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EVIDENCE OF MULTI-STEP REGULATION OF HSP72 EXPRESSION IN EXPERIMENTAL SEPSIS

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ABSTRACT—Heat shock proteins (Hsps) are a family of highly conserved proteins induced in response to various stresses. Hsps protect cells against subsequent lethal circumstances. Previous work from our laboratory has indicated that Hsp72 is not induced during experimental sepsis in rats, but the regulation of the induction of Hsp72 synthesis in this disease cascade has not been investigated. In the present study, we evaluated the expression of the *hsp72* gene, focusing on the activation and DNA-binding ability of heat shock factor 1 (HSF1), *hsp* mRNA accumulation, and Hsp72 synthesis in animal sepsis models induced by cecal ligation and puncture procedure. The results were compared with those of sham-treated and heat-shocked rats. It was shown that the expression of the *hsp72* gene in sepsis was a multi-step process, as previously documented in *in vitro* studies. Hsp72 synthesis was not induced during sepsis, whereas DNA binding of HSF was detectable, suggesting that the induction of Hsp72 is blocked downstream to HSF-DNA complex formation by the metabolic alteration occurring during sepsis. The dissociation failure of the constitutive heat shock element binding factor (CHBF) from the heat shock element may play an important role in this negative regulation.

KEYWORDS—cecal ligation and puncture, heat shock factor 1, heat shock element, hyperthermia, lymphocytes

INTRODUCTION

Sepsis and its sequelae, sepsis syndrome and septic shock, are life-threatening clinical complications and are mainly responsible for morbidity and mortality in the intensive care unit. Clinically, sepsis causes adverse metabolic alterations, tissue dysfunction, and perfusion abnormalities, and manifests variable stress conditions in the subjects, such as acidosis, hypoxemia, hypoglycemia, oliguria, hypotension, coagulopathy, multiple organ dysfunction, and ultimately death (1). Recently, significant advances in the delineation of the complex pathophysiology of sepsis have been made using small-animal models with cecal ligation and puncture (CLP) procedures (2). A biphasic dynamic change in the disease process has been found in both animal models and in patients with sepsis (3). Unfortunately, in spite of the advanced knowledge and early therapeutic intervention with antibiotics and the more recent anti-endotoxin antibody therapy (4), mortality remains high (5). Obviously, our knowledge in preventing the host cell damage responsible for multiple organ failure and death from sepsis is insufficient to deal with the disease.

While facing a stress condition, almost all living cells selectively synthesize a family of proteins, called heat shock proteins (Hsps), that protect the cells or the organism from subsequent damage (6). Heat shock protein genes (*hsp* genes) are highly conserved genes found in all organisms, and highly activated by various stresses (7). In addition to hyperthermia, Hsps have been proved to be induced by hypoxia, ischemia, inhibitors of energy metabolism, hemorrhagic shock, hypoglycemia, free radicals, heavy metals, and amino acid analogues, as well as during an inflammation process (7). In some physiological situations, such as in exercise, cells also respond to internal conditions and synthesize large amount of Hsps (8, 9).

Since the first report of Hsps in 1962, authors have examined the self-protective role of these proteins *in vivo* through cross-tolerance, which is the induction of Hsps in response to sublethal stress, thereby protecting cells against a subsequent lethal environment (10–12). Hotchkiss et al. reported that artificial induction of Hsps by hyperthermia protects mice against the lethal effects of endotoxin (13). It has also recently been reported that Hsp72 expression can be induced in the lungs by sodium arsenite administration, and that this can protect rats against sepsis (14). Induction of heat shock response has also been found to reduce mortality rates and organ damage in a sepsis-induced acute lung injury model (15). In our own previous study, whole-body hyperthermic pretreatment proved to decrease the mortality of CLP-induced sepsis in rats (16). These observations imply that Hsps indeed modulate the process of sepsis and significantly influence its outcome.

Recently, we as well as other authors have found that Hsps cannot be induced through the whole course of sepsis in CLP-induced animal models (15, 16). The basis for this phenomenon remains unclear; but it is reasonable to consider that the absence of Hsp induction may correlate with the poor outcome in the disease entity. In fact, many studies now focus on Hsps for novel therapeutic strategies in sepsis (17). The clinical outcome, using the protective potential of Hsps, seems promising

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(8, 16). An insight into such a mechanism would help open up new therapeutic strategies for sepsis treatment. In this study, we investigate the expression of the *hsp72* gene during the late phase of sepsis and compare it with that occurring in heat shock treatment.

