*, vol. 75, no. 6, pp. 2368–2375, 1993.

[102] X. Huang, R. Pawliczak, X. L. Yao et al., "Characterization of the human p11 promoter sequence," *Gene*, vol. 310, no. 1-2, pp. 133–142, 2003.

[103] X. L. Huang, R. Pawliczak, X. L. Yao et al., "Interferon-$\gamma$ induces p11 gene and protein expression in human epithelial cells through interferon-$\gamma$-activated sequences in the p11 promoter," *Journal of Biological Chemistry*, vol. 278, no. 11, pp. 9298–9308, 2003.

[104] X. L. Yao, M. J. Cowan, M. T. Gladwin, M. M. Lawrence, C. W. Angus, and J. H. Shelhamer, "Dexamethasone alters arachidonate release from human epithelial cells by induction of p11 protein synthesis and inhibition of phospholipase $A_2$ activity," *Journal of Biological Chemistry*, vol. 274, no. 24, pp. 17202–17208, 1999.

[105] L. H. K. Lim and S. Pervaiz, "Annexin 1: the new face of an old molecule," *The FASEB Journal*, vol. 21, no. 4, pp. 968–975, 2007.

[106] T. Nakano, O. Ohara, H. Teraoka, and H. Arita, "Glucocorticoids suppress group II phospholipase $A_2$ production by blocking mRNA synthesis and post-transcriptional expression," *Journal of Biological Chemistry*, vol. 265, no. 21, pp. 12745–12748, 1990.

[107] U. R. Juergens, F. Jäger, W. Darlath, M. Stöber, H. Vetter, and A. Gillissen, "Comparison of in vitro-activity of common used topical glucocorticoids on cytokine- and phospholipase inhibition," *European Journal of Medical Research*, vol. 9, no. 8, pp. 383–390, 2004.

[108] J. H. Kwon, J. H. Lee, K. S. Kim, Y. W. Chung, and I. Y. Kim, "Regulation of cytosolic phospholipase $A_2$ phosphorylation by proteolytic cleavage of annexin A1 in activated mast cells," *The Journal of Immunology*, vol. 188, no. 11, pp. 5665–5673, 2012.

[109] C. Guo, J. Li, L. Myatt, X. Zhu, and K. Sun, "Induction of G$\alpha$s contributes to the paradoxical stimulation of cytosolic phospholipase $A_2\alpha$ expression by cortisol in human amnion fibroblasts," *Molecular Endocrinology*, vol. 24, no. 5, pp. 1052–1061, 2010.

[110] C. Guo, Z. Yang, W. Li, P. Zhu, L. Myatt, and K. Sun, "Paradox of glucocorticoid-induced cytosolic phospholipase $A_2$ group IVA messenger RNA expression involves glucocorticoid receptor binding to the promoter in human amnion fibroblasts," *Biology of Reproduction*, vol. 78, no. 1, pp. 193–197, 2008.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2013, Article ID 586895, 10 pages
http://dx.doi.org/10.1155/2013/586895

*Research Article*

# Lung-Derived Mediators Induce Cytokine Production in Downstream Organs via an *NF-κB*-Dependent Mechanism

**E. K. Patterson,[1] L. J. Yao,[1] N. Ramic,[1] J. F. Lewis,[1,2] G. Cepinskas,[1] L. McCaig,[1] R. A. W. Veldhuizen,[1,2] and C. M. Yamashita[1,2]**

[1] *Lawson Health Research Institute, London, ON, Canada N6A 4V2*
[2] *Department of Medicine, Physiology and Pharmacology, Western University, London, ON, Canada*

Correspondence should be addressed to C. M. Yamashita; cyamash@uwo.ca

Received 29 May 2012; Revised 3 January 2013; Accepted 20 February 2013

Academic Editor: Celeste C. Finnerty



In the setting of acute lung injury, levels of circulating inflammatory mediators have been correlated with adverse outcomes. Previous studies have demonstrated that injured, mechanically ventilated lungs represent the origin of the host inflammatory response; however, mechanisms which perpetuate systemic inflammation remain uncharacterized. We hypothesized that lung-derived mediators generated by mechanical ventilation (MV) are amplified by peripheral organs in a "feed forward" mechanism of systemic inflammation. Herein, lung-derived mediators were collected from 129X1/SVJ mice after 2 hours of MV while connected to the isolated perfused mouse lung model setup. Exposure of liver endothelial cells to lung-derived mediators resulted in a significant increase in G-CSF, IL-6, CXCL-1, CXCL-2, and MCP-1 production compared to noncirculated control perfusate media ($P < 0.05$). Furthermore, inhibition of the NF-$\kappa$B pathway significantly mitigated this response. Changes in gene transcription were confirmed using qPCR for IL-6, CXCL-1, and CXCL-2. Additionally, liver tissue obtained from mice subjected to 2 hours of *in vivo* MV demonstrated significant increases in hepatic gene transcription of IL-6, CXCL-1, and CXCL-2 compared to nonventilated controls. Collectively, this data demonstrates that lung-derived mediators, generated in the setting of MV, are amplified by downstream organs in a feed forward mechanism of systemic inflammation.

## 1. Introduction

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) represent a spectrum of diseases characterized by the rapid onset of pulmonary infiltrates and progressive hypoxemia in the absence of significant left ventricular dysfunction [1]. Within the early phases of ALI, the role of mechanical ventilation and its influence on patient outcomes has been an area of specific interest [2]. It is now widely acknowledged that the use of excessive tidal volumes in patients with underlying ALI can further perpetuate lung dysfunction [3] while limiting injury through the use of lower tidal volumes has been the only therapeutic maneuver shown to improve survival [4]. Furthermore, the proinflammatory response associated with the mechanical stress of ventilation, known as biotrauma [5], represents one of the key mechanisms by which mechanical ventilation may be critical in determining patient outcomes.

Prior studies have demonstrated that the injured lung serves as the primary origin of proinflammatory mediators which may decompartmentalize into the systemic circulation [6–8]. Recent studies from our laboratory have shown that these lung-derived mediators are capable of eliciting the expression of surface adhesion molecules in liver endothelial cells both directly and in a tidal volume-dependent fashion [9, 10]. From a clinical perspective, it has been demonstrated that patients ventilated with low-tidal volumes had a reduction in plasma proinflammatory mediator levels compared to those patients ventilated by conventional strategies and, notably these levels correlated with a reduction in multiple organ failure [11]. Although such evidence implicates the lung as the primary source of mediators leading to systemic inflammation, the specific mechanisms that serve to perpetuate and propagate the ensuing proinflammatory signaling cascade remain uncharacterized.

For example, it remains unknown, whether the marked rise in plasma cytokines can be attributed entirely from a "spillover" phenomenon of a mechanically ventilated, injured lung to the systemic circulation or whether a primary inflammatory signal generated by the lung may be secondarily amplified by downstream peripheral organs. Therefore, characterization of the discrete signaling processes which drive persistent increases in systemic inflammatory mediators and the localization of their specific cellular origins may be critical in the development of effective therapeutic agents aimed at mitigating the inflammatory response resulting from mechanical ventilation.

