[Frontiers in Bioscience 16, 3004-3013, June 1, 2011]

Thymosin-alpha1 promotes the apoptosis of regulatory T cells and survival rate in septic mice

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1. ABSTRACT

Tregs are involved in immune disorder during sepsis; they can lead to a Th2 immune reaction. Their inhibitory effects can help alleviate inflammatory injury, but may also cause secondary immune inhibition. Thymosin-alpha1 is a polypeptide with powerful immunomodulatory activities. Current reports have shown that Thymosin-alpha1 conferred beneficial effects to septic patients. To explore the relationship between Thymosin-alpha1 and Tregs, in this study, we investigated the changing trend in $CD4^+CD25^+Foxp3^+$ T lymphocytes in a CLP septic mouse model. We also investigated the variation of apoptotic rate of $CD4^+CD25^+$ T lymphocytes, cytokine variation, and change of model survival rate when Thymosin-alpha1 intervening or not. We observed that the 72-h survival rate was improved, the percentage of $CD4^+CD25^+Foxp3^+$ T lymphocytes decreased and the apoptosis rate of $CD4^+CD25^+$ T lymphocytes increased after intervention of Thymosin-alpha1. At same time the expression of pro-inflammation cytokines IL-2, TNF-alpha and anti-inflammatory cytokines IL-10 and TGF-beta were regulated. In conclusion, Thymosin-alpha1 can effectively control the inflammatory response intensity and improve the 72-h survival rate of septic mice. Regulating Tregs may be another important role of Thymosin-alpha1 conditioning the immune reaction in sepsis.

2. INTRODUCTION

Severe sepsis and septic shock are leading causes of death in critically ill patients (1). The current research of sepsis focuses on finding answers to basic questions regarding infection, inflammation, immunology, coagulation, and tissue damage (2-5). Although developments in surgery, broad-spectrum antibiotics, instruments, and nutrient support have been growing in the past decades, mortality has yet not decreased significantly (6). Moreover, the advances in those therapies have caused some negative outcomes, such as the abuse of antibiotics and the large increase of medical costs (7, 8). The failure of anti-cytokine and anti-inflammatory therapies has made researches to reconsider the nature of sepsis (9). Now it is believed that specific immune dysfunction and non-specific inflammatory disorders are the most critical mechanisms of sepsis (10, 11). Also, the immune status is the key to the outcome of the disease (12, 13). Immunomodulation, a novel therapeutic strategy seeking not only to modulate non-specific inflammatory reactions, but also to stimulate specific immune function, has drawn significant attention in sepsis research, despite the existence of intense controversy (14-16).

Recent studies indicated that Tregs are involved in immune disorder during sepsis (17). Tregs are a subgroup of T lymphocytes with immunomodulatory activities. They inhibit activation of antigen presenting cells (APC) and T lymphocytes by cell-contact-dependent or cytokine secretion-dependent mechanisms, leading to a Th2 immune reaction (18). Tregs inhibit HLA-DR expression on $CD14^+$ monocytes by inducing Fas/FasL mediated apoptosis (19, 20), and thus, decrease immune mediated clearance. Previous studies in animal models have shown that Tregs are involved in both innate and acquired immune reactions during sepsis (21). The inhibitory effects of Tregs can help alleviate inflammatory injury, but may also cause secondary immune inhibition. It is worthwhile exploring how to maintain Tregs cell at an appropriate level to keep the balance of pro-inflammatory and anti-inflammatory reactions.

Thymosin-alpha1 is a polypeptide with powerful immunomodulatory activities (22, 23). It has frequently been used in anti-tumor and anti-viral immunotherapies and is now often administered to critically ill patients. Recent reports have shown that Thymosin-alpha1 conferred beneficial effects to septic patients, although underlying mechanisms remain unclear (24).

In this study, we studied the changing trend in $CD4^+CD25^+Foxp3^+$ T lymphocytes (the most representative Tregs population) in a CLP septic mouse model. We also investigated the variation of Tregs percentages in total $CD4^+$ T lymphocytes, apoptotic rate of $CD4^+CD25^+$ T lymphocytes, cytokine variation, and change of model survival rate after Thymosin-alpha1 intervention. Based on these results, we discussed the immune cross-talk among the sepsis, Thymosin-alpha1 and Tregs.

3. MATERIALS AND METHODS

3.1. Animal and grouping

SPF grade KM mice (weight 30-35 g; certification number: SCXK2007-003) were purchased from the Experimental Animals Center of the Second Military Medical University (Shanghai, China). The animals were randomly assigned into three groups according to the random number table: Healthy controls (HC), CLP sepsis group (CLP), and Thymosin-alpha1 intervention group (THI).

3.2. Surgical procedure of CLP

Based on the results of a pre-experiment, we adjusted the surgery procedure to achieve a 72-h survival rate of approximately 40%~50%. Animals were fasted with access to drinking water 12 h before the surgery. Animals were weighed and then anesthetized using 3% sodium pentobarbital (i.p., 1.5. ml/kg). The lower abdomen area was shaved and disinfected, and a surgical drape was applied before a median 1.0. cm incision was made in the lower abdomen. The cecum was carefully removed using forceps, and the distal 1/3 was ligated using a #4 thread. The ligated cecum was then punctured throughout twice at the middle using a #12 needle, and a little intestinal content was seen to be squeezed out. Then the cecum was relocated back into the abdominal cavity, and the abdomen was sutured. After the procedure, normal saline was administered subcutaneously (50 ml/kg) to replenish bodily fluid that was lost during the surgery. Mice were caged according to sampling time points (2 h, 6 h, 12 h, 24 h, 48 h and 72 h) with free access to food and drinking water. Sixteen mice were assigned in each subgroup. Mice were sampled and sacrificed at sampling time points if they survived.