MATERIALS AND METHODS

Animals

Experiments were performed on adult male Sprague-Dawley rats (weighing 270–350 gm) obtained from the National Experimental Animal Center (Nan-Kang, Taipei, Taiwan). The animals were divided into the following groups: intact rats (n = 6); heat-shocked rats 0 h post-treatment (n = 6); 2 h post-treatment (n = 6); 24 h post-treatment (n = 6)], sham-operated rats (n = 6); and CLP-operated rats (n = 10). Four CLP-operated rats died before sample collection, which is an acceptable mortality rate.

Experiments described in this study were approved by the Animal Committee of the Kaohsiung Medical College, and the authors adhered to the guidelines of the National Institutes of Health for the use of the experimental animals.

Heat shock treatment

The heat-shocked groups were heat shocked using a previously described method (16). The rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg weight), then whole-body heated using an electric pad to 42°C rectal temperature for 15 min. After heat-shock treatment, rectal temperature was maintained at 37°C until consciousness was completely regained. Attention was paid to ensure that the airway was free of obstruction. The heat-treated rats were allowed to recover and had free access to food and water. Rats of the sham-treated group were anesthetized, but not heat-treated.

Induction of sepsis

Sepsis was induced by cecal ligation and puncture (CLP) as described by Wichterman et al. (2) with modifications. Briefly, animals were fasted but permitted water for 6 h prior to the operation. Under light ether anesthesia, a laparotomy was performed through a midline abdominal incision. The cecum was pulled out and ligated just below the ileocecal valve. The ligated cecum was punctured twice at different sites with an 18-gauge needle, and the cecum gently compressed until feces were extruded. The bowel was then returned to the abdomen and the incision was closed in two layers. Control animals were sham operated (a laparotomy was performed and the cecum was manipulated but neither ligated nor perforated). After closing the abdomen, each animal received 5 mL/100 g normal saline subcutaneously to provide replacement for the extracellular fluid sequestered during peritonitis. After conclusion of these procedures, the animals were transferred to a recovery cage for 1 h and had free access to water. Animals were sacrificed 18 h after CLP. At this time point, animals were in late stage of sepsis (18).

Preparation of cell extracts and subcellular fractions

Lymphocytes were collected from CLP-operated and sham-treated animals 18 h post-operation by Ficoll-Paque solution and stored at −20°C. For Hsp72 detection, crude cell extract was used. The cell pellets were suspended in distilled water containing 1 mM phenylmethyl-sulfonylfluoride (PMSF) and sonicated using a Ultra-Sonic homogenizer (model XL2020, Farmingdale N.Y., USA). The protein was extracted from the supernatant after centrifuging the mixture (12,000 × g) for 5 min, and quantified by protein assay kit (Bio-Rad Co., Hercules, USA). For subcellular fraction preparation, lymphocyte pellets were resuspended in 150 μL of Nonidet P-40 (NP-40)-low-salt buffer (50 mM Tris-HCl pH8.0, 50 mM NaCl, 0.5 mM PMSF, 1% NP-40). After brief agitation, the lysed cells were centrifuged at 4°C for 1 min at 12,500 × g. The lipid layer was discarded, and the supernatant containing cytosolic and solubilized membrane proteins was used as a cytosolic fraction. The pellet was resuspended in 150 μL of NP-40-high-salt buffer (same as NP-40-low-salt buffer except for 450 mM NaCl), agitated briefly, and incubated on ice for 10 min. After centrifugation as above, the supernatant fraction (the soluble nuclear fraction) was removed and the precipitate was solubilized in 100 μL of Laemmli sample buffer and boiled for 5 min. The supernatant after centrifugation at 12,500 × g for 10 min was referred as a DNA-binding protein fraction.

Hsp72 detection by SDS-polyacrylamide gel electrophoresis and Western blotting

Equal amounts (50 μg) of protein extract were loaded to two sodium dodecyl sulfate (SDS) polyacrylamide gels (T:10%, C:2.5%), and electrophoresed at 30 mA/gel on a dual electrophoretic apparatus (Atto Co. Tokyo, Japan). After electrophoresis, the gels were processed for an immunoblotting study that was performed as described previously (8). A monoclonal antibody specific for Hsp72 (Boehringer Mannheim GmbH, Germany) was used as the primary antibody, and anti-mouse immunoglobulin G conjugated with peroxidase (Boehringer Mannheim GmbH, Germany) was used as the secondary antibody. The blots were developed with a BM Chemiluminescence Western blotting kit (Boehringer Mannheim GmbH, Germany) and autoradiographed. Detection of β-tubulin was executed synchronously as an internal marker.