One of the intracellular signaling pathways most widely recognized for its importance in inflammation is the nuclear factor kappa B (NF-$\kappa$B) signaling pathway. It has been well established that many receptors activate the NF-$\kappa$B pathway, the most extensively studied of which are the interleukin (IL), tumor necrosis factor (TNF), and toll-like receptor families [12]. The "canonical" activation of the NF-$\kappa$B pathway involves phosphorylation of p65 (RelA), and its translocation to the nucleus [13] leading to a number of proinflammatory responses including the upregulation of adhesion molecules (on both endothelial cells and leukocytes) and transcriptional regulation of a wide array of cytokines and chemokines [12]. Although activation of the NF-$\kappa$B pathway may be involved in the resolution of inflammation, particularly through its "alternative" pathway, we describe studies involving the acute phase of inflammation wherein the proinflammatory actions of NF-$\kappa$B activation predominate [12].

In the current study, it was hypothesized that inflammatory mediators generated by the lung in response to mechanical ventilation are secondarily amplified by downstream organs in a "feed forward" mechanism of systemic inflammation. Herein, we demonstrate that lung-derived mediators are definitively upregulated by liver tissues in both *in vitro* and *in vivo* models of mechanical ventilation-induced inflammation. Further studies examining specific intracellular pathways responsible for mediator amplification demonstrate that activation of the inflammation relevant NF-$\kappa$B signaling pathway in liver endothelial cells is in part responsible for these observations.

## 2. Materials and Methods

*2.1. Study Design.* In order to obtain inflammatory mediators generated and released specifically from the lung into the systemic circulation, the isolated perfused mouse lung (IPML) model was employed. Lungs were mechanically ventilated using the *ex vivo* IMPL setup and lung perfusate was obtained after a completion of the ventilation protocol. Subsequently, mouse liver endothelial cells were exposed to lung perfusate to determine whether subsequent increases in inflammatory mediators were observed and the signaling processes that may be involved such as the inflammation-associated NF-$\kappa$B pathway. Furthermore the physiological relevance of these *ex vivo* and *in vitro* studies was validated using an *in vivo* model of mechanical ventilation to observe similar findings in intact whole liver tissues.

*2.2. Animals.* Male mice were used for experiments (Charles River, Saint-Constant, Canada). All procedures were approved by the Animal Use Subcommittee at the University of Western Ontario in agreement with the guidelines of the Canadian Council of Animal Care. All animals were acclimatized a minimum of 72 hours prior to use in the experiments and had free access to water and standard chow, Lab Diet Rodent Diet 5001 (PMI Nutrition International, St Louis, MO).

*2.3. Ventilation-Induced Inflammation and the Isolated Perfused Mouse Lung (IPML) Model.* A model of ventilation-induced inflammation was employed as previously described to obtain lung-derived mediators [8, 10]. Using this technique, male 129X1/SVJ mice weighing between 25 and 30 grams were sacrificed and placed on the IPML and mechanically ventilated. Briefly, the pulmonary artery was initially isolated, cannulated, and secured using 4-0 silk. A second cannula was then inserted into the left ventricle and single pass of perfusate (RPMI 1640 lacking phenol red, +2% low endotoxin grade bovine serum albumin; Sigma, St Louis, MO) was utilized to clear the lung of the remaining blood. Subsequently, a continuous reperfusion of the pulmonary circulation was performed using approximately 10 mL of perfusate. This perfusate was used to replace the blood within the pulmonary vascular compartment, while bovine serum albumin was included to maintain the integrity of the pulmonary vessels. Animals were mechanically ventilated with room air for a period of 2 h with a tidal volume ($V_t$) of 12.5 mL/kg, respiratory rate of 30 breaths/min, positive end expiratory pressure (PEEP) of 3 cm $H_2O$ while using 5% $CO_2$ to maintain the pH of the bicarbonate-buffered RPMI. At the completion of the ventilation protocol, lung perfusate was collected and immediately stored at $-80^{\circ}$C. Lung perfusate was pooled and the levels of inflammatory cytokines in lung perfusate were determined using a Millipore Milliplex kit according to the manufacturer's protocol (Millipore, Billerica, MA) for ten inflammation relevant analytes using a multiplex assay. Samples were analyzed using the Luminex xMAP detection system on the Luminex$^{100}$ (Linco Research, St Charles, MO) as per manufacturer's instructions. New non-circulated perfusate media (control perfusate) were used as a blank control in the ELISA as well as a baseline or negative control in subsequent *in vitro* cell culture experiments.

*2.4. Mouse Liver Endothelial Cell Culture.* Mouse liver endothelial cells (MLEC) were a kind gift from Dr. Steven Alexander (Louisiana State Health Sciences Center, Shreveport, LA, USA). MLEC were cultured in (minimal essential media) MEM D-Valine (PromoCell, Heidelberg, Germany) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine (Invitrogen, Burlington, ON), MEM nonessential amino acids (Invitrogen), MEM vitamin mix (Invitrogen), and 1% penicillin/streptomycin (Invitrogen). Cells were passaged twice per week.

*2.5. Multiplex Enzyme-Linked Immunosorbent Assay (ELISA).* MLECs were seeded in a 24-well plate ($6 \times 10^4$ cells per well)

2 days prior to the experiment. The confluent MLEC monolayers were challenged for 8 h in a cell culture incubator with 0.25 mL of: (a) control uncirculated perfusate, (b) uncirculated perfusate containing cytomix using equal concentrations of TNF-$\alpha$, IL-1$\beta$ and interferon (IF)-$\gamma$ (10 ng/mL), (which has been used to simulate inflammatory conditions in cell culture [14]), or (c) lung perfusate. These conditioned media were then frozen at −80°C for later analysis. The obtained conditioned media were analyzed with the Millipore Milliplex kit and Luminex xMAP detection system as described above.

*2.6. Determination of NF-κB Activity in MLEC.* MLEC were plated two days prior to experimentation in 6-well (western blot) or 24-well (ELISA) plates at $1.5 \times 10^5$ or $6 \times 10^4$ cells per well, respectively. Control perfusate or lung perfusate was subsequently applied to MLEC cultures for 30 minutes. Following stimulation, cells were washed three times with cold phosphate buffered saline (PBS) and lysed in a buffer containing 0.5% sodium dodecyl sulfate (SDS), 1 mM ethylenediaminetetraacetic acid (EDTA), 50 mM Tris pH 7.5 plus 1 : 100 Protease Inhibitor cocktail (Sigma, St. Louis, MO). Cell lysates were subsequently boiled and subjected to western blot analysis using an anti-phospho-p65 antibody (Cell Signaling, Beverley, MA, USA) and anti-GAPDH (Cell Signaling, Danvers, MA) as previously described [15]. For the detection of phospho-p65 by ELISA, cells were lysed and processed according to the manufacturers instructions using the Pathscan phospho-p65(Ser536) ELISA kit (Cell Signaling). ELISA results were normalized to the total protein content per well as determined by the micro bicinchoninic acid (BCA) technique (Thermo Scientific, Nepean, ON).