3.3. Treatment for the THI group

The surgery procedure for the THI group was identical to the CLP group. After the surgery, Thymosin-alpha1 (100μg/kg, dissolved in normal saline, 50 ml/kg) was administered into the abdomen subcutaneously. Mice were caged according to the sampling time points (2 h, 6 h, 12 h, 24 h, 48 h and 72 h). Sixteen mice were assigned in each subgroup. Mice were sampled and sacrificed at sampling time points if they survived.

3.4. Treatment for HC group

The HC group was subdivided into healthy blank controls (HBC, n = 10) and Thymosin-alpha1 controls (HTC, n = 60). After fasting for 12 h (with access to drinking water), HBC animals were sacrificed for sample harvesting, whereas HTC animals were given Thymosin-alpha1 (100 μg/kg, dissolved in normal saline, 50 ml/kg) into the abdomen subcutaneously. At 2 h, 6 h, 12 h, 24 h, 48 h and 72 h after Thymosin-alpha1 administration, mice (n = 10 for each time point) were sacrificed for sample harvesting.

3.5. Survival rate

For calculation of survival rate, an additional 20 mice were added to the CLP group and the THI group. The

mice were combined with the other mice in their respective group for calculation of the survival rate within 72 h.

3.6. Sample collection

The mice that survived to the sampling time point were anesthetized using 3% sodium pentobarbital (i.p., 1.5. ml/kg). They were sacrificed by letting blood via heart. One centimeter of distal ileum was taken after laparotomy and then fixed in 10% formaldehyde for standard histological studies.

3.7. Flow cytometry

3.7.1. Measurement of $CD4^+CD25^+Foxp3^+$ T lymphocytes

EDTA-supplemented, freshly collected blood was centrifuged at 800 rpm for 5 min and then the supernatant was discarded. The cell pellet was resuspended and gently pipetted in erythrocyte lysis buffer (5× the volume of cell pellet) for 5 min. The lysate was centrifuged at 800 rpm at 4°C for 5 min, and the red supernatant was discarded. This step was repeated when the red blood cells were not lysed completely. The cell pellet was then resuspended and pipetted in 5 ml phosphate buffered saline (PBS) and centrifuged at 1200 rpm for 5 min. After washing, the cells were resuspended in PBS and counted. The cell concentration was adjusted to 1×10^6 cells/ml for staining. A total of 0.2.5μg of PE-Cy5 conjugated anti-mouse CD4 antibody (eBioscience , San Diego, CA, USA) and 0.1.25μg of PE anti-mouse CD25 antibody (eBioscience, San Diego, CA, USA) were added to the suspension, and the cells were incubated at 4°C for 30 min for cell surface staining. Afterwards, the cells were washed twice with PBS, fixed and permeabilized by incubation with 1 ml of 3:1 diluted Fixation/Permeabilizaiton solution (BD Biosciences, Pharmingen, San Diego, CA, USA) for 1 h at 4°C. The cells were then washed twice with 2 ml of permeabiliztion solution and resuspended in 100 μl of the same solution. FITC anti-mouse/rat Foxp3 antibody (eBioscience, San Diego, USA) was added, and cells were incubated for 1 h at 4°C for nuclear staining. Then, the cells were resuspended using 200 μl of Flow Cytometry Staining Buffer and analyzed by Flow Cytometry (BD FACSAria™, USA). $CD4^+CD25^+$ double-positive cells were gated out, and then the percentage of $Foxp3^+$ cells in this group was calculated based on the percentage of triple-positive cells in the T lymphocyte population. Non-stained negative controls were measured in parallel in each experiment.

3.7.2. Measurement of apoptosis in $CD4^+CD25^+$ double-positive cells

The blood samples were analyzed using the same protocol as described in 3.7.1. PE-Cy5 conjugated anti-mouse CD4 antibody and PE anti-mouse CD25 antibody were added to the cell suspension, and the cells were incubated at 4°C for 30 min for cell surface staining. Next, the cell pellets were resuspended in 100 μl of Binding Buffer and 10μl of Annexin V-FITC (20 μg/ml, Bender Medsystems, Vienna, Austria) and incubated for 30 min in the dark at room temperature. After this, 200 μl of Flow Cytometry Staining Buffer was added to the suspension and the cells were analyzed immediately using a flow cytometer. $CD4^+CD25^+$ double-positive cells were gated out and the percentage of Annexin V-positive cells was calculated, which indicated the apoptotic rate of $CD4^+CD25^+$ double-positive T lymphocytes.

3.8. Measurement of serum cytokines

The whole blood samples were kept standing for 15 min until coagulation. After high-speed centrifugation (3000 rpm, 20 min), the supernatant was collected, aliquoted and stored at -70°C for analysis. The concentrations of TNF-alpha, IL-2, IL-10 and TGF-beta were measured using specific ELISA kits (Diaclone, France) following the manufacturer's protocols. Briefly, standard curves and regression equations were calculated based on measurements of standard samples, and the cytokine concentrations in the plasma samples were calculated according to the regression equations.