HSF1 identification

The procedures for protein separation and Western blotting were essentially identical to those performed for Hsp72 detection. Prestained molecular weight standards were routinely run in parallel. For immunochemical detection, membranes were washed for 10 min in PBS and blocked in PBS with 5% nonfat dry milk for 1 h at room temperature. The membranes were then incubated at 4°C with anti-HSF1 antiserum (1:1000) which was a kind gift from Professor Morimoto R.I. The membranes were washed extensively in PBS-0.05% Triton X-100 and incubated at room temperature with a second antibody (anti-rabbit immunoglobulin G conjugated with peroxidase) at a 0.1% dilution in PBS containing 1% nonfat dry milk for 2 h. After several washes with PBS-0.05% Triton X-100, the blots were developed with a BM Chemiluminescence Western blotting kit (Boehringer Mannheim GmbH, Germany) and autoradiographed.

Gel mobility shift assay of DNA-protein complex

The procedure for gel mobility shift assays was modified from a previous study (19, 20). Whole cell extract was used as the source of protein, because the oligomer of HSF translocates rapidly to the nucleus after activation. Samples were prepared by whole-cell extraction buffer (20 mM HEPES, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM PMSF, 25% glycerol) and centrifugation. Eighty μg of whole cell extract was mixed with relatively large amount (0.1 ng) of a 5′-end-biotin-labeled, double-stranded heat-shock element (HSE) oligonucleotide (5′-CTAGAAGCTTC-TAGAAGCTTGTAG-3′) in 20 μL of 50 mM HEPES (pH 8.0), 100 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mg/mL BSA, 50 μg/mL poly[d(I-C)], and 10% glycerol. Following a 30-min incubation at room temperature, samples were loaded directly onto a 1.2% agarose and electrophoresed in a 1× TBE buffer at 100V. Protein-DNA complexes in gels were transferred to a nitrocellulose membrane by vacuum blotter for 90 min and the membrane was baked in a vacuum oven at 80°C for 1 h. Biotin-labeled nucleic acid was detected using a biotin streptavidin blotting kit (Boehringer Mannheim GmbH, Germany) and the blotted membrane was autoradiographed.

Total RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated by an RNA extraction buffer (BNAzol^{TM}B, Tel-Test Inc.). Aliquots of 0.1 μg of total RNA were employed as templates for RT-PCR using a one-step RT-PCR kit (Boehringer Mannheim GmbH, Germany). The final volume of the amplification reaction was 50 μL (10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.005% NP-40, 100 mM dNTPs, 1% DMSO, 250 nM of each primer, 2 units of Taq DNA Polymerase). The oligonucleotides, 5′-CTGGGCACCACCTACTCCTG-3′ and 5′-CTCC-TTCATTCTTGGTCAGCA-3′, used in amplification reaction were 360 bp apart. Beta-actin mRNA was chosen as the reference control with the oligonucleotides, 5′-CTACAATGAGCTGCGTGTGG-3′ and 5′-TAGCTCTTCT-CCAGGGAGGA-3′ as the primers. Twenty-two cycles of PCR were executed individually at the following settings: denaturation at 94°C for 60 sec, annealing at 54°C for 90 sec, extension at 73°C for 120 sec. RT-PCR products were visualized by electrophoresis on a 1.5% agarose gel containing a trace amount of ethidium bromide, and quantified by a densitometer and analysis software (Bio-1D V.97 software, Vilber Lourmat, France).

RESULTS

Hsp72 detection

Induction of Hsp72 during various conditions was detected by SDS-PAGE and Western blotting of the crude cell proteins from the lymphocytes obtained from intact, heat-shocked, sham-treated, and CLP-operated rats, and probed with a monoclonal antibody specific for Hsp72 and β-tubulin. The results showed that β-tubulin was observed in all samples. Hsp72 was undetectable in lymphocytes from sham-treated and CLP-operated as well as intact rats, while it was sharply induced 24 h after heat-shock treatment (Fig. 1).