*2.7. Real-Time Quantitative Polymerase Chain Reaction (qPCR).* $1.5 \times 10^5$ MLECs were placed on 35 mm dishes 2 days prior to exposure to 0.8 mL of the indicated perfusates (with or without NF-κB inhibitors) for 4 hours at 37°C. Total RNA was extracted from the cells using the RNeasy Plus Mini Kit (Qiagen, Toronto, ON, Canada). 1 $\mu$g of total RNA was reverse-transcribed using Superscript III reverse transcriptase (Invitrogen) following the manufacturer's protocol. qPCR was performed as described previously [10] with the exception that the Cq values were determined by linear regression in CFX Manager v2.1 (Biorad, Mississauga, ON). Cq data was exported into qbasePLUS (Biogazelle, Zwijnaarde, Belgium) for quantification of expression and statistical analysis. The gene-specific PCR efficiencies were determined using the "qpcR" package v1.36 in "R" v2.15.0 (http://www.r-project.org/) [16]. The data were fitted to a 5-parameter logistic curve using the smoothing option to determine reaction efficiencies using the Cy0 method. The control perfusate samples were used as the calibrator in each reaction for cultured cells, unventilated control livers were arbitrarily set to 1 for graphing after analysis. The target gene expression was normalized to the $\beta$-actin, GAPDH, and 18S RNA in all samples. Primer sequences were obtained from RTPrimerDB [17]: $\beta$-actin ID: 168, IL-6: 3269, TNF-$\alpha$: 3747, CXCL-2: 1068, or CXCL-1 [18], GAPDH: Fwd 5′-CAACGACCCCTTCATTGACCTC-3′ and Rev 5′-CCAATGTGTCCGTCGTGGAT-3′, 18s (a kind gift from Dr. Aaron Cox, Western University, London, ON, Canada): Fwd 5′-ACGATGCCGACTGGCGATGC-3′ and Rev 5′-CCCACTCCTGGTGGTGCCCT-3′.

*2.8. NF-κB Inhibitors.* For experiments involving NF-κB pathway inhibition, cells were preincubated with 15 $\mu$M IMD-0354 (Tocris Bioscience, Minneapolis, MN) or 20 $\mu$M caffeic acid phenethyl ester (CAPE) (Tocris) for 20 minutes, prior to exposure with lung perfusate that also contained the same concentration of the indicated inhibitor. A short preincubation period was used to ensure the NF-κB pathway would not be activated immediately upon exposure to the inflammatory mediators in the perfusate.

*2.9. In Vivo Model of Ventilation-Induced Inflammation.* C57BL/6 mice weighing between 20 and 30 grams were initially anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) and subsequently the left jugular vein and left carotid artery were exposed and cannulated with PE10 tubing which was secured in place with 5-0 silk. The arterial line was used to collect arterial blood samples (60 $\mu$L each time) for blood gas measurements (ABL 700, Radiometer, Copenhagen, Denmark), monitor hemodynamics, and deliver fluids (sterile 0.9% NaCl and 100 IU heparin/L) using an infusion pump at a rate of 0.5 mL/100 g/h. The venous line was used to deliver additional ketamine/xylazine anesthetic as needed and to deliver additional fluid (0.5 mL/100 g/h) continuously. Ketamine/xylazine was administered through the venous line to maintain a consistent level of anesthesia and avoid additional unnecessary animal handling. The trachea was exposed and a 14-gauge endotracheal tube was secured with 3-0 surgical silk. Animals were subsequently connected to the Harvard Mini-Vent volume-cycled mechanical ventilator (Harvard Instruments, Saint-Laurent, Canada) with the following parameters: $V_t$ = 10 mL/kg, PEEP = 3 cm $H_2O$, respiratory rate = 150 breaths/min (bpm), and $FiO_2$ = 1.0. After 15 minutes of ventilation, animals were assessed for initial inclusion criteria, which consisted of a ratio of arterial partial pressure of oxygen to fractional percentage of inspired oxygen ($PaO_2 : FiO_2$) of >400 mmHg.

Every fifteen minutes, for the subsequent 240 minutes measurements were taken of peak inspiratory pressure (PIP) blood pressure (BP), heart rate (HR) and recorded while temperature was constantly measured with a rectal probe attached to an Omega Engineering, HH-25TC thermocouple. After 4 h of ventilation, the animals were euthanized with an intravenous overdose of sodium pentobarbital (110 mg/kg). Liver samples were subsequently excised and snap frozen for later RNA extraction using Trizol reagent (Invitrogen) as per the manufacturer's protocol. qPCR was performed on extracted RNA samples as described above.

*2.10. Statistical Analysis.* Groups were analyzed by one-way analysis of variance (ANOVA) (cell culture samples) or Student's *t*-test (livers) using GraphPad Prism v4.03 (GraphPad Software Inc, La Jolla, CA), except qPCR statistics were

Table 1: Cytokine concentration in lung perfusate samples collected from animals sustaining ventilator-induced inflammation at the completion of 2 hours of mechanical ventilation using the isolated perfused mouse lung model. New uncirculated perfusate was used as the blank control for the ELISA. Values represent mean ± SEM.

| Mediator | Increase over control (pg/mL) |
|---|---|
| G-CSF | 21.87 ± 4.57 |
| IL-6 | 267.81 ± 28.35 |
| KC | 356.38 ± 57.98 |
| MIP2 | 198.70 ± 12.24 |
| MCP-1 | 2.01 ± 1.54 |
| TNF-$\alpha$ | 33.16 ± 4.55 |
| IFN-$\gamma$ | 1.44 ± 0.88 |
| IL-1$\beta$ | 0.51 ± 0.35 |
| IL10 | 0.95 ± 0.62 |
| Eotaxin | 17.46 ± 2.93 |

performed using qbasePLUS's internal statistical analysis by one-way ANOVA (cell culture samples) or Student's $t$-test (livers). Results were considered significant when $P < 0.05$.

## 3. Results

*3.1. Generating Lung-Derived Inflammatory Mediators.* In order to elicit ventilation-induced lung inflammation in mice and obtain lung-specific mediators in a perfused solution, we ventilated euthanized mice on the IPML apparatus. Analysis of the inflammatory cytokine concentrations in lung-derived perfusate collected at the completion of the mechanical ventilation protocol is shown in Table 1. The concentrations of lung-specific mediators from ventilated mice were comparable to previous observations made by our group using this protocol [10].

*3.2. Mouse Liver Endothelial Cell Response to Lung-Derived Mediators.* MLECs were exposed to control uncirculated perfusate, lung perfusate, or uncirculated perfusate plus cytomix for 8 h. MLECs exposed to lung perfusate expressed significantly greater concentrations of granulocyte colony stimulating factor (G-CSF), IL-6, chemokine (C-X-C motif) ligand 1 (CXCL-1), CXCL-2, and monocyte chemoattractant protein 1 (MCP-1) measured within the conditioned media compared to MLECs exposed to control perfusate and compared to concentrations in the perfusate before incubation on MLECs. The increases in cytokine concentrations is shown in Figure 1. Four of the analytes included in the assay demonstrated no significant change in concentrations after 8 h of incubation with lung perfusate (IF-$\gamma$, IL-1$\beta$, IL-10, and TNF-$\alpha$) as compared to the baseline concentrations, while eotaxin decreased significantly from baseline (data not shown). Incubation of MLEC with cytomix (10 ng/mL) demonstrated significant increases in MCP-1, granulocyte macrophage colony stimulating factor (GM-CSF), TNF-$\alpha$, IL-1$\beta$, and eotaxin, while the remaining analytes demonstrated no significant change from control.

