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ORIGINAL ARTICLE

Increased proportion of $CD4^+CD25^+Foxp3^+$ regulatory T cells during early-stage sepsis in ICU patients

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ICU;
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SIRS;
Soluble CD25 molecules

Background/Purpose(s): We investigated whether $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) are induced in patients suffering from early-stage septic shock and distinguish them from noninfectious patients with systemic inflammatory response.
Methods: The study included 37 patients with early-stage septic shock, 15 patients with noninfectious systemic inflammatory response syndrome (SIRS), and 24 heath controls. We prospectively assayed the fraction of Tregs expressing high levels of CD25 and forkhead box P3 (Foxp3) as well as the plasma levels of interferon-γ (IFN-γ), interleukin-4 (IL-4), and soluble CD25 in all the subjects studied.
Results: Compared with the control groups, the plasma levels of IFN-γ [66.10 (45.23–85.08) pg/mL vs. 20.97 (17.58–26.21) pg/mL, $p < 0.001$] and IL-4 [100.69 (77.41–127.68) pg/mL vs. 70.40 (64.14–80.15) pg/mL, $p < 0.001$] as well as the IFN-γ/IL-4 ratio [0.66 (0.62–0.67) vs. 0.30 (0.27–0.33), $p < 0.001$] were significantly elevated in the patients with early-stage septic shock, but there was no difference between patients with sepsis and patients with SIRS. We found that the proportion of $CD4^+CD25^+Foxp3^+$ T cells was significantly increased in the patients with early-stage septic shock [(66.82 ± 21.79%) vs. (51.79 ± 21.79%) vs. (56.45 ± 10.68%), $p = 0.003$] in comparison with the SIRS and control groups, which could be differentiated from the patients with SIRS. The plasma levels of soluble CD25 were also increased, and positively correlated with the proportion of Tregs in patients with early-stage septic shock (Spearman correlation coefficient = 0.390, $p = 0.003$).

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Conclusion: Our findings indicate that the proportion of $CD4^+CD25^+Foxp3^+$ T cells could be an indicator for the early diagnosis of sepsis. This proportion can also facilitate the evaluation of the patient's immune status and guide suitable immunoregulatory therapy.

Introduction

Sepsis is a major cause of death in intensive care units (ICUs), and the hospitalization and mortality rates for severe sepsis are increasing rapidly.[1] It is well known that sepsis undermines immune homeostasis by inducing an initial intense systemic inflammatory response that is rapidly followed by a negative feedback of anti-inflammatory processes.[2,3] The persistence of a marked compensatory anti-inflammatory response following sepsis is termed *immunoparalysis*. Several studies have reported that this inhibitory response may decrease the resistance of the host against secondary nosocomial infections, which negatively affects the patient's outcome.[4] A recent study showed that patients with severe sepsis suffer from immunosuppression toward the later stages of the disease.[5] However, data from the early stage of the disease remain unclear.

As an indicator of immunoparalysis, $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) play an important role in the immune response. These cells exert a pronounced anti-inflammatory effect through contact-mediated direct inhibition of other immune cells and produce high levels of soluble CD25, interleukin-4 (IL-4), and IL-10.[6,7] Tregs can cause an imbalance of the Th1/Th2 immune response [interferon-γ (IFN-γ)/IL-4] and induce $CD4^+$T cells to differentiate into Th2 cells. Under septic conditions, the presence of $CD4^+CD25^+Foxp3^+$ Tregs, which are the natural Tregs, indicates immunoparalysis.[8] It remains unclear as to whether $CD4^+CD25^+Foxp3^+$ Tregs are induced in patients with early-stage septic shock.

In this study, we assayed the proportion of $CD4^+CD25^+Foxp3^+$ Tregs and the plasma levels of IFN-γ, IL-4, and soluble CD25 in patients with early-stage septic shock. We attempted to find new targets to diagnose sepsis at an early stage and simultaneously evaluate the immune status of these patients.