3.9. Histological observation

1 cm distal ileum was collected from each mice after euthanasia. Ileum tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4μm/slide, and stained with HE by standard procedures. The histological slides with HE stain were read at 10 fields of high power (400×) using an Olympus BH2 microscope with computer-aided images analysis system (Qiu Wei Inc, Shanghai, China). The digital images were archived by a digital camera (Nikon 4500, Tokyo, Japan).

3.1.0 Statistical analysis

We used SPSS 10.0. for Windows version (SPSS Inc., IL, USA) for statistical analysis. The data were expressed as mean ±standard deviation (SD) when normally distributed. A *Barlett's* test was used for testing variance equality. If the variances were equal, a one-way analysis of variance (ANOVA) was undertaken to evaluate the difference, followed by a *post-hoc* LSD method for multi-comparisons. If the variances were not equal, a one-way approximate F test (F') was used for comparison. An independent *Chi-square* test was used for comparison of survival rates between the THI group and the CLP group. A P value of less than 0.05 was considered to be statistically significant.

4. RESULTS

4.1. Survival rate

There were no dead mice in HC group until the sample collection. As shown in the figure (Figure 1), Thymosin-alpha1 elevated the survival rate of sepsis mice significantly, compared to CLP group, there were visibly deference after operation 24 h. ($\chi^2 = 4.96$, $P = 0.026$ at 24 h; $\chi^2 = 4.59$, $P = 0.032$ at 48 h; $\chi^2 = 4.53$, $P = 0.033$ at 72 h).

4.2. Flow Cytometry

4.2.1. The percentage of Tregs in $CD4^+$ T lymphocytes

As shown (Figure 2), the percentage of $CD4^+CD25^+Foxp3^+$ T lymphocytes had no change with time in the healthy group after Thymosin-alpha1 injection ($P > 0.05$). In THI group, the $CD4^+CD25^+Foxp3^+$ T lymphocytes percentage did not change within the first 24 h, which increased gradually afterwards. In CLP group, the

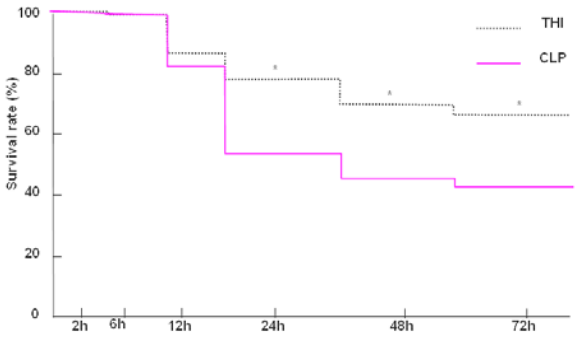

Figure 1. Thymosin-alpha1 elevated the survival rate of sepsis mice induced by CLP. The survival rate of THI mice was significantly higher than that of CLP group after 24 hours, (*) indicates a P value less than 0.05 ($\chi^2 = 4.96$, $P = 0.026$ at 24 h; $\chi^2 = 4.59$, $P = 0.032$ at 48 h; $\chi^2 = 4.53$, $P = 0.033$ at 72 h).

$CD4^+CD25^+Foxp3^+$ T lymphocytes percentage increased fast as early as 2 h and reached a higher level than THI group until the end of experiment ($P<0.05$ or $P<0.01$).

4.2.2. Apoptotic rate of $CD4^+CD25^+$ T lymphocytes

The apoptotic rate of $CD4^+CD25^+$ T lymphocytes was constantly low and did not fluctuate significantly in the HC group, regardless of Thymosin-alpha1 treatment. There was a mild increasing trend in the CLP group, whereas a significant increase occurred in the THI group. At 2 h and 6 h points, the apoptotic rates in the THI group were significantly lower than those in the CLP group ($P < 0.05$). However, at 12 h and later time points, the apoptotic rates in the THI group increased significantly, and statistically higher than those in the CLP group ($P < 0.01$). (Figure 3)

4.3. The change of serum cytokines

The concentrations of IL-2, TNF-alpha, IL-10 and TGF-beta in the HTC subgroup did not change significantly (Figure 4). Compared with the HBC subgroup, there was only a slight variation, but no statistically significant difference ($P > 0.05$). The IL-2 concentrations increased rapidly and peaked at 12 h and 24 h the in CLP and the THI groups, respectively. At 6 h and 12 h, IL-2 levels were significantly higher in the CLP group compared with the THI group ($P < 0.01$). The peak level was also higher in the CLP group than in the THI group ($P < 0.01$). The IL-2 levels decreased rapidly in the CLP group but slowly in the THI group ($P < 0.01$). TNF-alpha level in the THI group and the CLP group peaked at 12 h after the surgery and decreased rapidly in the CLP group and declined slowly in the THI group. At 2h and 6h, TNF-alpha level in the THI group was lower than in the CLP group ($P < 0.01$). However, TNF-alpha levels became higher in THI group than in the CLP group from 24 h to 72 h ($P < 0.01$). The peak levels of TNF-α in the THI group was slightly but not statistically lower than in the CLP group ($P > 0.05$). The changes in IL-10 and TGF-beta levels were similar in the THI group and the CLP group. They both increased gradually after surgery, and both were higher in the CLP group than in the THI group at all time points ($P < 0.05$ or $P < 0.01$).

