Int J Clin Exp Pathol 2015;8(2):1631-1639
www.ijcep.com /ISSN:1936-2625/IJCEP0004338

Original Article

Expression of S100 family proteins in neonatal rats with sepsis and its significance

Haiying Huang, Luoyang Tu

Department of Critical Care Medicine, Sir Run Run Shaw Hospital affiliated School of Medicine, Zhejiang University, 3 East Qingchun Rd. Jianggan District, Hangzhou, Zhejiang Province, People's Republic of China

Received December 1, 2014; Accepted January 28, 2015; Epub February 1, 2015; Published February 15, 2015

Abstract: Objective: This study aims to study the expression changes of S100 family proteins in neonatal rats with sepsis and investigate the effect and significance of S100 family proteins in pathogenesis and development of sepsis. Methods: The functions of S100 family proteins were analyzed with bioinformatics. The immune-associated proteins were chosen as the candidate proteins. Twenty neonatal SPF SD rats were randomly divided into two groups: sepsis model group and control group. The liver sample was stained with HE to evaluate the establishment of sepsis model. The expression amount of proinflammatory factor IL-1, IL-6 and TNF-α was detected with ELISA. The expression changes of S100A8, S100A9, S100A11 and S100A12 in sepsis model rats were detected with real-time PCR and Western blotting. After shRNA plasmid was transfected into THP-1 cells and the expression of S100A12 was silenced, the expression changes of proinflammatory factor IL-1, IL-6 and TNF-α in LPS-induced inflammation were studied in order to investigate the S100A12 mediated inflammatory process. Results: IL-1, IL-6 and TNF-α in the serum of rats with sepsis induced by LPS were 55.79 ± 3.80 ng/l, 48.76 ± 1.03 ng/l and 29.98 ± 2.27 ng/l respectively. S100A8, S100A9, S100A11 and S100A12 detected with real-time PCR in sepsis model group were 14.4 ± 1.37, 10.23 ± 1.81, 5.5 ± 1.64 and 9.97 ± 1.82 respectively. Compared with the control group, S100A8, S100A9, and S100A12 were significantly up-regulated. The shRNA silenced the expression of S100A12 which reduced the expression of proinflammatory factors after LPS stimulated the cells ($P < 0.05$). Conclusion: Compared with the control group, S100A8, S100A9, and S100A12 were significantly up-regulated in rat sepsis model group. After the expression of S100A12 in propylene glycol monomethyl ether acetate (PMA) induced human macrophages was silenced, the expression of proinflammatory factor IL-1, IL-6 and TNF-α was down-regulated.

Keywords: Sepsis, S100 family, LPS, THP-1

Introduction

Sepsis is a systemic inflammatory response syndrome caused by pathogenic microorganisms invading into circulatory system, growing and reproducing and producing endotoxin and exotoxin which can induce the injury of multiple organs and is a high-mortality disease in clinic. The routine use of antibiotics is unable to treat sepsis effectively indicating that sepsis may be a complex process in which a variety of immune-associated molecules act collaboratively. Therefore, in-depth study of its pathogenic mechanism is a difficult medical problem demanding prompt solution currently [1, 2].

Calcium binding protein is a large protein family with the functions of controlling cell cycle, cell differentiation, enzyme activation and muscle contraction. S100 protein family, one of its largest subfamilies, has more than twenty members. The proteins of this family have similar structure and functions and a high degree of homology and can play multiple biological effects by combining with Ca^{2+} and changing their conformation. Recent studies have demonstrated that multiple members of S100 protein family are involved in the occurrence and development of inflammation, especially acute inflammation. Therefore, in this experiment, we will use the bioinformatics database to screen out the inflammation-associated proteins in the S100 family and investigate the mechanism of S100 family proteins in the occurrence and development of sepsis by establishing the sepsis models in neonatal rats in the hope of providing a train of thought for disclosing the pathogenesis of sepsis [3, 4].

Materials and methods

Experimental animals and cells

Twenty male or female SPF SD rats, aged one week and weighing 25 ± 5 g, were purchased from Slac Laboratory Animal LLC. They were randomly divided into two groups with 10 rats in each group: sepsis model group and control group. The rats were fed in standard animal cages with five rats in each cage. All rats freely took food (breast-fed by mother rats) and drank water during the experiment. The laboratory was well ventilated and nature lighting day and night was achieved. The temperature was maintained at 18~25°C.

