

# Induction of acute hepatic injury by endotoxin in mice

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**Objective:** To investigate the changes of scavenger receptor (SR) and CD<sub>14</sub> in Kupffer cells in endotoxemia in order to uncover the mechanism of the liver to turn a defense organ into effector one in sepsis.

**Methods:** Mouse models of endotoxemia of different severity were reproduced by injection of different doses of lipopolysaccharide (LPS) via the tail vein. The expression of SR and CD<sub>14</sub> in the liver was assayed by immunohistochemistry and was subsequently analyzed with an image analysis system. The levels of TNF- $\alpha$  and IL-6 in liver tissue were determined with ELISA.

**Results:** The expression of SR in the liver in the high-dose group was markedly decreased one hour after injection of LPS, and also in the low-dose group at 3 hours. The expression of SR in the liver in the two groups was shown to be progressively decreased with the time prolonged. There was significant difference in average optical density (OD) values of SR between the two groups. The expression of CD<sub>14</sub> in the two groups was shown to be significantly increased one hour after injection of LPS, and more significantly with the time prolonged. But there was no significant difference in OD values of CD<sub>14</sub> between the two groups. The contents of intrahepatic proinflammatory mediators TNF- $\alpha$ , IL-6, ALT and TBIL were significantly increased after injection of LPS. Correlation analysis revealed that the changes of TNF- $\alpha$ , IL-6, ALT, and TBIL were negatively correlated with the expression of SR, and positively with the expression of CD<sub>14</sub>.

**Conclusion:** The up-regulation of CD<sub>14</sub> expression and down-regulation of SR expression on Kupffer cells might be one of the important mechanisms for the conversion of Kupffer cells from immune defensive to inflammatory response cells in acute hepatic injury.

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**Key words:** hepatic injury; endotoxemia; scavenger receptor; CD<sub>14</sub>; tumor necrosis factor- $\alpha$ ; interleukin

## Introduction

Liver is the largest defense and effector organ in sepsis.<sup>[1]</sup> Kupffer cells (KC) play a central role in the clearance of bacteria and their toxins from the body. Moreover, they also play a pivotal role in the pathogenesis of hepatic injury in sepsis.<sup>[2]</sup> It has been reported that scavenger receptor (SR) and CD<sub>14</sub> are the receptors responsible for regulation of clearance and inactivation of endotoxin by macrophages and activation of macrophages respectively.<sup>[3–5]</sup> The aims of this study were to investigate the expression of SR and CD<sub>14</sub> and their relations to TNF- $\alpha$ , IL-6, ALT and TBIL in endotoxemia in order to detect the mechanism for the decreased defense and the enhanced effector function of the liver in sepsis, which may provide evidence for the establishment of new therapeutic regimen for sepsis.

## Methods

### Animals

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Seventy-two Kunming mice of both sexes weighing  $20 \pm 2$  g were used. The animals were divided into control ( $n = 8$ ), low-dose (1 mg/kg,  $n = 32$ ), and high-dose (10 mg/kg,  $n = 32$ ) lipopolysaccharide (LPS) groups. They were sacrificed 1, 3, 5 and 8 hours after injection of LPS, 8 animals per each timepoint. One or 10 mg/kg LPS in 0.9% saline was injected into the mice via the tail vein. The animals were sacrificed immediately at 1, 3, 5 and 8 hours, respectively. Blood and liver tissue were taken and stored at  $-70$  °C for later use.

### Expression of SR and CD<sub>14</sub>

For standard immunohistochemistry examination, tissue samples were cut into 4  $\mu$ m paraffin sections, deparaffinized and dehydrated. Endogenous peroxidase activity was quenched with 0.3% H<sub>2</sub>O<sub>2</sub>-methanol. After being blocked with 10% normal goat serum, the sections were incubated for 1 hour at 37 °C and overnight at 4 °C with primary rat anti-mouse SR (ZF8, Serotec Ltd., UK) or rat anti-mouse CD<sub>14</sub> (Pharmingen, San Diego, CA, USA) mAb diluted in PBS at a final concentration of 1:600. Biotinylated rabbit anti-rat IgG diluted in 1% bovine serum albumin-PBS at a final concentration of 1:200 was then added and incubated for 30 min at 37 °C, followed by incubation with streptavidin/peroxidase complex diluted in PBS (1:200) for 30 min at 37 °C. The presence of SR and CD<sub>14</sub> was revealed by incubation with 3, 3'-diaminobenzidine (DAB). The sections were counter-stained with hematoxylin. The expression of SR and CD<sub>14</sub> was quantitatively determined with a PECBIAS image analysis system. The expression intensity of SR and CD<sub>14</sub> was expressed as mean OD values.