4.4. Histological observation

Peritoneal exudates, accompanied with unpleasant odor and distal cecum binded-up and encapsulated, aggravated significantly after the CLP surgery 24 h and deteriorated afterwards. In the ileum, there was hyperemia and edema, an increase in inflammatory cells, loss of mucosa, and hemorrhage-induced vomica. The pathological changes were similar in both the THI and the CLP groups. However, the pathological damage was significantly milder in the THI group than in the CLP group at all corresponding time points (Figure 5). There were no pathological changes in ileums of the HC group.

5. DISCUSSION

The present study demonstrated that, in septic mice, Thymosin-alpha1 intervention can decrease Tregs percentages in T lymphocyte populations, increase the apoptotic rate of $CD4^+CD25^+$ T lymphocytes, alleviate ileum pathological damage, and, more importantly, increase survival rates. At the same time, the inflammatory cytokine profile also changed towards an appropriate direction. All these results suggested that there might be some correlation between Thymosin-alpha1 and Tregs. Besides the pro-inflammatory reaction, an anti-inflammatory reaction was also initiated during sepsis (25). Our study shows that the balance between these two reactions differs at different stages of inflammation. At an early stage, pro-inflammatory reactions dominate, whereas anti-inflammatory, or mixed reactions, dominate at later stage. Accordingly, the change in the percentage of Tregs indicated a correlation between the balance shift of pro/anti-inflammatory reactions and Tregs-mediated Th1/Th2 balance shift (26). It's reasonable to believe that the increase in the percentage of Tregs is a self-protection mechanism against severe inflammatory reactions in sepsis. In the CLP model, the cause of disease cannot be effectively treated. Thus, the sepsis-inducing bacterial, virus, and cecum necrosis can constantly over-stimulate the organism, leading to off-balance Tregs modulation and immune derangement, which is dominated by anti-inflammatory reactions.

Thymosin-alpha1 intervention stabilized the fluctuation of Tregs percentage change and cytokine concentrations, especially during the later phase of sepsis. When the organism becomes immuneosuppressed, Thymosin-alpha1 can inhibit the release of anti-inflammatory cytokines and stimulate the release of pro-inflammatory cytokines. At the same time, Thymosin-alpha1 also inhibits the increase of Tregs , which is an important factor that induces immunosuppression (27). These findings indicate that Thymosin-alpha1 intervention fits very well in the basic context of immunmodulation therapy. Previous clinical studies showed that $CD4^+CD25^+$ T lymphocytes were much higher in peripheral blood of septic patients who were treated, but still died in