THP-1 human macrophages purchased from Shanghai Cell Bank of Chinese Academy of Sciences were cultured in 1640 complete medium (GIBIC), 15% fetal bovine serum (GIBIC) and 5% CO_2 incubator at 37°C.

Reagents and instruments

Lipopolysaccharide (LPS) was purchased from Sigma Company. IL-1, IL-6 and TNF-α ELISA kits were purchased from Wuhan Uscn Life Science Inc.. RNA extraction kit (RNeasy Plus Mini Kit) was purchased from QIAGEN. Reverse transcription kit (iScript cDNA Synthesis Kit) and real-time PCR fluorescent quantitative kit (SsoAdvanced SYBR Green Super mix) were purchased from Bio-Rad. S100A8, S100A9, S100A11 and S100A12 antibodies and S-100A12 (Calgranulin-C) shRNA Plasmid (including Plasmid Transfection Reagent, Plasmid Transfection Medium and Control shRNA Plasmid) were purchased from Santa Cruz Biotechnology. Horseradish peroxidase (HRP) labeled secondary antibody was purchased from Beijing Zhongshan Jinqiao Biotechnology Company Ltd.. ECL color kit and PVDF membrane (Polyvinylidene fluoride) were purchased from Millpore. Skimmed milk powder was purchased from Oxoid.

CO_2 constant temperature incubator was purchased from SANYO. MultiSkan FC ELIASA was purchased from Thermo Scientific. Fluorescent quantitative PCR test system (CFX96 Touch) was purchased from Bio-Rad.

Establishment of sepsis models in rats

LPS was injected intraperitoneally at a dosage of 0.1 μg/g (LPS was dissolved in saline with a concentration of 10 μg/ml). The saline was injected in the control group.

Detection of related proinflammatory factors with ELISA

IL-1, IL-6 and TNF-α in rat serum were detected quantitatively using IL-1, IL-6 and TNF-α ELISA kits. The specific procedure is as follows.

Standard holes, sample holes and blank holes were set respectively. The standard products of different concentration were added into seven standard holes respectively. The samples to be measured were also added. The ELISA plate was covered with tectorial membrane. They were incubated for two hours at 37°C. 100 μl of test solution (biotinylated primary antibody) was added into each hole. The ELISA plate was covered with tectorial membrane. They were incubated for one hour at 37°C. The liquid in the holes was discarded. Each hole was washed with 350 μl scrub solution, soaked with it for one to two minutes and washed repeatedly for three times. 100 μl of HRP labeled secondary antibody was added in each hole. The ELISA plate was covered with tectorial membrane. They were incubated for thirty minutes at 37°C. 90 μl of TMB zymolyte was added in each hole. The ELISA plate was covered with tectorial membrane. They were colored for 15 to 25 minutes in dark place at 37°C. When gradient blue appeared in the former three to four standard holes, the reaction was terminated. 50 μl of 2 M H_2SO_4 was added. OD value in each hole was measured immediately with ELIASA at 450 nm.

All data are expressed as the mean ± standard deviation (SD). The comparison between two groups was examined with t-test. Findings with $P < 0.05$ were considered to be statistically different, and those with $P < 0.01$ were considered to be statistically significant.

Paraffin embedding and HE staining

The liver tissue of the rats was cut into small pieces with a thickness of no more than 5 mm. These small pieces were marked, fixed in 10% neutral formalin for more than 24 hours, rinsed with tap water, on the next day dehydrated with gradient ethanol, embedded in paraffin and sliced (thickness was 3 to 5 μm).