Materials and methods

Study population

Patients with systemic inflammatory response syndrome (SIRS) and septic shock included in the study were from the respiratory ICU, emergency ICU, cardiac surgery ICU, and neurosurgery ICU of the Shanghai Ruijin Hospital and their data were collected between February 2009 and February 2010. The control group consisted of age-matched healthy individuals selected from the Medical Centre of Ruijin Hospital. Patients with an immune disease or those who had recently received (within the past 30 days) potent immunosuppressive agents were excluded. The study protocol was approved by the Institutional Review Board and the Hospital Committee on Ethics of Shanghai Jiao Tong University School of Medicine, and is consistent with the standards established by the Declaration of Helsinki.

SIRS group

The patients undergoing elective operations within 24 hours and meeting the diagnostic criteria for SIRS without signs of infection were included. At least two of the following criteria must be present to diagnose SIRS: $T > 38^\circ C$ or $< 36^\circ C$; heart rate $>$ 90 beats/minute; respiratory rate $>$ 20 breaths/minute or $PaCO_2 < 32$ mmHg; white blood cells $>$ 12 or $< 4 \times 10^9/L$.[9]

Sepsis group

The patients in ICU are diagnosed with sepsis if they meet the SIRS diagnostic criteria and had evidence of infection, such as positive findings in bacterial cultures of blood or other body fluids. The patients in early-stage septic shock are those who were diagnosed with sepsis within 24 hours. Such patients were also included in this study.

Control group

The control group included age-matched healthy individuals from the Medical Center of Ruijin Hospital.

Study protocol and methods

Peripheral arterial blood from patients with SIRS was collected between 7:00 and 8:00 AM within 24 hours after their operation. Blood was collected from patients with septic shock within 24 hours after the diagnosis. All the samples were anticoagulated with ethylenediaminetetraacetic acid.

Flow cytometry

Lymphocytes were separated from 2.5 mL of peripheral arterial blood by gradient centrifugation in a lymphocyte separation medium within 2 hours after the samples were harvested. The lymphocytes were counted and the cell concentration was diluted to 1×10^6/mL. A human regulatory T-cell staining kit (Human Regulatory T cell Staining Kit, eBioscience, San Diego, CA, USA) was used, and fluorescein isothiocyanate—conjugated CD4 antibody, APC-CD25 antibody, and PE-Foxp3 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were separately added to the diluted lymphocytes. The fluorescence intensity of the cell-surface antibodies was measured by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA), while blank controls and isotype controls were used to eliminate autofluorescence and nonspecific fluorescence. The fluorescence intensity was portrayed as a scattered two-dimensional dot plot, which was

stored in the computer. The $CD4^+CD25^+Foxp3^+$ T-cell ratio was analyzed using CellQuest software ($CD4^+CD25^+Foxp3^+$ T cells % = $CD4^+CD25^+Foxp3^+$ T cells/$CD4^+CD25^+$ T cells × 100%).

Enzyme-linked immunosorbent assay

Human IL-4 Quantikine HS (R&D Systems, Inc., Minneapolis, MN, USA), human IFN-γ Quantikine Pharmpak (R&D Systems, Inc., Minneapolis, MN, USA), and human IL-2sRα Quantikine ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA), were used to determine the plasma levels of IL-2sRa, IFN-γ, and IL-4, respectively.

Statistical analysis

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows was used for statistical analyses and a two-sided $p < 0.05$ was considered statistically significant. The Kolmogorov—Smirnov test was used to check for normal distribution. Comparisons between patients with sepsis, patients with SIRS, and/or healthy volunteers were performed using Student *t* test as normally distributed values, Mann—Whitney *U* test, and Kruskal—Wallis test when indicated. Data are expressed as mean ± standard deviation or median ± interquartile range when appropriate. Spearman rank correlation test was used to test relationship between two variables.

Results

General information

The age factor was not significantly different among patients in the sepsis group (61.67 ± 11.87) years, the SIRS group (67.06 ± 12.57) years, and the control group (56.54 ± 6.37) years. There were 15 cases in the SIRS group (8 men and 7 women), including 7 cases with cardiac valve replacement, 6 cases with coronary artery bypass graft, 1 case with cardiac tumor resection, and 1 case with subtotal resection of thyroid. There were 37 patients (26 men and 11 women) in the sepsis group, including 28 cases of pneumonia in which the primary pathogens were Gram-negative bacterium. A total of 24 age-matched healthy individuals, including 14 men and 10 women, were included as the control group. No significant difference was found among groups with respect to gender composition. All clinical data from the patients in the sepsis group, the SIRS group, and the control group are shown in Table 1.