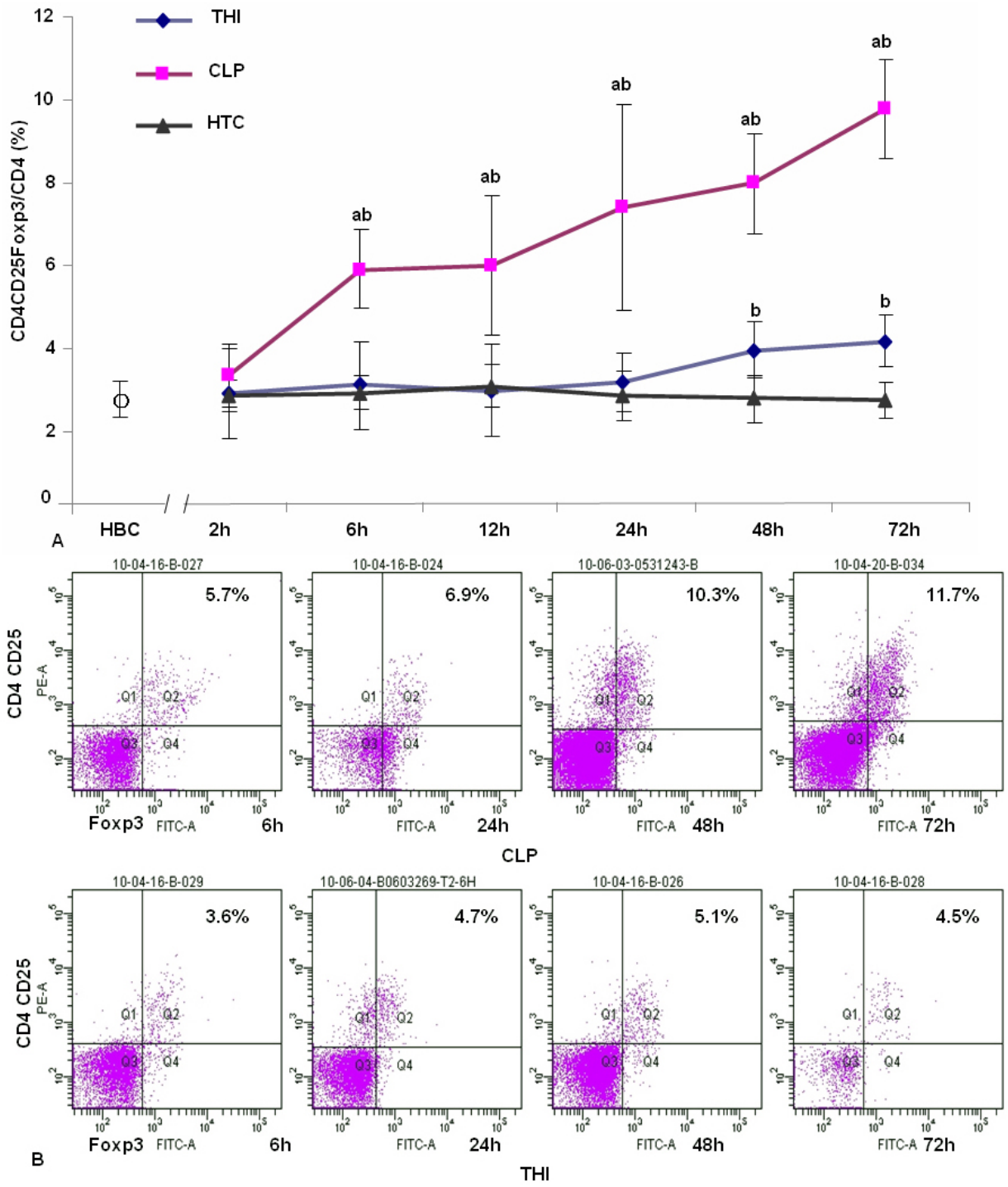

Figure 2. The percentage of $CD4^+CD25^+Foxp3^+$ T cells in $CD4^+$T lymphocyte population. (A) The percentage of $CD4^+CD25^+Foxp3^+$ T cells in $CD4^+$T lymphocytes population almost did not change in the HTC group, and the numbers were also not different from the HBC group ($P > 0.05$). In the THI group, the percentage did not change significantly within the first 24 h, but increased gradually afterwards. In the CLP group, the percentage increased soon as early as 2 h after operation. Compared with THI group, the percentage of $CD4^+CD25^+Foxp3^+$ T lymphocytes were keeping higher remarkable after 6 h and later ($P < 0.05$ or $P<0.01$), (^{a}) indicates a P value less than 0.01 comparing with THI, (^{b}) indicates a P value less than 0.01 comparing with HTC. (B) There were picked out many typical photos of flow cytometry to demonstrate the deference between the THI and CLP groups.