### TNF- $\alpha$ and IL-6 levels in liver tissue

Liver specimens were homogenated at 4 °C. The final supernatant was determined with the method described by Bradford et al.<sup>[7]</sup> The levels of TNF- $\alpha$  and IL-6 in hepatic homogenates were measured with ELISA kits designed for the determination of mouse cytokines (Endogen Inc, Woburn, MA, USA) according to the manufac-

turer's instructions. The assay was run in duplicate. The presence of the cytokines was detected at 450 nm (reference 550 nm) using a microplate reader. The cytokine concentrations were automatically computed according to the standard curve.

### ALT and TBIL level in serum

The levels of serum ALT and TBIL were tested with auto-biochemical analyzer.

### Histopathological study

The liver specimens were fixed in formalin, paraffin-embedded, sectioned, and stained with HE.

### Statistical analysis

The data were expressed as  $\bar{x} \pm s$ . The difference between each group was analyzed by *t* test and the correlative significance was defined as  $P < 0.05$ .

## Results

### Expression of SR

Massive expression of SR was observed in the liver in sham animals, being mainly distributed on the surface of Kupffer cells. The intra-hepatic expression of SR was shown to be significantly decreased in the two groups 3 hours after injection of LPS, and less with prolonged experimental time. Imaging analysis indicated that the mean OD values of SR in the 10 mg/kg group were significantly decreased 1 hour after injection of LPS compared with those in the sham group ( $P < 0.01$ ). The mean OD values in the 1 mg/kg group were also markedly decreased at 3 hours ( $P < 0.01$ ). The intra-hepatic expression of SR was inhibited by LPS in a time and dose-dependent manner. As shown by imaging analysis, the mean OD values of SR expression in the 10 mg/kg group were obviously lower than those in the 1 mg/kg group 1 hour after injection of LPS. The inter-group difference was more obvious with prolonged experimental time (Table 1).

### Expression of CD<sub>14</sub>

**Table 1.** Changes of average OD values of SR, CD<sub>14</sub> and dose-dependent response to LPS in hepatitis induced by endotoxin

Groups (mg)	Controls	After injection of LPS (h)			
		1	3	5	8
SR	1943 ± 76				
1		187 ± 76	1600 ± 86**	1480 ± 52**	1260 ± 60*
10		1820 ± 102***	1300 ± 82***	1030 ± 82***	794 ± 69***
CD <sub>14</sub>	970 ± 85				
1		1100 ± 116*	1590 ± 106**	1590 ± 106**	2121 ± 165**
10		1151 ± 152*	1633 ± 91**	1633 ± 91**	2252 ± 145**

Compared with controls, \*  $P < 0.05$ , \*\*  $P < 0.01$ ; compared with 1 mg/kg LPS group, #  $P < 0.01$ .

**Table 2.** Changes in TNF- $\alpha$ , IL-6 levels and dose-dependent response to LPS

Groups (mg)	Controls	After injection of LPS (h)			
		1	3	5	8
TNF- $\alpha$	47 ± 5.7				
1		92 ± 11**	77 ± 7**	51 ± 8	51 ± 9
10		113 ± 9*** <sup>▲</sup>	91 ± 10*** <sup>▲</sup>	57 ± 6*	53 ± 7
IL-6	53.9 ± 7.4				
1		61.7 ± 5.9*	90.6 ± 5.3**	75.2 ± 7.3*	67.8 ± 7.6*
10		67 ± 6.22**	116.1 ± 12*** <sup>▲▲</sup>	100.6 ± 6.8*** <sup>▲▲</sup>	94.3 ± 6.8*** <sup>▲▲</sup>

Compared with controls, \*  $P < 0.05$ , \*\*  $P < 0.01$ ; compared with 1 mg/kg LPS group, <sup>▲</sup>  $P < 0.05$ , <sup>▲▲</sup>  $P < 0.01$ .