The paraffin slices were placed in haematoxylin and stained for fifteen minutes. Then the slices were rinsed with tap water for fifteen minutes

Table 1. Molecular functions and subcellular localization of main members of S100 family proteins

Gene	Molecular function	Subcellular location	Associated disease
S100A1	ATPase binding; identical protein binding; S100 protein binding; calcium ion binding; protein homodimerization activity	Cytoplasm	Cardiomyopathy
S100A2	calcium ion binding; identical protein binding		Breast cancer
S100A3	calcium ion binding; zinc ion binding	Cytoplasm	
S100A4	calcium ion binding; zinc ion binding;	Cytoplasm	Metastatic carcinoma
S100A5	calcium ion binding; poly (A) RNA binding; RAGE receptor binding		
S100A6	calcium-dependent protein binding; calcium ion binding; ion transmembrane transporter activity; protein homodimerization activity; S100 protein binding; protein homodimerization activity; tropomyosin binding; zinc ion binding	Nucleus envelope. Cytoplasm. Cell membrane; Peripheral membrane; Cytoplasmic side	melanoma
S100A7	calcium ion binding; RAGE receptor binding; zinc ion binding	Cytoplasm; Secreted	Immune related
S100A8	arachidonic acid binding; calcium ion binding; microtubule binding; RAGE receptor binding; Toll-like receptor 4 binding; zinc ion binding	Cytoplasm; Cell membrane	Immune related Inflammation
S100A9	antioxidant activity; arachidonic acid binding; calcium ion binding; microtubule binding; RAGE receptor binding; signal transducer activity; Toll-like receptor 4 binding; zinc ion binding	Cytoplasm; Cell membrane	Inflammation
S100A11	calcium-dependent protein binding; calcium ion binding; protein homodimerization activity; S100 protein binding	Cytoplasm; Nucleus	Prostate cancer Colorectal cancer
S100A12	calcium ion binding; copper ion binding; RAGE receptor binding; zinc ion binding	Secreted; Cytoplasm; Cell membrane; Peripheral membrane protein	Immune related

Main retrieval database: uniprot database (http://www.uniprot.org/); IPI database (http://www.ebi.ac.uk/IPI).

Table 2. The primers used in real-time PCR

Gene	Accession NO.	Primer (5'-3')
S100A8	NM_013650.2	For: TGCCCTCAGTTTGTGCAGAATA Rev: CCAACGCAAGGAACTCTTCGA
S100A9	NM_009114.3	For: TTACTTCCCACAGCCTTTGC Rev: AGGACCTGGACACAAACCAG
S100A11	NM_016740.3	For: GCGCCTCGCTCAGCTC Rev: TCGATGCACCGCTCAGTC
S100A12	NM_009114.3	For: CTTCCACCAATACTCAGTTCG G Rev: GCAATGGCTACCAGGGATATG
GAPDH	NM_001115114.1	For: TCACCACCATGGAGAAGGC Rev: GCTAAGCAGTTGGTGGTGCA

S100A8: S100 calcium binding protein A8; S100A9: S100 calcium binding protein A9; S100A11: S100 calcium binding protein A11; S100A12: Calgranulin; CGAPDH: glyceraldehyde phosphate dehydrogenase; For: forward; Rev: reverse.

Table 3. Synthetic system of inverse transcription

Components	Volume per Reaction
5×iScript reaction mix	4 μl
iScript reverse transciptase	1 μl
RNA template	1 μg
Nuclease-free water	Up to 20 μl

iScript reaction mix was purchased from Bio-Rad. Nuclease-free water was purchased from Invitrogen.

Table 4. Synthetic system of PCR

Components	Volume per Reaction
SsoAdvanced SYBR Green Super mix	5 μl
Forward primer (10 μM)	0.4 μl (400 nM)
Reverse primer (10 μM)	0.4 μl (400 nM)
cDNA template	100 ng
Nuclease-free water	Up to 10 μl

Sso Advanced SYBR Green Super mix was purchased from Bio-Rad.

and faded in 1% hydrochloric acid ethanol solution until turning red. The slices were rinsed again with tap water and restored into blue. It could be observed under microscopy that cell nuclei were stained blue and cytoplasm or connective tissue fiber was colorless. The slices were put into 50%, 70% and 80% ethanol orderly for five minutes seperatively and counterstained in 0.5% eosin ethanol solution for two minutes. To place the slices in 95% ethanol, wash away the excess red and blot up the excess ethanol with blotting-paper. After transparent processing, mounting was performed with neutral gum.

Expression and functions of S100 family proteins with bioinformatics

The molecular functions of S100 family proteins were shown in **Table 1** [5-13]. To search the molecular functions, subcellular localization and related diseases of S100 family proteins with uniprot database (http://www.uniprot.org/), IPI database (http://www.ebi.ac.uk/IPI) and KEGG PATHWAY database (http://www.genome.jp/kegg/pathway.html).