The IFN-γ and IL-4 levels are not significantly different between early-stage sepsis and SIRS

The plasma levels of IFN-γ and IL-4 were measured as indicators of Th1 and Th2 cellular activity, which is regulated by Tregs. The IFN-γ and IL-4 levels in the plasma from the sepsis group were significantly higher than those in the control group: IFN-γ, 66.10 (45.23—85.08) pg/mLversus 20.97 (17.58—26.21) pg/mL, $p < 0.001$; IL-4, 100.69 (77.41—127.68) pg/mLversus 70.40 (64.14—80.15) pg/mL, $p < 0.001$. Compared with the healthy control group, the levels of these two cytokines in the SIRS group were also significantly higher: IFN-γ, 66.92 (61.20—79.23) pg/mLversus 20.97 (17.58—26.21) pg/mL, $p < 0.001$; IL-4, 101.81 (94.04—118.68) pg/mLversus 70.40 (64.14—80.15)pg/mL, $p < 0.001$. However, there was no significant difference between the sepsis group and the SIRS group. We also calculated the IFN-γ/IL-4 ratio in which significant differences between the sepsis group and the control group [0.66 (0.62—0.67) vs. 0.30 (0.27—0.33), $p < 0.001$] as well as

Table 1 Characteristics of the septic, SIRS, and control groups

Characteristics	Control ($n = 24$)	SIRS ($n = 15$)	Sepsis ($n = 37$)	*p*
Age (years)	56.54 ± 6.37	67.06 ± 12.57	61.67 ± 11.87	0.02
Gender: female/male	10/14	7/8	11/26	0.04
Diagnosis				
Pneumonia	—	—	28	
Pancreatitis	—	—	6	
Others	—	—	3	
Microbiological diagnosis				
Acinetobacter baumannii	—	—	13	
Pseudomonas aeruginosa	—	—	7	
Staphylococcus aureus	—	—	5	
Klebsiella pneumoniae	—	—	5	
Escherichia coli	—	—	5	
Enterococcus faecalis	—	—	1	
Hemolytic staphylococcus	—	—	1	
Surgical operation				
Cardiac valve replacement	—	7	—	
CABG	—	6	—	
Cardiac tumor resection	—	1	—	
Subtotal resection of thyroid	—	1	—	

CABG = coronary artery bypass graft surgery; SIRS = systemic inflammatory response syndrome.

between the SIRS group and the control group were noted. However, there was no significant difference between the sepsis group and the SIRS group. All statistical data are shown in Table 2.

The fraction of $CD4^+CD25^+Foxp3^+$ T cells was significantly elevated at an early stage in patients with septic shock

We assessed the expression of CD4 and CD25, which are the characteristic surface markers of Tregs, and found it to be 1.75% (0.59—2.24%) in the sepsis group, 1.08% (0.63—1.90%) in the SIRS group, and 1.07% (0.80—1.43%) in the control group (Fig. 1). Although the proportion of CD4 and CD25 was higher in the sepsis group than in the control and SIRS groups, the difference was not statistical significant. However, after the proportion of $CD4^+CD25^+Foxp3^+$T cells in $CD4^+CD25^+$ T cells was determined using flow cytometry, the percentage of $CD4^+CD25^+Foxp3^+$ T cells (Tregs) in $CD4^+CD25^+$ T cells was evaluated. We found that there was a significant increase in the percentage of Tregs in the sepsis group [74.40% (53.05—84.68%) vs. 51.76% (28.46—64.88%) vs. 60.45% (33.24—64.28%), $p = 0.003$] in comparison with the other two groups (data shown in Fig. 2). No significant difference in the percentage of Tregs was found between the SIRS group and the control group.