*3.3. Lung-Derived Mediator Effects on NF-κB Activation and Gene Expression in MLEC.* Based on the observation that

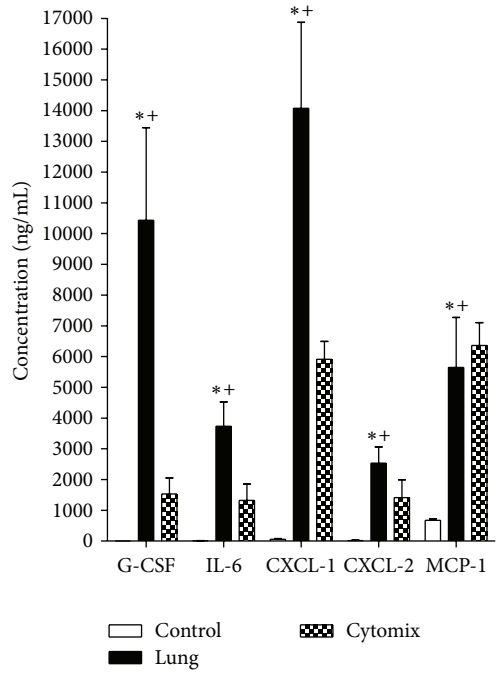



Figure 1: Absolute increase in selected cytokine concentrations in MLEC media. G-CSF, IL-6, CXCL-1, CXCL-2, and MCP-1 concentrations from MLECs exposed to control uncirculated perfusate (open bar), lung perfusate (solid bar), and cytomix (checkered bar). ($^*P < 0.05$ concentration after 8 h incubation on MLEC versus concentration before incubation on MLEC (0 h), $^+P < 0.05$ versus control perfusate after both were incubated on MLEC cultures for 8 h, ±SEM). Where open bars are not apparent (G-CSF, IL-6, CXCL-1, CXCL-2), the increase is too small to print.

incubation with lung perfusate elicited the production of further inflammatory mediators, we investigated the role of the inflammation-relevant NF-κB signaling pathway in this process. Incubation of MLEC with lung perfusate resulted in a significant increase in NF-κB-subunit p65 phosphorylation compared to cells incubated with control perfusate media (Figure 2). Figures 2(a) and 2(b) depict a representative western blot and quantification of phospho-p65 from MLEC stimulated with either lung perfusate or TNF-$\alpha$ as a positive control. Similarly, in independent experiments, activation of NF-κB was also confirmed employing an ELISA approach to detect phospho-p65 (Ser536) (Figure 2(c)) with cytomix used as a positive control. p65 phosphorylation, detected by ELISA, was significantly increased in lung perfusate and cytomix exposed cells compared to control perfusate alone.

Based on the above observations, two structurally different NF-κB inhibitors, IMD-0354 (IMD) and caffeic acid phenethyl ester (CAPE), were employed. These compounds have previously been determined to interfere with NF-κB activation at two different points along the NF-κB signaling cascade [19, 20]. Initial experiments were performed to determine the effective and minimally cytotoxic concentrations of both inhibitors. The obtained results indicated that IMD and CAPE were effective in suppressing NF-κB activation at 15 and 20 $\mu$M, respectively (data not shown). Treating

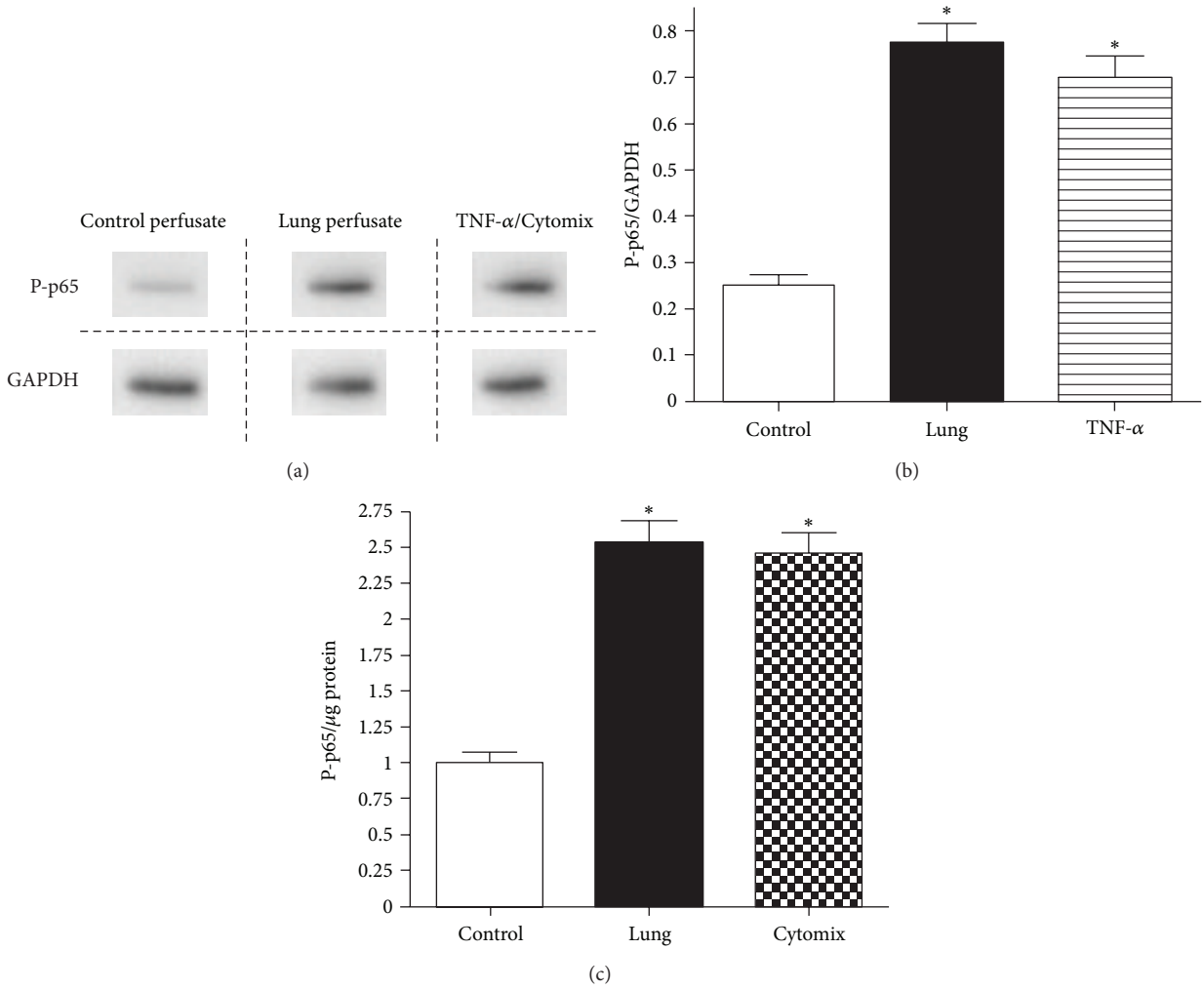



Figure 2: Activation of NF-$\kappa$B in MLEC exposed to control uncirculated perfusate, lung perfusate, and TNF-$\alpha$/cytomix. (a) Representative western blot and (b) densitometry of P-p65 relative to GAPDH expression. (c) Activation of NF-$\kappa$B employing phospho-p65 (Ser536) ELISA. ($^*P < 0.05$ versus control, ±SEM).