Detection of expression of related genes in liver tissue with real-time PCR

The RNA in liver tissue was extracted according to the instruction of total RNA extraction kit. The RNA precipitation was dissolved with Rnase-free water and the concentration of RNA was measured with ultraviolet spectrophotometer. According to the procedure of reverse transcription kit, total RNA was extracted from the tissue to obtain cDNA by reverse transcription which was tested with real-time PCR. To search the mRNA sequences of S100A8, S100A9, S100A11 and S100A12 in NCBI database and design the primers of real-time PCR. All primers were synthesized by Invitrogen Corporation. The specific sequences were shown in the **Table 2**. The synthetic system of inverse transcription was shown in **Table 3**, the synthetic system of PCR was shown in **Table 4**.

In real-time PCR, gene amplification Ct value was measured which was negatively correlated with DNA initial copy number. The ΔCt value of the samples was analyzed using relative quantification method with GAPDH as an endogenous reference. ΔCt equals target gene Ct value minus endogenous reference gene Ct value. ΔCt is data analysis value.

shRNA plasmid transfection and LPS stimulation

THP-1 cells were induced to be macrophages after stimulated by propylene glycol monomethyl ether acetate (PMA) for four days and the macrophages began to grow adherently. When cells grew to 50-70%, they were transfected with S100A12 shRNA plasmid.

shRNA plasmid DNA was diluted with shRNA plasmid transfection medium according to the

Table 5. IL-1, IL-6 and TNF-α detected with ELISA[a]

	Control	LPS +	
		S100A12 shRNA	Control shRNA
IL-1a	15.79 ± 5.32	26.51 ± 11.73	35.72 ± 9.70*[b]
IL-6	19.83 ± 1.06	18.23 ± 3.90	46.17 ± 5.05**
TNF-α	11.92 ± 8.30	24.37 ± 5.14	38.09 ± 12.07*

[a]to be calculated according to standard curve (ng/l); [b]*P < 0.05, **P < 0.01.

instruction. The cell culture medium was not added with antibiotics. The diluted shRNA plasmid DNA was added in the six-hole plate and incubated for five hours at 37°C. Then it was cultured sequentially in complete culture medium with 1 ml 20% fetal bovine serum and antibiotics for 24 hours. The medium was replaced with fresh complete culture medium.

LPS was dissolved in sterile saline and stimulated the cells for 4 to 6 hours with a final concentration of 1 μg/ml.

Western blotting

The tissue or cell samples were decomposed with RIPA lysate while added with protease inhibitor cocktail and mixed by pipetting. After the samples were placed on ice for 30 minutes, probe-type ultrasonic was used to decompose the cells on ice with short shocks of appropriate frequency. The decomposed mixture was centrifugated at 4°C for 20 minutes at a speed of 13000 r/min. The supernatant was removed to a new centrifugal tube and the protein concentration was determined using protein assay kit.

After SDS-PAGE electrophoresis, the gel was immersed in transfer buffer and balanced for 10 minutes. Transfer "sandwich" was assembled, transfer buffer was added and the electrode of 100 V was plugged in for 45 to 60 minutes. After the trarsmembrane, PVDF membrane was rinsed with TBS for 10 to 15 minutes, placed in TBS/T blocking buffer containing 5% (w/v) skimmed milk powder and shaked at room temperature for one hour. It was incubated at room temperature for two hours after added with appropriate dilution degree of primary antibody (diluted with TBST containing 1% (w/v) skimmed milk powder). Then the membrane was rinsed with TBST for three times (5 to 10 minutes at a time). The membrane was incubated at room temperature for one hour with HRP labeled secondary antibody (1:10000) diluted with TBST containing 0.05% (w/v) skimmed milk powder and rinsed for three times with TBST (5 to 10 minutes at a time). To expose the membrane and take a photograph to save the experimental result.

Results

IL-1, IL-6 and TNF-α detected with ELISA

The sepsis model was established by intraperitoneal injection of LPS in neonatal rat. Twelve hours later after injection of LPS, tail vein blood was collected to detect the content of IL-1, IL-6 and TNF-α in the serum. The standard curve was made according to the standard products in ELISA kit and the results were obtained by calculation with light absorption value at 490 nm corresponding to the standard curve.