The plasma levels of soluble CD25 were significantly increased in patients with early-stage septic shocks

The plasma levels of soluble CD25 (IL-2sRa) were determined to investigate whether the increase in IL-2sRa in the plasma of the sepsis group was accompanied by an increase of $CD4^+CD25^+Foxp3^+$ Tregs. The plasma level of CD25 in the sepsis group was significantly higher than that in the SIRS group and the control group [408.76 (259.85—548.00) pg/mLvs. 345.94 (243.09—520.77) pg/mLvs. 140.33 (110.56—176.12) pg/mL, $p < 0.001$]. There was also a significant difference between the SIRS group and the control group (Fig. 3A).

Positive correlation between soluble CD25 and Tregs

We also found that the levels of CD25 cells in the plasma and the proportion of $CD4^+CD25^+Foxp3^+$T cells in $CD4^+CD25^+$ T cells were significantly and positively correlated (Fig. 3B; Spearman correlation coefficient = 0.390, $p = 0.003$).

Discussion

Our study showed that IFN-γ and IL-4 levels were highly increased in patients with early-stage septic shock when compared with the control, which suggests that the activity of Th1 and Th2 cells has increased. Although IL-4 was higher, IFN-γ/IL-4 ratio was still elevated. Therefore, patients who are at the early stage of the sepsis have increased levels of protective Th1 immunity, which was similar to the result of a study involving mice with early-stage septic shock.[10] A previous study showed that Tregs mediated the Th2 predominance in the later stage of the sepsis.[11] Our results suggested IL-4 levels were increased not only in the later stage of the sepsis but also in the early stage. So both pro- and anti-inflammatory responses were activated in patients with early-stage septic shock. The increased IFN-γ/IL-4 ratio suggests that the Th1 and Th2 immune responses are imbalanced. However, in early stages, the change in the inflammatory cytokines cannot differentiate sepsis from SIRS.

The principal finding of our study is that that the patients in early sepsis have a significant increase in the proportion of Tregs in their peripheral blood, as previously demonstrated in severe intra-abdominal infections.[10] Although Tregs comprise only a small fraction of the T-lymphocyte population in the immune system, they appear to potently regulate the activation of other cells in the immune system, making them an important participant in the inhibition of the immune response during sepsis. The patients with septic shock may be benefitted from the limitation of exacerbated infection-induced pathology, yet it increases the risk of secondary infection.

We found that the proportion of Tregs in early sepsis is much higher than that in control and SIRS groups, whereas no significant difference was found between the SIRS and control groups. Previous studies also found the same results, but the correlation between the proportion of Tregs and the severity of sepsis in patients remains unclear.[8,12] It is suggested that the proportion of Tregs may distinguish early-stage sepsis from SIRS. Thus, it may be possible for patients with septic shock to receive more suitable immune regulation treatment at an earlier time, and the manipulation of Treg cells may offer an innovative therapy for patients with septic shock. However, further study is required to understand the conditions under which such a therapy may be effective.

Table 2 Levels of IFN-γ and IL-4 in the septic, SIRS, and control groups

Factor	Normal	SIRS	Sepsis
IFN-γ[a] (pg/mL)	20.97 (17.58—26.21)	66.92 (61.20—79.23)*	66.10 (45.23—85.08)**
IL-4[a] (pg/mL)	70.40 (64.14—80.15)	101.81 (94.04—118.68)*	100.69 (77.41—127.68)**
IFN-γ/IL-4	0.30 (0.27—0.33)	0.66 (0.65—0.67)*	0.66 (0.62—0.67)**

SIRS versus normal: *$p < 0.001$; sepsis versus normal: **$p < 0.001$.
IFN = interferon; IL = interleukin; SIRS = systemic inflammatory response syndrome.
[a] Values are expressed as median (interquartile range).