MLEC with either IMD or CAPE significantly mitigated the production of proinflammatory mediators released by MLEC after incubation with lung perfusate as shown in Figure 3.

To confirm that these changes occurred at the level of gene transcription, selected mediators were chosen for qPCR analysis in MLECs exposed to lung perfusate (Figure 4). Gene transcription of IL-6, CXCL-1 and CXCL-2 were significantly reduced by treating MLEC with either IMD or CAPE prior to exposure to lung perfusate, whereas neither inhibitor had a significant effect on the gene expression of TNF-$\alpha$, although there was a trend of reduced TNF-$\alpha$ expression.

*3.4. Hepatic Inflammatory Cytokine Expression In Vivo.* Physiological parameters for animals undergoing 2 hours of mechanical ventilation are shown in Figure 5. Over the course of mechanical ventilation, there was a decrease in $PaO_2$ at both 120 and 240 minutes of mechanical ventilation compared to the baseline $PaO_2$; however, this decrease was not statistically significant. In contrast, the PIP, also shown in Figure 5, increased over the course of ventilation and was significantly increased at 60 minutes and thereafter compared to the baseline (time 0) PIP. Additionally, blood pressure and partial pressure of $CO_2$ did not vary significantly from the baseline (data not shown). The lack of a significant change in the majority of these parameters suggested that a significant degree of lung dysfunction was not elicited by this ventilation protocol. Figure 6 depicts the qPCR analysis of selected inflammatory mediators expressed in mouse livers. qPCR demonstrated a significant increase in CXCL-1, CXCL-2, IL-6, and TNF-$\alpha$ gene transcription in livers of mechanically ventilated animals compared to non-ventilated controls, a phenomenon consistent with our observations made *in vitro*.

## 4. Discussion

The results of the current study present a novel finding of an NF-$\kappa$B-dependent mechanism of proinflammatory cytokine amplification by liver endothelial cells secondary to mechanical ventilation. Previous studies have consistently demonstrated that the NF-$\kappa$B signaling cascade represents

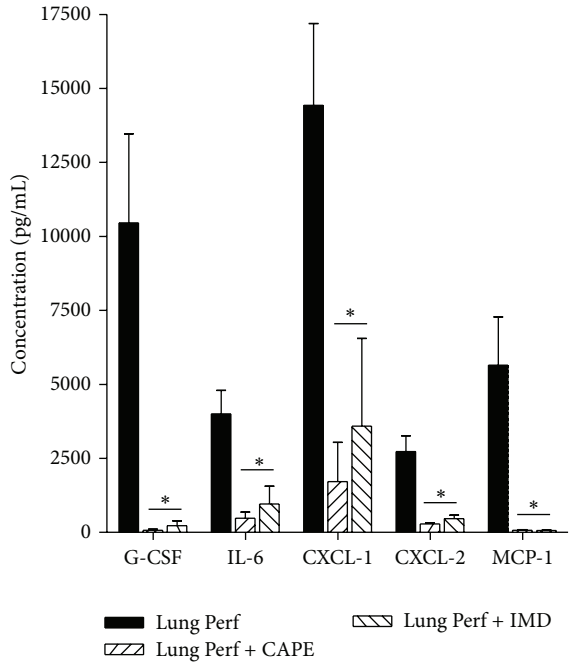



Figure 3: NF-$\kappa$B inhibitor effect on cytokine concentrations in MLEC-exposed media. G-CSF, IL-6, CXCL-1, CXCL-2, and MCP-1 after 8 h exposure to lung perfusate and treatment with CAPE or IMD. $^{*}P < 0.05$ versus lung perfusate, ±SEM.

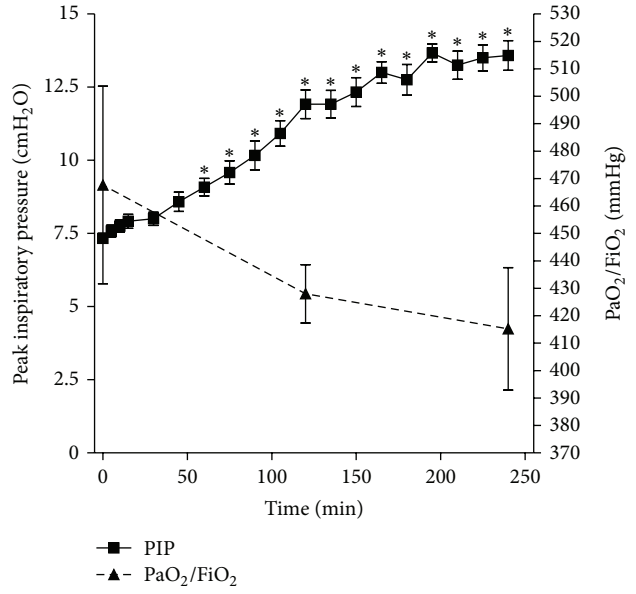



Figure 5: Physiological parameters during *in vivo* ventilation. Peak inspiratory pressure (PIP) (solid line, left axis) was determined every 15 minutes; arterial partial pressure of oxygen over fraction of inspired oxygen ($PaO_2/FiO_2$) (dashed line, right axis) was determined at time 0, 120, and 240 minutes of *in vivo* mechanical ventilation. $^{*}P < 0.05$ versus time 0, ±SEM.

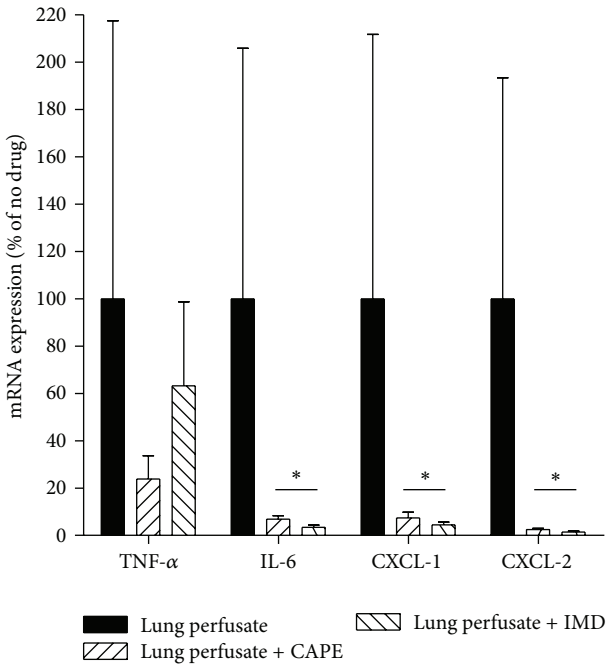



Figure 4: Quantitative PCR of TNF-$\alpha$, IL-6, CXCL-1, and CXCL-2 expressed in MLEC in response to lung perfusate alone or treatment with CAPE or IMD during lung perfusate exposure. $^{*}P < 0.05$ versus Lung perfusate, ±SEM.