Table 5 shows that the proinflammatory factor IL-1, IL-6 and TNF-α were significantly increased after induction with LPS for 12 hours which indicated that LPS started the process of body's immune response through pattern recognition receptors (PRRs) of the innate immune cells.

HE stain result of liver tissue

After induction with LPS for 24 hours, rat liver tissue was collected, embedded in paraffin and stained with HE. The result is shown in **Figure 1**. In control group, the liver area morphology of normal rats is normal and hepatic lobule and hepatic arteries are clear, whereas in sepsis group, inflammatory invasion, severe liver damage, blurry tissue boundary and unclear hepatic lobule are displayed obviously which indicates that LPS caused a systemic inflammatory response and it was manifested obviously in liver tissue.

Expression changes of S100A8, S100A9, S100A11 and S100A12 at mRNA level detected with real-time PCR

After induction with LPS for 24 hours, rat liver tissue was collected for liquid nitrogen grinding.RNA kit was used to extract tissue total RNA and obtain cDNA by reverse transcription. Expression of S100A8, S100A9, S100A11 and S100A12 at mRNA level was detected with real-time PCR. It can be seen that in control group S100A8, S100A9, S100A11 and S100A12 were at a low level and in sepsis

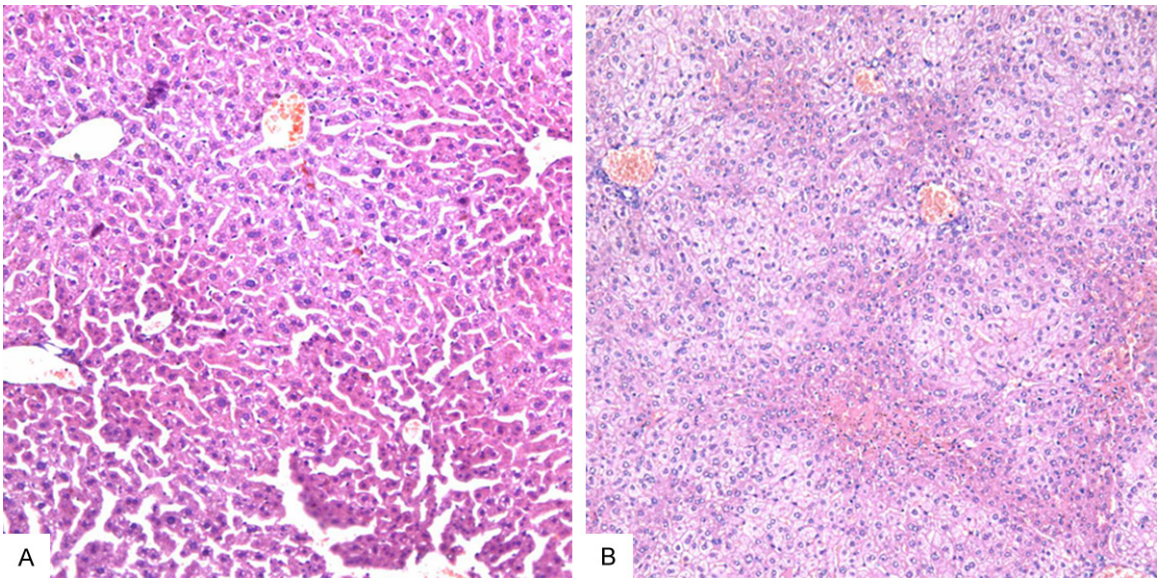

Figure 1. HE stain of rat liver tissue. A: Control group; B: Sepsis group. 400×.

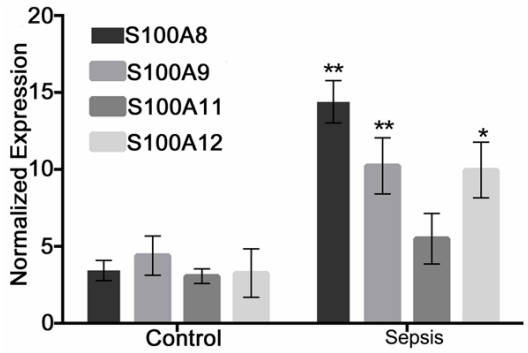

Figure 2. Expression changes of S100A8, S100A9, S100A11 and S100A12 at mRNA level detected with real-time PCR. Normalized fold expression is gene relative expression amount automatically calculated by software through homogenization processing of endogenous reference. *$P < 0.05$, **$P < 0.01$.

model group S100A8 and S100A9 were markedly upregulated ($P < 0.01$) indicating that they were actively involved in the related process of inflammation and S100A11 changed very little compared with control group ($P = 0.17$) which has no statistical significance. S100A12 was also markedly up-regulated ($P < 0.05$) after LPS induction due to mediating immune response pathway (**Figure 2**).

