Original Article

Effect of transforming growth factor- β 1 on monocyte Toll-like receptor 4 expression in septic rats

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BACKGROUND: Sepsis is a tough problem in critical ill patients. This study aimed to investigate the dynamic changes of monocyte Toll-like receptor (TLR) 4 expression in peripheral blood of septic rats and to determine the effects of transforming growth factor (TGF) - β ₁ on TLR4 expression.

METHODS: Altogether 132 clean level SD rats were randomly divided into a control group ($n=12$), a sepsis model group ($n=60$), and a TGF- β ₁ intervention group ($n=60$). In the sepsis model group and TGF- β ₁ intervention group, the rats were subdivided into five groups (2-hour group, 6-hour group, 12-hour group, 24-hour group, and 48-hour group), with 12 rats in each group. Cecal ligation puncture (CLP) was performed in the sepsis model group and TGF- β ₁ intervention group to establish models of sepsis. The rats in the sepsis model group were injected with 1 mL normal saline at the caudal vein 0.5 hour after the model establishment; the rats in the TGF- β ₁ intervention group were injected with 20 ng/mL or 250 g TGF- β ₁ 0.5 hour after the model establishment. Flow cytometry was used to detect the change of monocyte TLR4 in peripheral blood, and enzyme-linked immunosorbent assay (ELISA) was used to detect the change of TNF- α level in peripheral blood.

RESULTS: At 6-12 hours after CLP, the monocyte TLR4 in peripheral blood started to decrease, and reached the lowest level at 12 hours. Compared to the control group, the monocyte TLR4 expression at 6 and 12 hours was lowered significantly ($P<0.05$). Compared to the sepsis model group at 2, 24 and 48 hours after CLP, the monocyte TLR4 expression in the TGF- β ₁ intervention group decreased dramatically ($P<0.05$), but there were no differences between the two groups at 6 and 12 hours respectively. Compared to the control group, the concentration of NF- κ B in liver tissue increased significantly 6 hours after CLP ($P<0.05$). After use of TGF- β ₁, the concentration of NF- κ B was decreased significantly but still higher than that of the control group. Compared to the control group, the concentration of TNF- α in peripheral blood was increased significantly at 2-48 hours after CLP ($P<0.05$). After use of TGF- β ₁, TNF- α was further increased.

CONCLUSION: During sepsis, TGF- β ₁ can decrease the monocyte TLR4 expression and NF- κ B in liver tissue, but facilitate the formation of proinflammatory mediator TNF- α . This finding indicates that TGF- β ₁ may play a role in promoting inflammatory response during sepsis, but this regulation is not via direct regulation of monocyte TLR4 in peripheral blood.

KEY WORDS: Sepsis; TGF- β ₁; TLR4; Monocyte; TNF- α

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INTRODUCTION

Sepsis is the most concerned clinical problem in critical ill medicine. Toll-like receptors (TLRs) play an important role in releasing massive inflammatory mediators in sepsis.^[1,2] TLR4 is a member of the TLRs

family, which involves in LPS recognition and signal transduction. Transforming growth factor (TGF) - β as a multifunctional growth factor is secreted by platelet, macrophage, cartilage cell, vascular smooth muscle cell, etc, and is widely expressed in many tissues and

organs.^[3,4] Its regulation in sepsis is also considered to be vital although its effect is still in controversy and its mechanism is unclear. In this study cecal ligation puncture (CLP) on rats was made to duplicate a sepsis model to determine the effect of TGF- β_1 on the dynamic changes of monocyte TLR4 expression and TNF- α expression as well as NF- κ B in the liver of septic rats.

METHODS

Animals

A total of 132 clean level SD rats, weighing between 22-250 g, were randomly divided into a control group ($n=12$), a sepsis model group ($n=60$) and a TGF- β_1 intervention group ($n=60$). In the control group, the rats were killed after anesthesia. Cecal ligation puncture (CLP) was performed in the sepsis model group and TGF- β_1 intervention group to establish models of sepsis. The rats in the sepsis model group were injected with 1 mL/250 g normal saline at the caudal vein 0.5 hour after the model establishment, and the rats in the TGF- β_1 intervention group were injected with 20 ng/mL or 250 g TGF- β_1 0.5 hour after the model establishment. In the sepsis model group and TGF- β_1 intervention group, the rats were divided into five sub-groups, namely 2-hour group, 6-hour group, 12-hour group, 24-hour group, and 48-hour group, with 12 rats in each group.