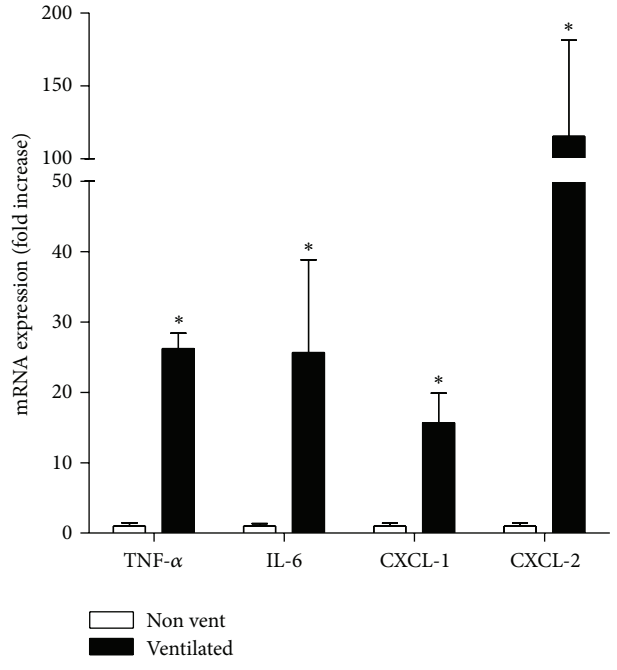



Figure 6: Quantitative PCR for TNF-$\alpha$, IL-6, CXCL-1, and CXCL-2 in liver tissues extracted from nonventilated (nonvent) and *in vivo* mechanically ventilated (ventilated) mice. Values represent fold-increase over expression in nonventilated mice. $^{*}P < 0.05$ versus non-ventilated mice ±SEM.

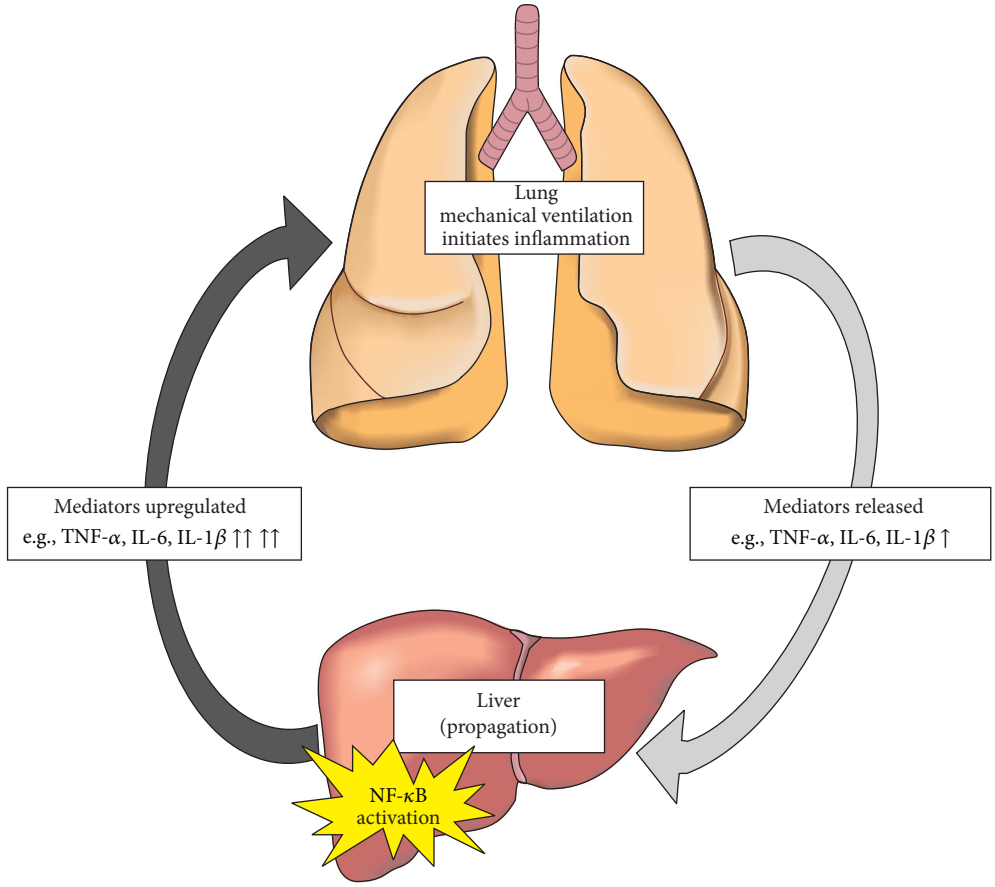



Figure 7: Proposed inflammatory pathway diagram. Inflammation initiated in the lung releases inflammatory mediators (light grey arrow, right side) which then translocate to peripheral organs (e.g., liver). These organs amplify the inflammatory signal, through an NF-$\kappa$B-dependent pathway, leading to further release of inflammatory mediators, which then travel back to the lung, and/or other peripheral organs (dark grey arrow) where the signal is further propagated in a feed-forward mechanism of acute inflammation.

a key regulatory process controlling the transcription of many proinflammatory mediators as it is estimated that over 400 activators of this inflammatory pathway [21] have been identified including physical stress [22], oxidant stress [23], and proinflammatory cytokines [24]. Thus, while it may not be unexpected that lung perfusate obtained from ventilated mice that is rich in multiple proinflammatory cytokines is capable of activating the NF-$\kappa$B pathway in liver endothelial cells, we highlight unique aspects which we believe are relevant in the context of systemic inflammation subsequent to the initiation of mechanical ventilation.

Firstly, through the use of the IMPL model, we show that *specific* mediators originating from a lung generated in response to mechanical ventilation are capable of inducing NF-$\kappa$B signaling in endothelial cells of a peripheral organ. The IMPL model allows the pulmonary circulation to be isolated from the systemic circulation, thereby facilitating the collection of mediators generated directly by the lung as a result of mechanical (ventilation) stress. Although other aspects of the IPML model may have contributed to the inflammatory mediators in perfusate, such as surgery and lack of blood, current literature suggests that the vast majority of these mediators are induced by the cell stretch due to ventilation [25–27]. From a clinical standpoint, although the absolute rises in serum cytokines have been directly correlated with outcomes in the setting of ARDS [11], the specific origin of these mediators has been incompletely characterized. Therefore, based on the results of this study we speculate that although the injured lung serves as the primary origin of the systemic inflammatory response, the signal is promptly propagated by peripheral organs in a maladaptive "feed-forward" mechanism of systemic inflammation. Figure 7 depicts an illustration of this pathway.