The positive products of CD<sub>14</sub> expression were mainly located on the surface of Kupffer cells. Little intrahepatic expression of CD<sub>14</sub> was observed in the sham group. The intra-hepatic expression of CD<sub>14</sub> was markedly increased in the two groups 3 hours after injection of LPS, and diffusely distributed in the liver at 8 hours. The results of imaging analysis indicated that the mean OD values of CD<sub>14</sub> expression in the two groups were significantly higher than those in the sham group 1 hour after injection of LPS ( $P < 0.05$ ), and were much increased with prolonged experimental time. There were no significant differences between the two groups (Table 1) (Fig. 1).

#### TNF- $\alpha$

The levels of TNF- $\alpha$  in the liver tissue in both 1 and 10 mg/kg groups reached a peak at 1 hour after injection of LPS ( $P < 0.01$ ) and progressively decreased afterwards. At 5 hours, TNF- $\alpha$  level, though returning to control level in the 1 mg/kg group, was still markedly higher in the 10 mg/kg group ( $P < 0.05$ ). Intra-hepatic TNF- $\alpha$  levels in the 10 mg/kg group were significantly

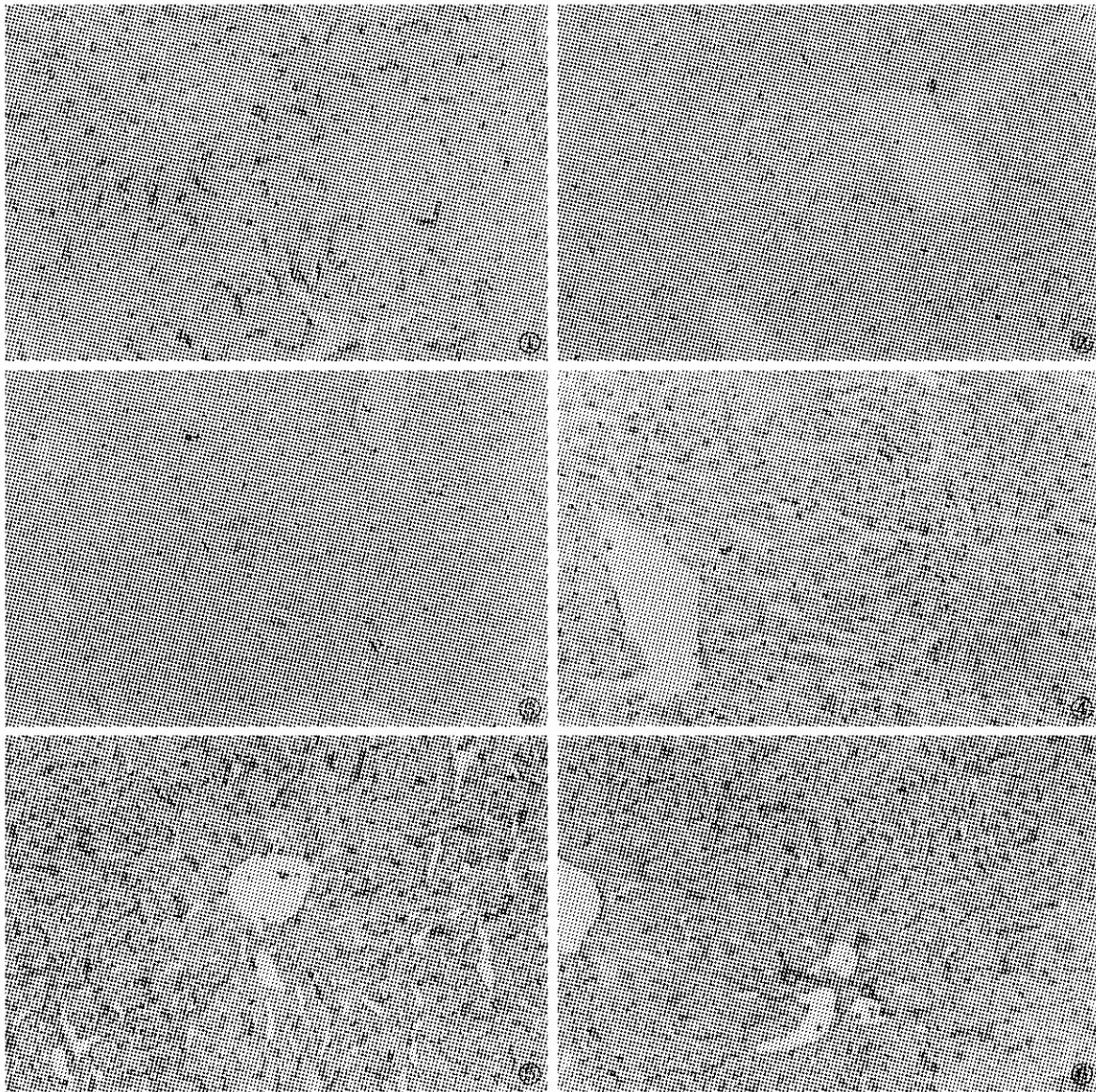
higher than those in the 1 mg/kg group 1 and 3 hours after injection of LPS (Table 2).