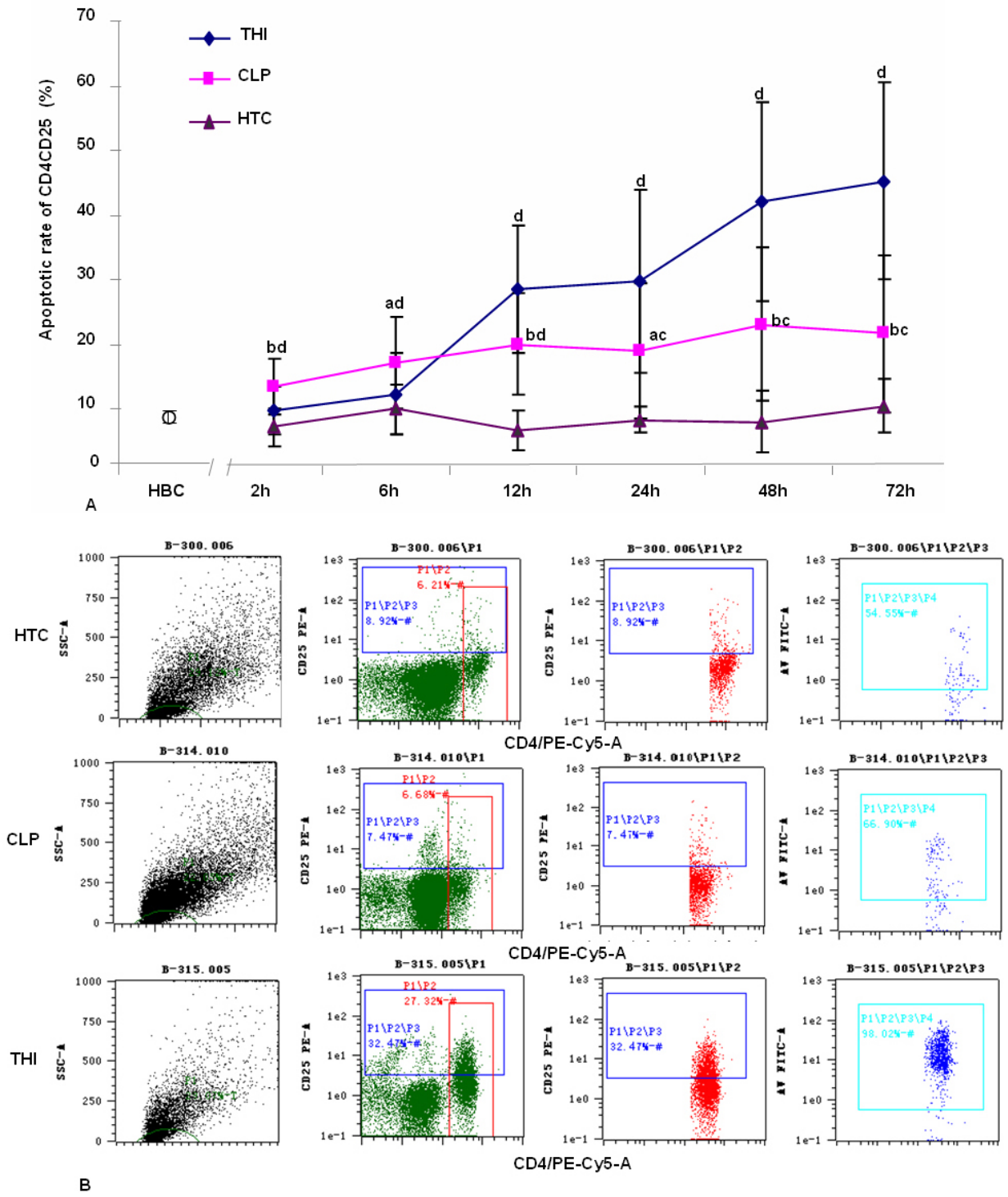

Figure 3. Apoptotic rates of $CD4^+CD25^+$ T lymphocytes. (A) the apoptotic rates of $CD4^+CD25^+$ T lymphocytes was constantly low and did not fluctuate significantly in the HC group, including HTC and HBC. There was a mild increasing trend in the CLP group, whereas a significant increase occurred in the THI group after surgery 6h. The apoptotic rates in the THI group were significantly higher than those in the CLP group at 12h and later. ($P < 0.01$), ([a]) indicates a P value less than 0.01 comparing with THI, ([b]) indicates a P value less than 0.01 comparing with HTC. (B) Displayed the calculating process of the apoptotic rate of $CD4^+CD25^+$ T lymphocytes by flow cytometry, and one sample of HTC, THI and CLP group laid out respectively.

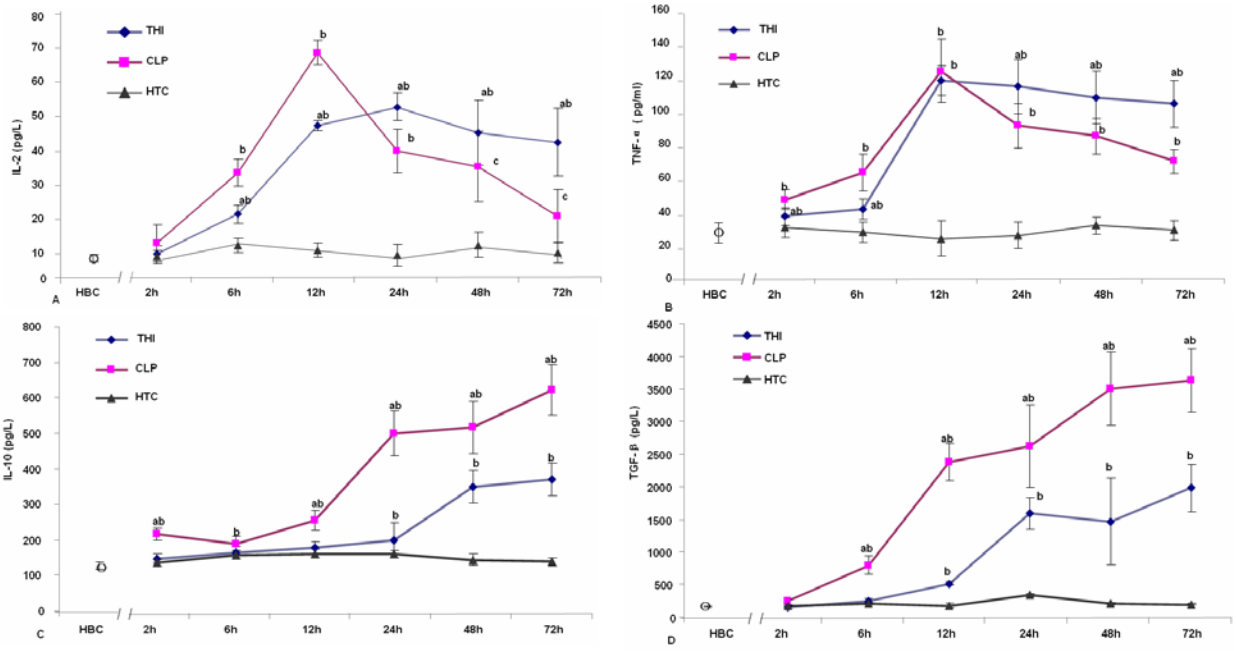

Figure 4. Concentrations of serum cytokines. The concentrations of IL-2, TNF-alpha, IL-10 and TGF-beta in the HTC subgroup did not change significantly. Compared with the HBC subgroup, there was only a slight variation, but no statistically significant difference ($P > 0.05$). (A) The IL-2 concentrations increased rapidly and peaked at 12 h and 24 h the in CLP and the THI groups, respectively. At 6 h and 12 h, IL-2 levels were significantly higher in the CLP group compared with the THI group ($P < 0.01$). The peak level was also higher in the CLP group than in the THI group ($P < 0.01$). The IL-2 levels decreased rapidly in the CLP group but slowly in the THI group ($P < 0.01$), ([a])indicates a P value less than 0.01 comparing with CLP, ([b]) indicates a P value less than 0.01 comparing with HTC. (B) TNF-alpha level in the THI group and the CLP group peaked at 12 h after the surgery and decreased rapidly in the CLP group and slowly in the THI group. At 2h and 6h, TNF-alpha level in the THI group was lower than in the CLP group ($P < 0.01$). However, TNF-alpha levels became higher in THI group than in the CLP group at 24 h and 72 h ($P < 0.01$). The peak level of TNF-alpha in the THI group was slightly, but not statistically ($P > 0.05$) lower than in the CLP group, ([a]) indicates a P value less than 0.01 comparing with CLP, ([b]) indicates a P value less than 0.01 comparing with HTC. (C and D) The changes in IL-10 and TGF-beta levels were similar in the THI group and the CLP group. They both increased gradually after surgery, and both were higher in the CLP group than in the THI group at all time points, ([a])indicates a P value less than 0.01 comparing with THI, ([b]) indicates a P value less than 0.01 comparing with HTC.