Secondly, while this lung-derived perfusate contains elevated levels of multiple inflammatory mediators, equivalent or greater concentrations of cytomix (TNF-$\alpha$, IL-1$\beta$, IF-$\gamma$) failed to elicit an equal magnitude of responses. These findings would suggest that the effects observed in our model may be an aggregate effect of multiple mediators present in lung perfusate samples which are generated specifically through the effects of mechanical ventilation. Furthermore, the downstream increase in inflammatory mediators originating from liver cells was not simply a global, nonspecific effect. Rather, although liver endothelial cells are capable of producing a wide spectrum on inflammatory mediators [28], the rise in mediators appeared to be restricted to a significant increase in 5 out of 10 analytes measured including G-CSF, IL-6, CXCL-1, CXCL-2, and MCP-1. Notably, TNF-$\alpha$ was

not significantly elevated in the cell culture model, although TNF-$\alpha$ gene transcription was significantly up-regulated in lung perfusate treated cells. This may be related to the known properties of the TNF-$\alpha$ gene which is rapidly transcribed upon stimulation, but has subsequent translation tightly controlled [29]. Although some mediators were not significantly increased upon exposure to the MLEC cultures (IF-$\gamma$, IL-1$\beta$, IL-10, and TNF-$\alpha$), this is not to suggest these mediators are not important or do not contribute to inflammation. These findings not only underscore the complexity of the systemic inflammatory response secondary to mechanical ventilation, but also may explain why previous therapeutic interventions targeting isolated cytokines have not resulted in improvement in patient outcomes [30].

Using the *in vivo* model of ventilation-induced inflammation highlights several interesting observations. Although the use of mechanical ventilation is obligatory in the setting of ALI and ARDS to maintain host survival, the *in vivo* model adopted in the current study employed the use of mechanical ventilation alone to study its downstream effects on systemic inflammation. Despite the absence of marked changes in host physiology (oxygenation), significant proinflammatory changes were noted in liver tissues suggesting that systemic manifestations of mechanical ventilation may not only occur in the absence of physiological lung dysfunction but that preexisting lung injury may not be an obligatory requirement for potentially deleterious systemic manifestations.

Clinical studies in patients with ARDS have consistently demonstrated that stepwise increases in inflammatory cytokines in patients with ARDS have been correlated with greater adverse outcomes [31, 32]. For example, Ranieri et al. showed that patients exposed to protective modalities of MV had lower pulmonary and systemic inflammation compared to patients on conventional ventilation strategies [33]. Furthermore, other studies have also demonstrated that patients ventilated with lower tidal volumes had a lower plasma level of IL-6, as well as soluble TNF-$\alpha$ and IL-1 receptor antagonists compared to those ventilated with conventional strategies, thereby providing evidence that mechanical ventilation independently leads to systemic inflammation [11]. The current study adds to the growing body of evidence that injudicious use of mechanical ventilation can contribute adversely toward a maladaptive systemic inflammatory response by peripheral organs, and furthermore, may provide insight into potential mechanism by which therapeutic approaches, such as low tidal volume mechanical ventilation, have been successful in improving patient outcomes.

Our data would suggest that the adoption of either a primary or complementary strategy of mitigating peripheral organ responses early in the course of ARDS through the blockade of maladaptive pathways such as NF-$\kappa$B signaling in peripheral organs may be an effective approach to consider. Alternately, strategies aimed at minimizing the translocation of lung-derived mediators into the systemic circulation may represent a more "proximal" upstream approach; however, the specific mechanisms responsible for the release of these mediators remains as yet undetermined.

Although we describe a potential mechanism whereby inflammatory signals originating the in the lung are subsequently amplified by cells of a downstream organ, we recognize that our model does have inherent limitations. Firstly, we chose to utilize liver endothelial cells as the cell type of interest due to the immediate proximity and exposure of this cell layer to lung-derived mediators which may circulate *in vivo*. Therefore, our findings are limited to this specific cell type and we have not accounted for the contribution of other tissue specific cells within the liver such as hepatocytes or Kuppfer cells, for example. The contribution of other cell types from liver and other organs may account for why we did not observe significant increases in several mediators previously shown to be important in patient outcomes (e.g., IL-1$\beta$, TNF-$\alpha$). Nonetheless, the use of whole liver tissues employed in the *in vivo* model of mechanical ventilation indicates that increases in IL-6, for example, may be expressed throughout the liver and not restricted to any one cell type. Secondly, our investigation focused primarily on proinflammatory effect, the contribution of anti-inflammatory mediators in this process may also be important to evaluate in future studies. Thirdly, it remains unknown whether similar links exist between the lung and other downstream organs such as the kidneys, heart or brain and whether an amplification of inflammatory mediators from theses other systemic organs contribute to a greater or lesser extent toward systemic inflammation. Whether the NF-$\kappa$B signaling cascade represents a common pathway of proinflammatory signaling within each organ or whether other organ specific proinflammatory signaling pathways exists remains to be characterized. Future studies to determine the generalizability of our findings beyond a single downstream organ are therefore warranted.

In the current study, we demonstrate that inflammatory mediators generated by the lung in response to mechanical ventilation decompartmentalize to the systemic circulation in a murine model of ventilator-induced inflammation. Subsequently, we show that the levels of these inflammatory mediators are significantly amplified upon exposure to liver endothelial cells thereby resulting in a maladaptive upregulation of the systemic inflammatory response. The results of *in vitro* experiments illustrating this phenomenon are further confirmed in an *in vivo* model of ventilation induced inflammation whereby a significant increase in transcriptional activity in these mediators is observed in the liver. Ultimately, we show that the propagation of the systemic inflammatory response by the liver occurs through an NF-$\kappa$B-dependent mechanism and that inhibition of this signaling pathway can, in part, mitigate these responses. The significance of these findings will require further studies to determine whether blockade of the NF-$\kappa$B pathway in peripheral organ tissues would provide a rational means of therapeutic intervention.

## Authors' Contribution

E. K. Patterson conceived experiments, performed experiments, and wrote the paper; L. J. Yao performed experiments;

N. Ramic performed experiments; J. F. Lewis conceived experiments and reviewed the paper; G. Cepinskas conceived experiments and reviewed the paper; L. McCaig conceived experiments and performed experiments; R. A. W. Veldhuizen conceived experiments and reviewed the paper; C. M. Yamashita conceived experiments, performed experiments, and wrote the paper.

## Acknowledgments

The authors wish to thank Ms. Shannon Seney for her technical assistance with the multiplex assays and data collection. Granting support for these studies was provided by the Parker B. Francis Foundation (CMY), the Program of Experimental Medicine, Department of Medicine, Western University (CMY/ RAWV), The Heart and Stroke Foundation of Ontario NA6914 (GC) The Ontario Thoracic Society (CMY), and Canadian Institutes of Health Research (RAWV).

## References

[1] M. A. Matthay and R. L. Zemans, "The acute respiratory distress syndrome: pathogenesis and treatment," *Annual Review of Pathology*, vol. 6, pp. 147–163, 2011.

[2] A. S. Slutsky and L. N. Tremblay, "Multiple system organ failure: is mechanical ventilation a contributing factor?" *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 6, pp. 1721–1725, 1998.

[3] L. N. Tremblay and A. S. Slutsky, "Pathogenesis of ventilator-induced lung injury: trials and tribulations," *American Journal of Physiology*, vol. 288, no. 4, pp. L596–L598, 2005.

[4] R. G. Brower, M. A. Matthay, A. Morris, D. Schoenfeld, B. T. Thompson, and A. Wheeler, "Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome," *The New England Journal of Medicine*, vol. 342, no. 18, pp. 1301–1308, 2000.

[5] L. N. Tremblay and A. S. Slutsky, "Ventilator-induced injury: from barotrauma to biotrauma," *Proceedings of the Association of American Physicians*, vol. 110, no. 6, pp. 482–488, 1998.