#### IL-6

The levels of IL-6 in the liver in both 1 and 10 mg/kg groups were significantly higher than those in the sham group 1 hour after injection of LPS ( $P < 0.05$  and  $P < 0.01$ ) and peaked at 3 hours, and progressively decreased afterwards. The IL-6 levels in both groups were still markedly higher than those in the sham group at 8 hours. Intrahepatic IL-6 levels in the 10 mg/kg group were significantly higher than those in the 1 mg/kg group at 3, 5, and 8 hours after injection of LPS ( $P < 0.01$ ) (Table 2) (Figs. 2–6).

#### ALT and TBIL

The levels of ALT, TBIL in serum in the 10 mg/kg group were significantly increased 1 hour after injection of LPS compared with those in the sham group ( $P < 0.05$  and  $P < 0.01$ ). The levels of ALT, TBIL in the 1 mg/kg group were also markedly increased at 3 hours ( $P < 0.05$  and  $P <$



**Fig. 1.** Intrahepatic expression of SR in the sham group, showing diffuse distribution of brown granules (original magnification  $\times 200$ ).

**Fig. 2.** 1 mg/kg group. Intrahepatic expression of SR is shown to be markedly decreased 8 hours after LPS injection (original magnification  $\times 200$ ).

**Fig. 3.** Intrahepatic expression of SR in the 10 mg/kg group 8 hours after LPS injection, showing sporadic stained granules (original magnification  $\times 200$ ).

**Fig. 4.** Intrahepatic expression of CD<sub>14</sub> in the sham group, showing few brown granules on the cell surface (original magnification  $\times 200$ ).

**Fig. 5.** Intrahepatic expression of CD<sub>14</sub> in the 1 mg/kg group 8 hours after injection of LPS, showing diffuse distribution of brown granules on cell surface (original magnification  $\times 200$ ).

**Fig. 6.** Intrahepatic expression of CD<sub>14</sub> in the 10 mg/kg group 8 hours after LPS injection, showing diffuse distribution of stained granules on cell surface (original magnification  $\times 200$ ).

**Table 3.** Changes in plasma ALT, TBIL levels and dose-dependent response

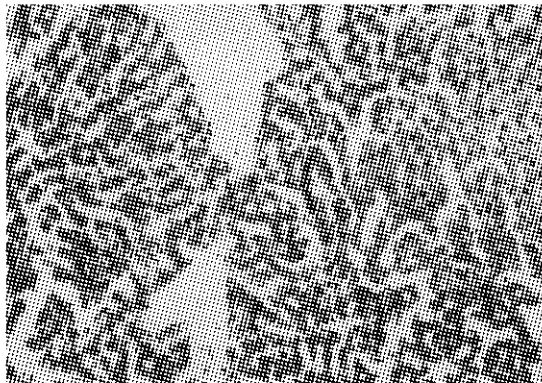
Groups (mg)	Controls	After injection of LPS (h)			
		1	3	5	8
ALT	14.4 ± 1.1				
1		15.19 ± 1.30	15.69 ± 0.98*	18.85 ± 1.6**	22.8 ± 1.45**
10		19.28 ± 1.65**	26.7 ± 1.87***	35.7 ± 3.9***	38.5 ± 3.19***
TBIL	1.57 ± 0.12				
1		1.71 ± 0.13	1.96 ± 0.15**	2.4 ± 0.15**	2.83 ± 0.15**
10		2.55 ± 0.35***	3.1 ± 0.28***	3.87 ± 0.3***	4.21 ± 0.47***

Compared with controls, \* $P < 0.05$ , \*\* $P < 0.01$ ; compared with 1 mg/kg LPS group, # $P < 0.01$ .