Establishment of the model

The rats were fasted for 24 hours before surgery, but were accessible to water. After intraperitoneal anesthesia with 2% pentobarbital sodium (40 mg/kg), the rats were fixed on the operation pad. After a sterile hole towel was put over the rat's belly, a 2 cm long incision was made along the abdominal midline to expose the cecum. The root of the cecum was ligated using silk suture to avoid obstruction. Later a 18-0 needle was used to puncture the cecum 3 times and leave a 2 mm wide rubber slice through both sides of cecal wound to prevent the wound closure, put the cecum back into the abdominal cavity after the puncture, and close the abdominal incision layer by layer. Five mL of Ringer's solution was injected into the abdominal cavity. The rats were free to eat and drink after the procedure. The mortality rate of the rates was 92.5% after modeling.

Detection of monocyte TLR4 expression in peripheral blood

A flow cytometer (FACS-VANTAGE SE) was used to detect the expression of monocyte TLR4 in

peripheral blood. Monocyte was picked out from forward and lateral scattering, and the TLR4 expression on the monocyte was documented by monocyte TLR4 expression percentage.

Detection of TNF- α and NF- κ B

The peripheral blood of the rats was precipitated by centrifugation, and blood serum was stored in a freezer for subsequent testing. Enzyme-linked immunosorbent assay (ELISA) was used to test the sample strictly according to the instructions of the test kit. OD value was read at 450 nm by an UV spectrophotometer, standard curve was produced by ELISA software, and the concentration of test sample was calculated by the OD value.

Statistical analysis

Mean standard deviation was used to express the acquiring data from this study, which were calculated by the SPSS 11.5 statistics software. The three groups were compared by ANOVA. The relevance between TNF- α and TGF- β_1 was used in correlation analysis. A P value <0.05 was considered statistically significant.

RESULTS

Changes of TLR4 expression in monocyte in peripheral blood

At 2 hours after CLP, the monocyte TLR4 expression in peripheral blood decreased, and reached the lowest level at 12 hours. Compared to the control group, the monocyte TLR4 expression at 6 and 12 hours was significantly decreased ($P<0.05$). Compared to the sepsis model group at 2, 24 and 48 hours after CLP, the monocyte TLR4 expression in the TGF- β_1 intervention group decreased dramatically ($P<0.05$), but there were no differences between the two groups at 6 and 12 hours respectively (Table 1).

Change of NF- κ B in liver tissue

At 6-48 hours after CLP procedure, the NF- κ B concentration in the liver tissue increased more significantly than in the control group ($P<0.05$). The NF- κ B concentration in the liver tissue decreased more significantly in the TGF- β_1 intervention group than in the sepsis model group ($P<0.05$) (Table 2).

Change of TNF- α concentration in rat peripheral blood

The concentration of TNF- α was increased more

Table 1. The monocyte TLR4 expression in peripheral blood at different time points

Groups	2h	6h	12h	24h	48h
Control	52.68±10.52	52.68±10.52 [#]	52.68±10.52 [#]	52.68±10.52	52.68±10.52
CLP	43.89±11.12	36.72±5.07 [*]	27.10±8.46 [*]	54.45±2.82	43.55±16.49
TGF-β ₁	22.90±9.34 ^{*#}	30.49±2.39 [*]	24.86±2.39 [*]	28.38±5.10 ^{*#}	10.70±3.77 ^{*#}

Compared to the control group at the same time point, ^{*}P<0.05; compared to the CLP group at the same time point, [#]P<0.05

Table 2. The change of NF-κB in liver tissue at different time points

Groups	2h	6h	12h	24h	48h
Control	63.0±23.0	63.7±11.0	71.5±13.5	57.8±25.7	75.1±9.4
CLP	121.6±18.9	151.4±13.8 [*]	166.9±18.7 [*]	229.0±30.9 [*]	235.6±23.9 [*]
TGF-β ₁	118.1±10.3	124.4±11.3 [▲]	127.1±17.2 [▲]	147.5±19.5 [▲]	203.3±19.7 [▲]

Compared to the control group at the same time point, ^{*}P<0.05; compared to the CLP group at the same time point, [▲]P<0.05

Table 3. Changes of TNF-α concentration in peripheral blood at different time points

Groups	2h	6h	12h	24h	48h
Control	53.39±2.14 [#]	53.39±2.14 [#]	53.39±2.14 [#]	53.39±2.14 [#]	53.39±2.14 [#]
CLP	78.43±3.46 [*]	67.32±4.23 [*]	72.54±5.66 [*]	63.94±4.89 [*]	66.30±3.51 [*]
TGF-β ₁	90.75±7.15 ^{*#}	90.69±6.66 ^{*#}	130.78±7.60 ^{*#}	93.36±7.21 ^{*#}	97.00±5.42 ^{*#}

Compared to the control group at the same time point, ^{*}P<0.05; compared to the CLP group at the same time point, [#]P<0.05

significantly in peripheral blood than in the control group at 2-48 hours after CLP ($P<0.05$, Table 3). The TNF-α concentration in serum sample of the TGF-β₁ intervention group was not correlated with the positive rate of TLR4 expression in monocytes ($r=0.127$, $P>0.05$).