Expression changes of S100A8, S100A9, S100A11 and S100A12 at protein level detected with western blot

After induction, the rat liver tissue was collected for detecting the expression changes of

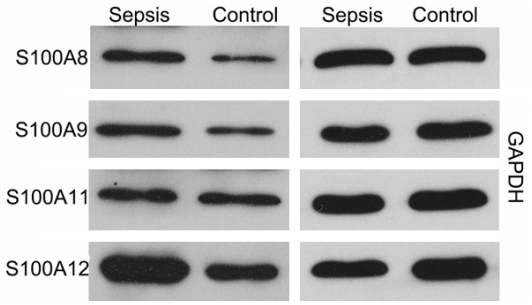

Figure 3. Expression changes of S100A8, S100A9, S100A11 and S100A12 at protein level.

S100A8, S100A9, S100A11 and S100A12 at protein level with western blot. The result was consistent with that of real-time PCR. That is to say, after LPS induction, S100A8, S100A9 and S100A12 showed a significant up-regulation (**Figure 3**).

Expression of proinflammatory factors after the silence of S100A12

In order to further study the effect of S100A12 in the generating process of sepsis, we chose the shRNA method to silence the expression of S100A12 in THP-1 cells (induced to be human macrophages by PMA). Then the cells were stimulated by LPS (1 μg/ml LPS stimulating the cells for 4 hours). It can be seen that 24 hours later after S100A12 shRNA was transfected, endogenous S100A12 expression was significantly down-regulated suggesting the success of gene silence (**Figure 4**).

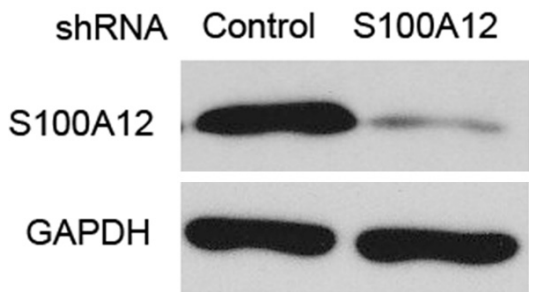

Figure 4. Silence effect of S100A12.

The cells transfected with control shRNA and cells transfected with S100A12 shRNA were stimulated by LPS for 4 hours, the supernatant of cell culture fluid was collected and IL-1, IL-6 and TNF-α in the supernatant were detected with ELISA. As shown in **Table 5**, in the control group, these proinflammatory factors were at a low level and in sepsis model group they were significantly up-regulated. Above all, we observed that the concentration of IL-1, IL-6 and TNF-α showed a down-regulation after S100A12 expression was silenced (compared with the cells transfected with control shRNA). It suggests that S100A12 played an important role in mediating the inflammatory response.

Discussion

The incidence of sepsis is about 0.3%. Each year approximately 18 million people suffer from severe sepsis in the world with an increase of 0.8%-1.5% each year [14]. Sepsis has been considered to be excessive inflammatory response for a long time which is treated only according to the principle of antagonism of excessive inflammation in addition to the treatment of primary disease. However, the clinical data shows that the effect of simple anti-inflammatory therapy for sepsis is unsatisfactory. Studies have proved that interaction between cytokine plays an important role in the pathogenesis of sepsis and the interaction anti-inflammation can cause the inflammatory signals to be amplified step by step in the transmission process showing amplification effect similar to "waterfall" which results in large amounts of inflammatory factors such as proinflammatory factor IL-1β, IL-4, IL-6, IL-8 and TNF-α and reactive oxygen species (ROS) produced and secreted into the blood [15]. The proinflammatory factors can further act on immune system and activate inflammatory cells resulting in the release of more cytokines. Therefore, sepsis should be a complex network interaction regulating process and not a single factor induced immune response. There must be a variety of immune cells and proteins involved in this process. Therefore, to investigate the link between inflammation-associated proteins is very important for understanding the occurrence and development mechanisms of sepsis.