**Table 4.** Correlation between TNF- $\alpha$  and IL-6 in liver tissue; ALT and TBIL in plasma; and average OD values of SR and CD<sub>14</sub> ( $r$  values)

Item	TNF- $\alpha$		IL-6		ALT		TBIL	
	1 mg	10 mg	1 mg	10 mg	1 mg	10 mg	1 mg	10 mg
CD <sub>14</sub>	0.659	0.810	0.924	0.868	0.839	0.930	0.897	0.876
SR	-0.573	-0.718	-0.871	-0.893	-0.84	-0.949	-0.917	-0.746

Compared with CD<sub>14</sub> OD values, \* $P < 0.05$ ; 1 mg: 1  $\mu$ g/kg LPS dose; 10 mg: 10  $\mu$ g/kg LPS dose.



**Fig. 7.** Mostly cellular denaturation, necrosis and lobular disorder in liver tissue following injection of high-dose LPS (10 mg/kg) at 8 hours, HE, original magnification  $\times 200$ .

0.01), and much increased with prolonged experimental time. There were significant differences between the 1 and 10 mg/kg groups (Table 3).

### Histopathological study

Results of liver histopathological study showed inflammatory cells infiltration in the 1 mg/kg group and hepatocyte spotty necrosis in the 10 mg/kg group 3 hours after injection of LPS, respectively. In the 1 mg/kg group, hepatocyte de-

generation and local necrosis were the major features, whereas in the 10 mg/kg group, cellular necrosis and lobular disorder in the liver tissue were the main characteristics 8 hours after injection of LPS (Fig. 7).

### Correlation analysis

Correlation analysis indicated that changes of TNF- $\alpha$ , IL-6, ALT and TBIL levels were correlated positively with the OD values of CD<sub>14</sub> and negatively with the OD values of SR in the liver, respectively (Table 4).

### Discussion

In our study, SR expression in the liver in the two groups was decreased gradually with prolonged experimental time. The intrahepatic expression of SR was down-regulated in endotoxemia or endotoxic shock in a dose-dependent manner and the expression of CD<sub>14</sub> in the liver was up-regulated in the development of endotoxemia or endotoxic shock. These results are consistent with those reported by Li et al.<sup>[8]</sup> We conclude that the defense function of the liver is progressively decreased with the gradual down-regulation of SR in the liver in the development of sepsis or septic shock, and

that the activation of macrophages by LPS is progressively enhanced with the gradual up-regulation of CD<sub>14</sub> in the liver in sepsis or septic shock. In our study, however, LPS-induced CD<sub>14</sub> expression in the liver was not increased in a dose-dependent manner, showing no significant differences between the two groups, possibly because of the effect of CD<sub>14</sub> as a LPS-sensitive receptor.<sup>[9]</sup> So low doses of LPS could induce a profound expression of CD<sub>14</sub>.

Sepsis or septic shock remains one of the major causes of death in surgical intensive care units.<sup>[10]</sup> Binding of LPS to macrophages could induce the release of cytokine and inflammatory mediators, leading to organ damage.<sup>[11]</sup> In this study, the intrahepatic levels of inflammatory cytokines TNF- $\alpha$  and IL-6 in both 1 and 10 mg/kg groups were significantly increased 1 hour after LPS administration. The levels of IL-6 peaked at 3 hours and remained to be higher than those in the sham group at 8 hours. The intrahepatic levels of TNF- $\alpha$  and IL-6 were significantly higher in the 10 mg/kg group than in the 1 mg/kg group. These findings indicated that inflammatory response is progressively enhanced in the liver in sepsis or septic shock in a dose-dependent manner.

Correlation analysis showed that changes in the levels of TNF- $\alpha$ , IL-6 in the liver are correlated positively with the OD values of CD<sub>14</sub> and negatively with the OD values of SR, respectively. Antibody to CD<sub>14</sub> could markedly inhibit the production of cytokines such as TNF- $\alpha$ , IL-1 and IL-6 in endotoxemia,<sup>[12,13]</sup> and blocking expression of SR on Kupffer cells could promote cytokine production.<sup>[14]</sup> These suggest that there might be an intrinsic relationship between enhanced intrahepatic inflammatory responses and expression of SR and CD<sub>14</sub> in the liver in the development of sepsis or septic shock.