comparison with patients who survived (13). This also supports the idea that a decrease of Tregs in the later phase of sepsis is beneficial to recovery. Our results clearly showed that Thymosin-alpha1 can maintain immune homeostasis in septic mice, but does not affect any observed physical parameters in healthy controls. It is well known that Thymosin-alpha1 is a biological substance normally present in organisms. Thymosin-alpha1can induce differentiation and maturation of T lymphocytes, inhibit apoptosis of T lymphocytes (28, 29), and stimulate the immune system towards Th1 differentiation (30). Our study indicated that these immunomodulatory effects of Thymosin-alpha1 may be highly correlated with a change in Tregs levels.

Further analysis showed that the increase of Tregs percentages is due to, not only the increase of absolute number of cells, but also the increase of the relative number after the loss of total $CD4^+$ T lymphocytes (Figure 6). It was previously reported that activation-induced cell death (AICD) of $CD4^+CD25^+$ Tregs exists in autoimmune diseases. This might be an important mechanism by which organisms maintain certain levels of immune tolerance and avoids excessive immunosupression (10, 19). In sepsis, CD25 (alpha-chain of IL-2R) is highly expressed on Tregs cells and competitively binds IL-2 away from other effector cells. Those effector cells terminate proliferation and initiate apoptosis due to the lack of growth stimuli (18, 31). In sepsis, the expression of the Tregs-expressing molecule Foxp3 increases significantly (32), as does the secretion of IL-10 and TGF-beta. They can induce the differentiation of $CD4^+CD25^+$ T lymphocytes to Tregs cells, which becomes hyperactive in sepsis. It is even believed by some researchers that Tregs

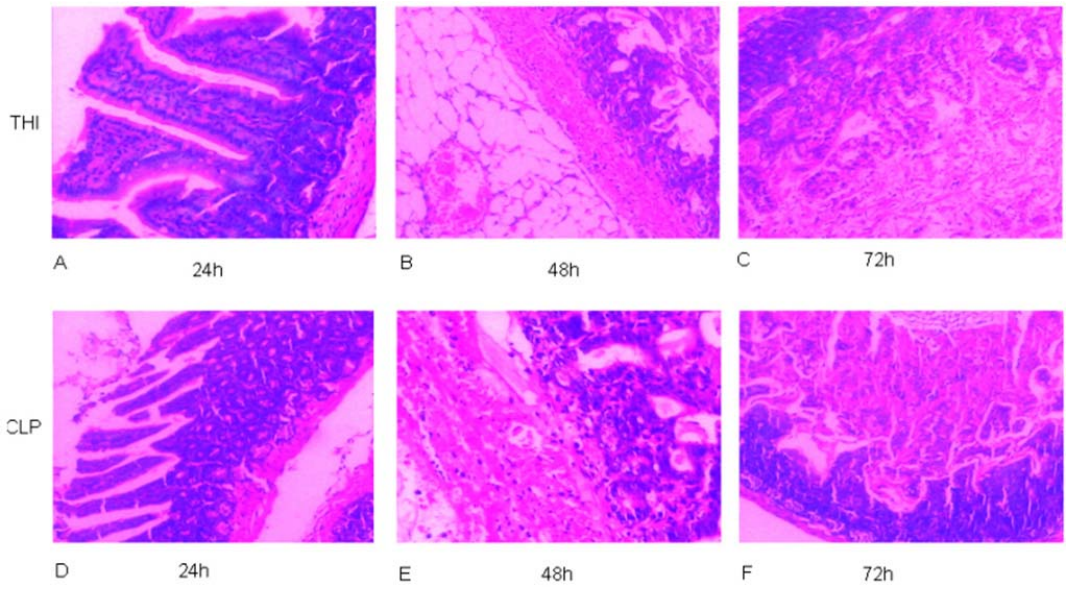

Figure 5. Pathological determination of ileum.Distal ileum pathological determination under microscopy (HE stain, 400×). (A), It was only a small amount of intestinal neutrophils, and no other abnormalities; (B),The intestinal neutrophils exudation was mild; (C), Portions of the muscles of necrosis and mucous membrane layer off; (D), with a large number of intestinal mucoca edema and infiltration and the neutrophilic exudation; (E),The intestinal neutrophils exudation increased, and large mass of muscles necrosis; (F), The intestinal mucosa exudation of neutrophils exaggerated, large mass of muscles necrosis and the intestinal wall structural damaged.