DISCUSSION

TLR4 can be found in many tissues, its mRNA also can be found in monocytes, neutrophils, dendritic cells, vascular endothelial cells, and intestinal epithelial tissue. Different immune cells show different TLR4 expressions after infection even in the immune cells subtypes.^[5] As one of the phagocytes, monocytes play an important role in the host defense. The activation of monocytes and release of excessive inflammatory mediators are the key factors of sepsis and its progress.

Although some studies have indicated that the level of TLR4 expression in monocytes increases in septic patients, there is still no consensus about whether TLR4 on monocyte surface is upregulated in cases of sepsis.^[6,7] The present study showed that TLR4 expression was decreased on monocyte surface in peripheral blood, and there were statistical significances at 6 and 12 hours after CLP compared to the control group.^[8] Decreased TLR4 expression in monocytes may be due to the fact that, during sepsis, TLR4 transmits LPS simulation signal into cells, activates downstream signal reaction after a series of signaling pathways, stimulates the transcription of nuclear NF-κB, and induces the rapid

activation and secretion of inflammatory mediators. Yang et al^[9] found that LPS signal, which was induced by TLR4, could increase the NF-κB activity by 35 times. To counteract the excessive inflammatory response, the body mobilizes the anti-inflammatory response system to counteract further expression of inflammation. A decreased TLR4 expression may indicate compensatory anti-inflammatory response, and it may also be related to LPS tolerance. It was found that the down-regulated TLR4 expression in rat's macrophage was related to endotoxin tolerance.^[10] Fujihara et al^[11] also found a decreased TLR4 expression on monocyte surface in peripheral blood of septic patients, indicating the monocyte tolerance to LPS.

A recent study^[10] found that TGF-β has pleiotropic immunomodulatory effects. Others found that when the TGF-β₁ gene was removed from mice, there was a multiple organ inflammatory response showing lymphocyte infiltration and autoimmune diseases. The death of mice indicates the immunosuppressive effect of TGF-β. TGF-β takes part in inflammatory response, and after LPS stimulation, inflammatory response of the mice with TGF-β gene deficiency is out of control.^[12] These studies indicated that TGF-β can suppress immune response, reduce the release of proinflammatory factors, and exert counteracting effect on excessive inflammatory response in sepsis. However some studies came to an opposite conclusion. Garcia-Lazaro et al^[13] found that TGF-β to immune cells may suppress the function of inflammatory effectors, TGF-β to liver cells seems to promote LPS-

stimulated secretion of inflammatory cytokines and to predispose for lethal endotoxemic shock. Wang et al^[14] found that the high expression of TGF- β_1 in keratinocytes could delay the wound healing. But we can't conclude that TGF- β_1 is involved in the process of pro-inflammatory response, because NF- κ B decreases in the liver tissue. NF- κ B is a key regulatory factor, and can produce a variety of inflammatory mediators, and this indicates that the inflammation response is not in an aggravating process. The increase of TNF- α in blood may be associated with other causes, as Ruemmele et al found that by regulating TLR4, intestinal epithelium cells have endogenous secretion of TNF- α .

In our study, TGF- β_1 decreased the expression of monocyte TLR4 in peripheral blood and NF- κ B in the lung tissue, while increased the expression of TNF- α . The TNF- α concentration in serum sample of the TGF- β_1 intervention group was not correlated with the TLR4 expression or NF- κ B ($r=0.127$, $P>0.05$). O'Mahony et al^[15] found that combined with ligand, the expression of TLR2 or TLR4 in monocyte was not affected. Brunialti et al^[16] and associates found that in patients with severe sepsis and septic shock, TLR2 and TLR4 still expressed despite the decrease of cytokine production. This indicated that the change of inflammatory factors was not caused by the change of TLR expression, which may be related to intracellular signaling or regulation of other inflammatory factors. However, the decreased TLR4 may indicate a feedback regulation.

The pathogenesis of sepsis is very complicated. It is an effective network involving inflammatory cells and factors, therefore the mechanism can not be explained by single factor or single effect. This study indicates that TGF- β_1 may play a role in promoting inflammatory response in the process of sepsis, which may not be regulated by TLR4 expression.

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