In our study, the levels of ALT and TBIL in serum was shown to increase with prolonged experimental time. The liver tissue showed different injuries characterized by infiltration of inflammatory cells, hepatocyte denaturation, and necrosis. The degrees of inflammatory response and hepatic injury were dose-effect related to endotoxins. Correlation analysis revealed that changes of ALT and

TBIL in serum were correlated negatively with the OD values of SR and positively with the OD values of CD<sub>14</sub>. However, LPS-induced CD<sub>14</sub> expression in the liver was not increased in a dose-dependent manner, showing no significant differences between the two groups. It is possibly because CD<sub>14</sub> is a LPS-sensitive receptor<sup>[4,9]</sup> and a low dose of LPS could induce profound expression of CD<sub>14</sub>. Therefore, large doses of LPS could activate other low affinity LPS receptor on Kupffer cells besides CD<sub>14</sub>,<sup>[15,16]</sup> resulting in more severe LPS-induced hepatic damage. In conclusion, the down-regulation of SR and up-regulation of CD<sub>14</sub> might be an important mechanism for the liver to turn a defense organ into effector one in sepsis.

### Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

### References

- 1 Jiang JX, Chen HS, Diao YF, et al. Distribution of endotoxins in tissues and circulation and its effects following hemorrhagic shock. *Chin Med J* 1998;11: 118–122.
- 2 Yoshioka K, Kakumu S, Arao M. Immunohistochemical studies of intrahepatic tumor necrosis factor in chronic liver disease. *J Clin Pathol* 1990;43:298–302.
- 3 Van der Luan LJW, Dopp EA, Haworth R, et al. Regulation functional involvement of macrophage scavenger receptor MARCO in clearance of bacteria in vivo. *J Immunol* 1999;162:939–947.
- 4 Jiang JX. LBP/CD<sub>14</sub> and their relationship with endotoxin actions. *Prog Physiol Sci* 1997;28:86–88.
- 5 Haziot A, Hijiya N, Goyert SM. Role of CD<sub>14</sub> in infection; studies in CD<sub>14</sub>-deficient mice. *Prog Clin Biol Res* 1998;397:255–260.
- 6 Schimke J, Mathison J, Morgiewicz J, et al. Anti-CD<sub>14</sub> mAb treatment provides therapeutic benefit after in vivo exposure to endotoxin. *Proc Natl Acad Sci USA* 1998;95:13875–13880.
- 7 Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;238:248–254.

- 8 Li SW, Wu CX, Shi YJ. Experimental study on expression of CD<sub>14</sub> mRNA and protein of liver cells up-regulated by endotoxin. *J Chin Liver Dis* 2001;9:103-104.
- 9 Feng JM, Liu YS, Shi JQ, et al. The role of LPS in the CD<sub>14</sub> expression and the activation of Kupffer cells. *Chin J Burns* 2002;18:107-110.
- 10 Murphy K, Haudek SB, Thompson M, et al. Molecular biology of septic shock. *New Horiz* 1998;6:181-193.
- 11 Tobias PS, Tapping RI, Gegner JA. Endotoxin interactions with lipopolysaccharide-responsive cells. *Clin Infect Dis* 1999;28:476-481.
- 12 Vonasmuth EJ, Dentener MA, Bazil V, et al. Anti-CD<sub>14</sub> antibodies reduces responses of cultured human endothelial cells to endotoxin. *Immunology* 1993;80:78-83.
- 13 Leturcq DJ, Moriarty AM, Talbott G, et al. Antibodies against CD<sub>14</sub> protect primates from endotoxin-induced shock. *J Clin Invest* 1996;98:1533-1538.
- 14 Mendez C, Ronald V. Scavenger receptor blockade augments LPS-induced TNF production by alveolar macrophages. *Surg Forum* 1995;46:41-44.
- 15 Malhotra R, Bird M. L-selectin: a novel receptor for lipopolysaccharide and its potential role in bacterial sepsis. *BioEssays* 1997;19:919-923.
- 16 Laherty SF, Golenbock DT, Milham FH, et al. CD11/CD18 leukocyte integrins: new signaling receptors for bacterial endotoxin. *J Surg Res* 1997;73:85-89.

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