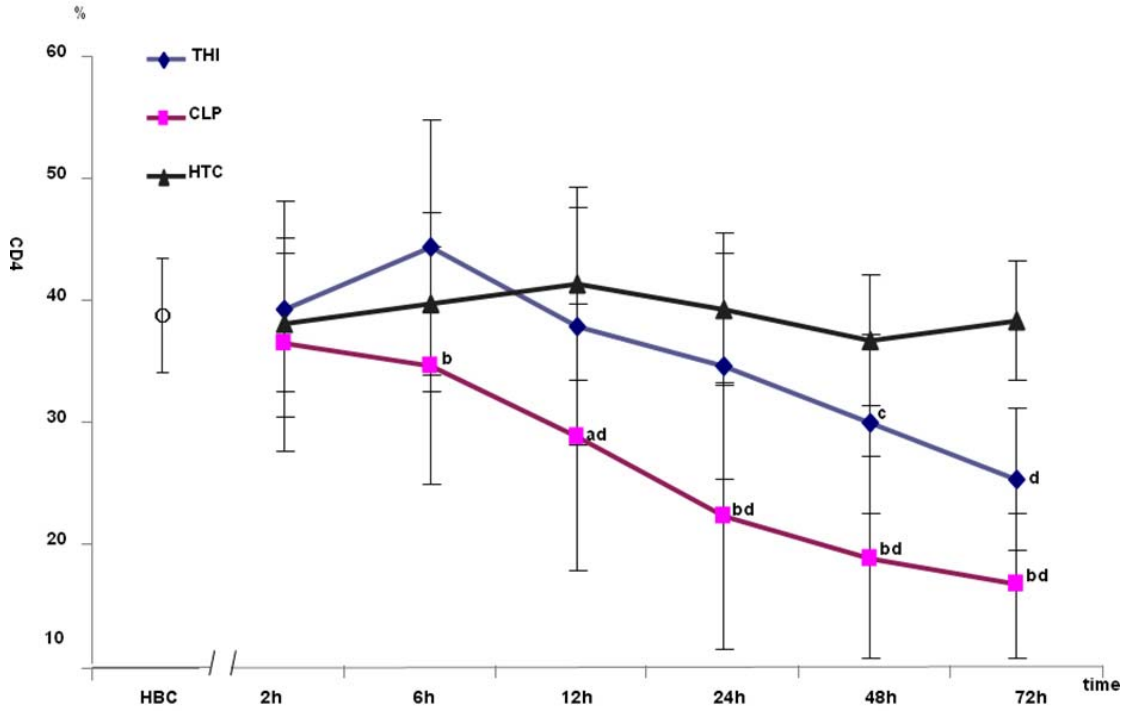

Figure 6. Changes of percentage of CD4+ T lymphocytes. There was no change of CD4+ T lymphocytes percentage of total T lymphocytes in HTC mice during the experiment. CD4+ T lymphocytes decreased in sepsis mice, both of THI and CLP groups, but there were deferent degree in two groups. CD4+ T lymphocytes decreased quickly after operation 2h, and then declined sustaining later. Yet compare with HTC, the percentage of CD4+ T lymphocytes did not decreased obviously until post operation 48h. The percentage of CD4+ T lymphocytes were significantly lower in CLP group than in THI group ($P<0.05$ or $P<0.01$). (^{a,b}) Indicate a P value less than 0.05 or 0.01 comparing with THI, (^{c,d}) indicates a P value less than 0.05 or 0.01 comparing with HTC.

can be anti-apoptotic in sepsis (33, 34). However, we are the first to show that Thymosin-alpha1 stimulates Tregs apoptosis in sepsis, especially in the later phase of the disease. This is especially significant because these results make immunomodulation-based therapies possible through manipulating Tregs levels in sepsis.

It is noteworthy that the Thymosin-alpha1-mediated Tregs apoptosis started mildly at the early phase of sepsis, and becomes gradually stronger along the advancement of the disease. Thymosin-alpha1 does not strengthen inflammation early during sepsis, but it can effectively inhibit the Treg-induced inmmunosuppresion later. Some previous immunomodulation-based bedside and bench studies drew similar conclusions (22, 24, 28). However, it remains unclear if the Thymosin-alpha1-mediated modulation of Tregs is through a direct or indirect mechanism. To answer this question, further studies are needed, including *in vitro* cell culture-based experiments.

6. CONCLUSION

Thymosin-alpha1 can effectively control the inflammatory response intensity and improve the survival rate of septic mice. Promoting the apoptosis of Tregs and reducing the proportion of Tregs in blood, may be another important role of Thymosin-alpha1 conditioning the immune reaction in sepsis.

7. ACKNOWLEDGEMENTS

Jian Wan and Yi Shan are co-first authors, Hongwei Shan and Zhaofen Lin are both corresponding authors. This work was partly financially supported by a grant from Shanghai Public Health Board (No. 08GWZX1103). The authors thank Shanghai Changzheng Hospital Experimental Centre for technical assistance during this study. The authors also thank Dr. Jie Zou for critical reading of the manuscript. All authors have no conflicts of interest relevant to this article.

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Abbreviations: Tregs, regulatory T cells; SPF, specific pathogen-free; HC, healthy control group; HBC, healthy blank control group; HTC, Thymosin-alpha1 healthy control group; CLP, cecal ligation and puncture/sepsis induced by cecal ligation and puncture group; THI, Thymosin-alpha1 intervention sepsis group; ELISA, enzyme-linked immunosorbent assay; HE, Hematoxylin-eosin; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor; EDTA, ethylene diamine tetracetic acid; FITC, fluorescein isothiocyanate

Key Words: Thymosin-alpha1, regulatory T cells, Sepsis, Survival Rate, Apoptosis; Inflammatory Cytokine, Immunodulation, Mouse

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