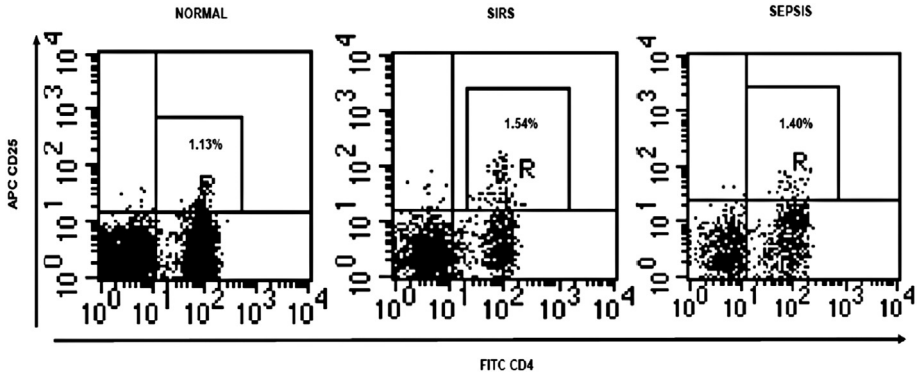

Figure 1. The fraction of $CD4^+CD25^+$ T cells among $CD4^+$ T cells in 37 patients with septic shock [1.75% (0.59—2.24%)], 15 patients with SIRS [1.08% (0.63—1.90%)], and 24 healthy individuals [1.07% (0.80—1.43%)] was detected by flow cytometry. Using Kruskal—Wallis test, the overall difference of $CD4^+CD25^+Foxp3^+$ T cells (regulatory T cells) among $CD4^+CD25^+$ T cells in the three groups found not to be statistically significant. $CD4^+CD25^+$ T cells are in the R frame. FITC = fluorescein isothiocyanate; SIRS = systemic inflammatory response syndrome.

In our experiment, 80% of the bacteria collected were Gram negative. It is unknown whether the type of bacteria plays a role in the regulation of Tregs in patients with septic shock. Cook et al found that various Toll-like receptors are expressed on the surface of Tregs, including lipopolysaccharide (LPS) receptors such as TLR4.[13] It was previously shown *in vivo* and *in vitro* that stimulation with LPS increased the surface markers for Tregs, which indicates the proliferation and inhibition of apoptosis of these cells.[14]

CD25 could be produced by activated Tregs,[15] and $CD4^+CD25^+$ T cells can express CD25 using certain enzymes (e.g., bromelain).[16] We found that the CD25 levels in plasma were increased, as described in a previous study.[17] We also found that the plasma levels of CD25 were significantly and positively correlated with the proportion of