[6] Y. Imai, J. Parodo, O. Kajikawa et al., "Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome," *JAMA*, vol. 289, no. 16, pp. 2104–2112, 2003.

[7] E. A. Truscott, L. A. McCaig, L. J. Yao, R. A. W. Veldhuizen, and J. F. Lewis, "Surfactant protein-A reduces translocation of mediators from the lung into the circulation," *Experimental Lung Research*, vol. 36, no. 7, pp. 431–439, 2010.

[8] M. G. Walker, J. M. Tessolini, L. McCaig, L. J. Yao, J. F. Lewis, and R. A. Veldhuizen, "Elevated endogenous surfactant reduces inflammation in an acute lung injury model," *Experimental Lung Research*, vol. 35, no. 7, pp. 591–604, 2009.

[9] N. Markovic, L. A. McCaig, J. Stephen et al., "Mediators released from LPS-challenged lungs induce inflammatory responses in liver vascular endothelial cells and neutrophilic leukocytes," *American Journal of Physiology*, vol. 297, no. 6, pp. G1066–G1076, 2009.

[10] M. G. Walker, L. J. Yao, E. K. Patterson et al., "The effect of tidal volume on systemic inflammation in acid-induced lung injury," *Respiration*, vol. 81, no. 4, pp. 333–342, 2011.

[11] G. U. Meduri, S. Headley, G. Kohler et al., "Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS: plasma IL-1$\beta$ and IL-6 levels are consistent and efficient predictors of outcome over time," *Chest*, vol. 107, no. 4, pp. 1062–1073, 1995.

[12] T. Lawrence, "The nuclear factor NF-kappaB pathway in inflammation," *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 6, Article ID a001651, 2009.

[13] M. S. Hayden and S. Ghosh, "NF-$\kappa$B, the first quarter-century: remarkable progress and outstanding questions," *Genes and Development*, vol. 26, no. 3, pp. 203–234, 2012.

[14] D. K. Nakayama, D. A. Geller, C. J. Lowenstein et al., "Cytokines and lipopolysaccharide induce nitric oxide synthase in cultured rat pulmonary artery smooth muscle," *American Journal of Respiratory Cell and Molecular Biology*, vol. 7, no. 5, pp. 471–476, 1992.

[15] S. J. Jeong, C. A. Pise-Masison, M. F. Radonovich, U. P. Hyeon, and J. N. Brady, "A novel NF-$\kappa$B pathway involving IKK$\beta$and p65/RelA Ser-536 phosphorylation results in p53 inhibition in the absence of NF-$\kappa$B transcriptional activity," *The Journal of Biological Chemistry*, vol. 280, no. 11, pp. 10326–10332, 2005.

[16] C. Ritz and A. N. Spiess, "qpcR: an R package for sigmoidal model selection in quantitative real-time polymerase chain reaction analysis," *Bioinformatics*, vol. 24, no. 13, pp. 1549–1551, 2008.

[17] S. Lefever, J. Vandesompele, F. Speleman, and F. Pattyn, "RTPrimerDB: the portal for real-time PCR primers and probes," *Nucleic Acids Research*, vol. 37, no. 1, pp. D942–D945, 2009.

[18] M. A. Hegeman, M. P. Hennus, C. J. Heijnen et al., "Ventilator-induced endothelial activation and inflammation in the lung and distal organs," *Critical Care*, vol. 13, no. 6, article R182, 2009.

[19] Y. Onai, J. I. Suzuki, T. Kakuta et al., "Inhibition of I$\kappa$B phosphorylation in cardiomyocytes attenuates myocardial ischemia/reperfusion injury," *Cardiovascular Research*, vol. 63, no. 1, pp. 51–59, 2004.

[20] G. F. Sud'ina, O. K. Mirzoeva, M. A. Pushkareva, G. A. Korshunova, N. V. Sumbatyan, and S. D. Varfolomeev, "Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties," *FEBS Letters*, vol. 329, no. 1-2, pp. 21–24, 1993.

[21] P. A. Baeuerle and T. Henkel, "Function and activation of NF-kappa B in the immune system," *Annual Review of Immunology*, vol. 12, pp. 141–179, 1994.

[22] H. Zhao, T. Hiroi, B. S. Hansen, and J. J. Rade, "Cyclic stretch induces cyclooxygenase-2 gene expression in vascular endothelial cells via activation of nuclear factor kappa-$\beta$," *Biochemical and Biophysical Research Communications*, vol. 389, no. 4, pp. 599–601, 2009.

[23] F. Alessandrini, I. Beck-Speier, D. Krappmann et al., "Role of oxidative stress in ultrafine particle-induced exacerbation of allergic lung inflammation," *American Journal of Respiratory and Critical Care Medicine*, vol. 179, no. 11, pp. 984–991, 2009.

[24] R. Wu, W. Dong, M. Zhou et al., "Ghrelin attenuates sepsis-induced acute lung injury and mortality in rats," *American Journal of Respiratory and Critical Care Medicine*, vol. 176, no. 8, pp. 805–813, 2007.

[25] L. N. Tremblay and A. S. Slutsky, "Ventilator-induced lung injury: from the bench to the bedside," *Intensive Care Medicine*, vol. 32, no. 1, pp. 24–33, 2006.

[26] P. E. Parsons, M. D. Eisner, B. T. Thompson et al., "Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury," *Critical Care Medicine*, vol. 33, no. 1, pp. 1–6, 2005.

[27] N. E. Vlahakis, M. A. Schroeder, A. H. Limper, and R. D. Hubmayr, "Stretch induces cytokine release by alveolar epithelial cells in vitro," *American Journal of Physiology*, vol. 277, no. 1, pp. L167–L173, 1999.

[28] M. K. Connolly, A. S. Bedrosian, A. Malhotra et al., "In hepatic fibrosis, liver sinusoidal endothelial cells acquire enhanced immunogenicity," *Journal of Immunology*, vol. 185, no. 4, pp. 2200–2208, 2010.

[29] T. Raabe, M. Bukrinsky, and R. A. Currie, "Relative contribution of transcription and translation to the induction of tumor necrosis factor-$\alpha$ by lipopolysaccharide," *The Journal of Biological Chemistry*, vol. 273, no. 2, pp. 974–980, 1998.

[30] K. J. Bosma, R. Taneja, and J. F. Lewis, "Pharmacotherapy for prevention and treatment of acute respiratory distress syndrome: current and experimental approaches," *Drugs*, vol. 70, no. 10, pp. 1255–1282, 2010.

[31] P. Agouridakis, D. Kyriakou, M. G. Alexandrakis et al., "The predictive role of serum and bronchoalveolar lavage cytokines and adhesion molecules for acute respiratory distress syndrome development and outcome," *Respiratory Research*, vol. 3, article 25, 2002.

[32] K. D. Liu, D. V. Glidden, M. D. Eisner et al., "Predictive and pathogenetic value of plasma biomarkers for acute kidney injury in patients with acute lung injury," *Critical Care Medicine*, vol. 35, no. 12, pp. 2755–2761, 2007.

[33] V. M. Ranieri, P. M. Suter, C. Tortorella et al., "Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial," *JAMA*, vol. 282, no. 1, pp. 54–61, 1999.