Calcium binding protein is a large protein family with the functions of controlling cell cycle, cell differentiation, enzyme activation and muscle contraction. S100 protein family, one of its largest subfamilies, is a group of acidic proteins with low molecular weight (9-14 kDa) and has more than twenty members. The proteins of this family have similar structure and functions and a high degree of homology and can play multiple biological effects by combining with Ca^{2+} and changing their conformation. S100 protein family was initially found in bovine brain tissue by Moore in 1965 and was named due to being completely dissolved in neutral saturated ammonium sulfate [16]. The biological functions of S100 protein family mainly refer to post-translational modification of proteins, regulation of cell growth and differentiation, cell skeleton establishment, regulation of Ca^{2+} homeostasis and participating in inflammatory reaction. In the family members, S100A8, S100A9 and S100A12 have been proved to have relationship with inflammatory diseases and exacerbate inflammatory response cooperating with inflammatory factors.

In our study, we established sepsis models in neonatal rats and the expression amount of IL-1, IL-6 and TNF-α in the serum was detected with ELISA. The results showed that IL-1, IL-6 and TNF-α in sepsis model group were significantly higher than those in normal control group. This confirms that numerous inflammatory factors went into the blood stream in the generating process of sepsis and amplified the signals by interaction and concatenation connection eventually causing body injury. Liver injury is a common reaction in patients with sepsis in clinic and liver is also the organ of inflammation earlier manifestation, so we detected the changes of S100A8, S100A9, S100A11 and S100A12 in rat liver tissue at mRNA level using real-time PCR. The experiment proved that S100A8, S100A9 and

S100A12 were significantly up-regulated compared with control group and S100A11 changed little probably due to S100A11 mainly participating in the occurrence and development process of tumors.

The experimental results show that S100A12 was highly expressed in rat sepsis model. We choose S100A12 to explore the mechanisms in depth. The relative molecular weight of S100A-12 is 10575, it consists of 92 amino acids and the gene is located on human chromosome 1q21 [17, 18]. It is mainly expressed and secreted by neutrophils and is an important proinflammatory factor which is widely distributed in human somatic cells. Studies have found that the protein plays an important role in microbial resistance, regulation of cell apoptosis and participating in inflammation and immune reactions. S100A12 activates and participates in multiple signal transduction pathways by combining with Ca^{2+} including calmodulin kinase II, protein kinase C (PKC), phospholipase C (PLC), Ca^{2+} regulation and mitogen-actived protein kinas (MAPK).

We chose the macrophages closely associated with the pathogenesis of sepsis as the research object, but because it is difficult to isolate and culture the human macrophages, we used the adherent cells with the characteristics of macrophages which were induced from THP-1 by PMA. In the preliminary experiment, when low dose of LPS (0.5 μg/ml) was used to stimulate human macrophages for 6 hours, the secrection of proinflammatory factor IL-1β, IL-4 and IL-6 could be detected in the supernatant of culture medium. In order to quickly obtain the inflammatory environment, in the experiment we used 1 μg/ml of LPS to stimulate the cells transfected with control shRNA and cells transfected with S100A12 shRNA and detected the concentration of proinflammatory factors in cell culture medium 4 hours later. The result shows that proinflammatory factors were all significantly up-regulated after stimulation of LPS compared with control group (without stimulation of LPS) which is consistent with prior result. However, we used shRNA to silence the expression of S100A12 and found that the up-regulation trend of proinflammatory factors was inhibited. After stimulation of LPS, the proinflammatory factor concentration of cells transfected with control shRNA was higher than that of cells transfected with S100A12 shRNA ($P < 0.05$). This result suggests that S100A12 has an effect of positive regulation of proinflammatory factors in inflammatory reaction and this effect plays an important role in the occurrence and development of sepsis.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Haiying Huang, Department of Critical Care Medicine, Sir Run Run Shaw Hospital Affiliated School of Medicine, Zhejiang University, 3 East Qingchun Rd. Jianggan District, Hangzhou, Zhejiang Province, People's Republic of China. Tel: 86-571-86090172; E-mail: haiyinhuang@126.com

References

[1] González-Reimers E, Santolaria-Fernández F, Martín-González MC, Fernández-Rodríguez CM and Quintero-Platt G. Alcoholism: a systemic proinflammatory condition. World J Gastroenterol 2014; 20: 14660-14671.