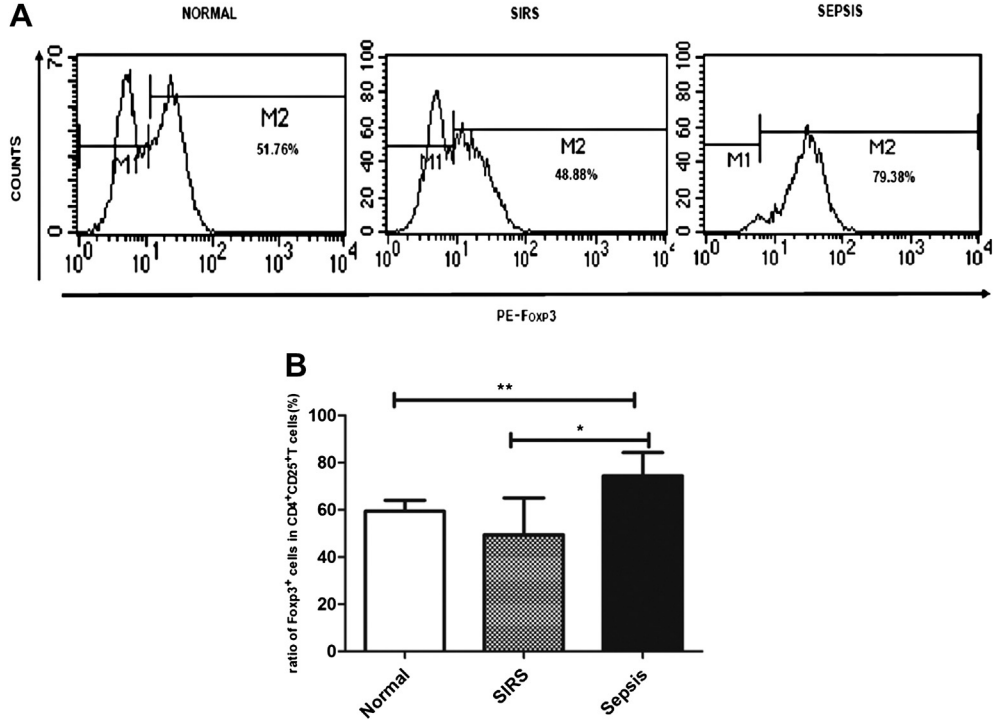

Figure 2. The fraction of $CD4^+CD25^+Foxp3^+$ T cells among $CD4^+CD25^+$ T cells in 37 patients with septic shock (66.82 ± 21.79%), 15 patients with SIRS (51.79 ± 21.79%), and 24 healthy individuals (56.45 ± 10.68%) was detected by flow cytometry. (A) The M2 peak represents the fraction of $CD4^+CD25^+Foxp3^+$ T cells among the $CD4^+CD25^+$ T cells. (B) The ratio of forkhead box P3 ($Foxp3^+$) T cells among $CD4^+CD25^+$ T cells is increased in patients with septic shock in comparison with healthy individuals and patients with SIRS (SIRS vs. sepsis: $*p < 0.05$; sepsis vs. normal: $**p < 0.05$). SIRS = systemic inflammatory response syndrome.

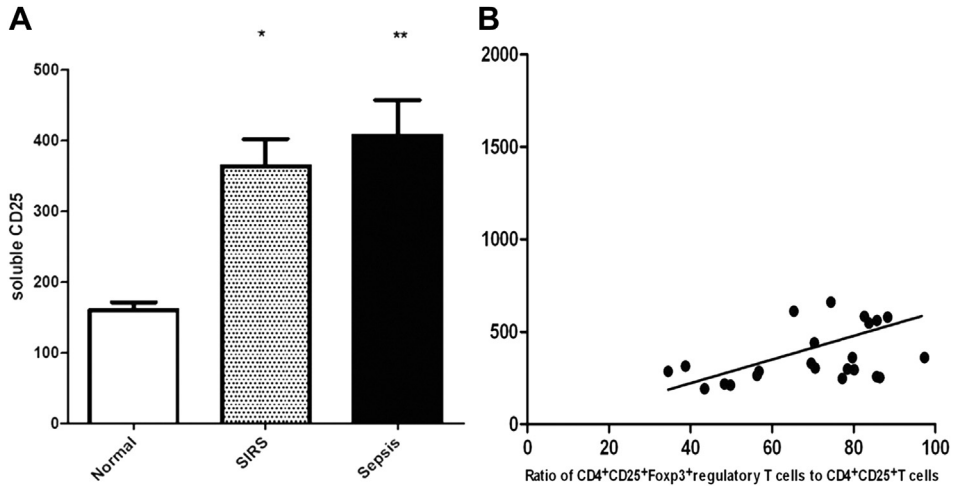

Figure 3. (A) The plasma levels of soluble CD25 (IL-2sRa) were detected by enzyme-linked immunosorbent assay. Differences among the three groups were evaluated using a nonparametric Kruskal—Wallis test. Differences between two groups were evaluated using Mann—Whitney U test (SIRS vs. normal: $*p < 0.001$; sepsis vs. normal: $**p < 0.001$). (B) The level of IL-2sRa (pg/mL) in plasma and the ratio of $CD4^+CD25^+Foxp3^+$ regulatory T cells to $CD4^+CD25^+$ T cells showed a significantly positive correlation (Spearman correlation coefficient = 0.390, $p = 0.003$). SIRS = systemic inflammatory response syndrome.

Tregs. Thus, the plasma level of CD25 may reflect the presence of Tregs, suggesting that CD25 may be a convenient marker for the diagnosis of septic shock in patients.

In conclusion, the results of our study indicate that the proportion of $CD4^+CD25^+Foxp3^+$ T cells can be helpful in the early diagnosis of sepsis. The presence of Tregs reflects the status of immune-suppression in patients with septic shock. Therefore, determining the proportion of $CD4^+CD25^+Foxp3^+$ T cells may allow patients to receive suitable immune-regulation treatment at an early stage. Tregs may also offer an innovative therapy for septic patients in the future.

Limitations

First, the number of the subjects enrolled in this study is not adequate, especially the patients with SIRS, which may bias the analysis. Second, the cause of septic shock in our patients is only due to bacterial infection and thus other infections such as fungi- or virus-induced sepsis were not explored. Third, although we tried to find out whether the sepsis occurred within 24 hours, we could not ensure that all the patients were really in this stage of the disease. Finally, the lack of function assay was another limitation of this study.

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References

1. Dombrovskiy VY, Martin AA, Sunderram J, Paz HL. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. *Crit Care Med* 2007;**35**:1244—50.
2. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *New Engl J Med* 2003;**348**:138—50.
3. Hotchkiss RS, Coopersmith CM, McDunn JE, Ferguson TA. The sepsis seesaw: tilting toward immunosuppression. *Nat Med* 2009;**15**:496—7.
4. Frazier WJ, Hall MW. Immunoparalysis and adverse outcomes from critical illness. *Pediatr Clin North Am* 2008;**55**:647—68. xi.
5. Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA* 2011;**306**:2594—605.
6. Venet F, Chung CS, Monneret G, Huang X, Horner B, Garber M, et al. Regulatory T cell populations in sepsis and trauma. *J Leukoc Biol* 2008;**83**:523—35.
7. Chatila TA. Role of regulatory T cells in human diseases. *J Allergy Clin Immunol* 2005;**116**:949—59.
8. Monneret G, Debard AL, Venet F, Bohe J, Hequet O, Bienvenu J, et al. Marked elevation of human circulating $CD4^+CD25^+$ regulatory T cells in sepsis-induced immunoparalysis. *Crit Care Med* 2003;**31**:2068—71.
9. Muckart DJ, Bhagwanjee S. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions of the systemic inflammatory response syndrome and allied disorders in relation to critically injured patients. *Crit Care Med* 1997;**25**:1789—95.
10. Fu Q, Cui N, Yu W, Du C. Percentages of $CD4^+$ T regulatory cells and HLA-DR expressing monocytes in severe intra-abdominal infections. *Scand J Infect Dis* 2010;**42**:475—8.
11. MacConmara MP, Maung AA, Fujimi S, McKenna AM, Delisle A, Lapchak PH, et al. Increased $CD4^+$ $CD25^+$ T regulatory cell activity in trauma patients depresses protective Th1 immunity. *Ann Surg* 2006;**244**:514—23.
12. Hein F, Massin F, Cravoisy-Popovic A, Barraud D, Levy B, Bollaert PE, et al. The relationship between $CD4^+CD25^+CD127^-$ regulatory T cells and inflammatory response and outcome during shock states. *Crit Care* 2010;**14**:R19.
13. Cook DN, Hollingsworth Jr JW, Schwartz DA. Toll-like receptors and the genetics of innate immunity. *Curr Opin Allergy Clin Immunol* 2003;**3**:523—9.

14. Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med* 2003;**197**:403—11.
15. Pedersen AE, Lauritsen JP. CD25 shedding by human natural occurring $CD4^{+}CD25^{+}$ regulatory T cells does not inhibit the action of IL-2. *Scand J Immunol* 2009;**70**:40—3.
16. Secor Jr ER, Singh A, Guernsey LA, McNamara JT, Zhan L, Maulik N, et al. Bromelain treatment reduces CD25 expression on activated $CD4^{+}$ T cells *in vitro*. *Int Immunopharmacol* 2009;**9**:340—6.
17. Saito K, Wagatsuma T, Toyama H, Ejima Y, Hoshi K, Shibusawa M, et al. Sepsis is characterized by the increases in percentages of circulating $CD4^{+}CD25^{+}$ regulatory T cells and plasma levels of soluble CD25. *Tohoku J Exp Med* 2008;**216**:61—8.