[2] Tanaka K, Koike Y, Shimura T, Okigami M, Ide S, Toiyama Y, Okugawa Y, Inoue Y, Araki T, Uchida K, Mohri Y, Mizoguchi A and Kusunoki M. In Vivo Characterization of Neutrophil Extracellular Traps in Various Organs of a Murine Sepsis Model. PLoS One 2014; 9: e111888.

[3] Ji YF, Huang H, Jiang F, Ni RZ and Xiao MB. S100 family signaling network and related proteins in pancreatic cancer. Int J Mol Med 2014; 33: 769-776.

[4] Vento G, Lio A, Tirone C, Aurilia C, Tana M, Piras A, Ricci C, Perelli S, Romagnoli C, Posteraro B, Iavarone F, Cabras T, Fanali C, Messana I and Castagnola M. Association of high levels of α-defensins and S100A proteins with Candida mannan detection in bronchoalveolar lavage fluid of preterm neonates. Pediatr Res 2013; 74: 19-25.

[5] Engelkamp D, Schäfer BW, Erne P and Heizmann CW. S100 alpha, CAPL, and CACY: molecular cloning and expression analysis of three calcium-binding proteins from human heart. Biochemistry 1992; 31: 10258-10264.

[6] Duarte-Costa S, Castro-Ferreira R, Neves JS and Leite-Moreira AF. S100A1: a major player in cardiovascular performance. Physiol Res 2014.

[7] Lee SW, Tomasetto C, Swisshelm K, Keyomarsi K, Sager R. Down-regulation of a member of the S100 gene family in mammary carcinoma cells and reexpression by azadeoxycytidine treatment. Proc Natl Acad Sci U S A 1992; 89: 2504-2508.

[8] Gregory SG, Barlow KF, McLay KE, Kaul R and Swarbreck D. The DNA sequence and biological annotation of human chromosome 1. Nature 2006; 441: 315-321.
[9] Engelkamp D, Schäfer BW, Mattei MG, Erne P and Heizmann CW. Six S100 genes are clustered on human chromosome 1q21: identification of two genes coding for the two previously unreported calcium-binding proteins S100D and S100E. Proc Natl Acad Sci U S A 1993; 90: 6547-6551.
[10] Tulchinsky E, Ford HL, Kramerov D, Reshetnyak E, Grigorian M, Zain S and Lukanidin E. Transcriptional analysis of the mts1 gene with specific reference to 5' flanking sequences. Proc Natl Acad Sci U S A 1992; 89: 9146-9150.
[11] Ota T, Suzuki Y, Nishikawa T and Otsuki T. Complete sequencing and characterization of 21,243 full-length human cDNAs. Nat Genet 2004; 36: 40-45.
[12] Brodersen DE, Nyborg J and Kjeldgaard M. Zinc-binding site of an S100 protein revealed. Two crystal structures of Ca2+-bound human psoriasin (S100A7) in the Zn2+-loaded and Zn2+-free states. Biochemistry 1999; 38: 1695-1704.
[13] Goyette J and Geczy CL. Inflammation-associated S100 proteins: new mechanisms that regulate function. Amino Acids 2011; 41: 821-842.
[14] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J and Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29: 1303-1310.
[15] Kasahara E and Inoue M. Cross-talk between HPA-axis-increased glucocorticoids and mitochondrial stress determines immune responses and clinical manifestations of patients with sepsis. Redox Rep 2015; 20: 1-10.
[16] Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965; 19: 739-744.
[17] Mefford HC, Sharp AJ, Baker C, Itsara A and Jiang Z. Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. N Engl J Med 2008; 359: 1685-1699.
[18] Goyette J, Yan WX, Yamen E, Chung YM, Lim SY, Hsu K, Rahimi F, Di Girolamo N, Song C, Jessup W, Kockx M, Bobryshev YV, Freedman SB and Geczy CL. Pleiotropic roles of S100A12 in coronary atherosclerotic plaque formation and rupture. J Immunol 2009; 183: 593-603.