Dynamic distribution of HSF1

As shown in Fig. 2, under normal conditions, HSF1 is localized in cytoplasm and barely observed in the nuclear fraction (left panel). After heat-shock treatment, HSF1 immediately translocated from cytoplasm to nucleus and started to bind with DNA fragments (central panel). At 24 h after heat-shock treatment, the HSF1 in the nuclear fraction and cytoplasm disappeared, and most was detected in DNA-binding fraction (right panel). The results from the sham-treated rats showed that the distribution of HSF1 was identical to that of the intact control group; but the dynamic change of HSF1 from the CLP-induced sepsis group was similar to that at 0 h after heat shock (Fig. 3). These results indicate that the HSF1 of lymphocytes obtained from CLP-induced sepsis is activated and translocated into the nucleus and also in part bound to DNA.

Gel mobility-shift assay of DNA-protein complex

In order to ascertain that the HSF1 was activated and integrated with heat shock element (HSE) to form a HSE-HSF complex, a gel mobility shift assay was performed. As shown in Fig. 4, there were two classes of HSE-binding factors, a constitutive (CHBF) and an inducible HSE-binding factor (IHBF), easily distinguished by gel mobility shift assays. The HSE-CHBF complex has a faster migration in 1.2% agarose gel compared to HSE-IHBF complexes. In the intact rats, both the constitutive HSE-CHBF and the HSE-IHBF complexes were barely visible. When the animals were exposed to heat stress, the HSE-IHBF complex increased drastically, while CHBF remained bound with HSE. However, the band of CHBF-HSE complexes faded away as the time passed, e.g. 24

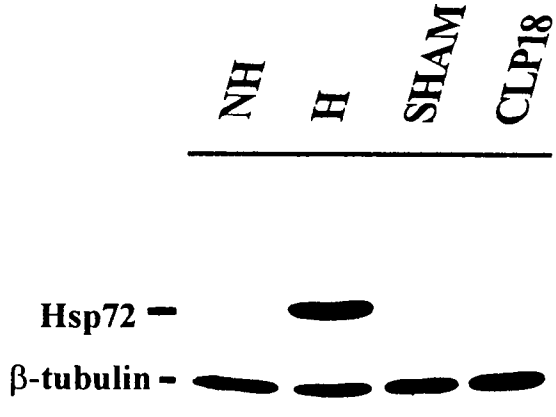

FIG. 1. **Hsp72 detection in the lymphocytes by Western blotting and immuno-chemical study.** The biosynthesis of Hsp72 in CLP was compared with sham, heated and non-heated conditions. NH: intact rat; H: 24hr after heat shock treatment; SHAM: sham operated rat; CLP18: 18hr after cecal ligation and puncture operation (late sepsis).

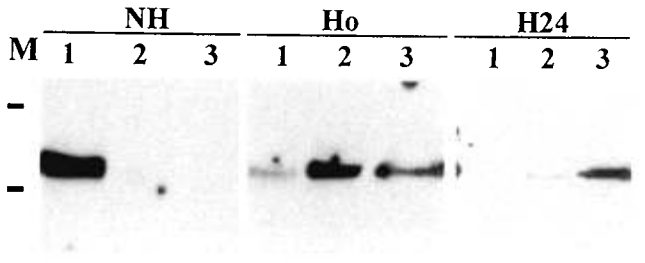

FIG. 2. **Dynamic subcellular distribution of HSF1 in the lymphocytes before and after heat shock treatment.** Left panel indicates result obtained from intact rat (NH), while those from 0hr and 24 h after heat shock treatment are shown in middle (Ho) and right (H24) panels, respectively. Lane 1: cytoplasm extract fraction; 2: nuclear fraction; 3: DNA-binding protein fraction. M: molecular marker (97.4 Kd and 67 Kd, from top to bottom).

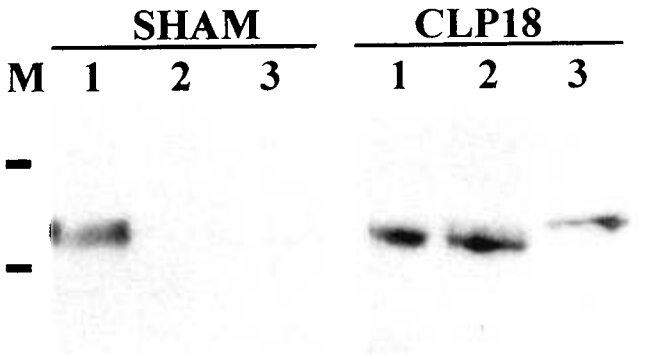

FIG. 3. **Dynamic subcellular distribution of HSF1 in the lymphocytes obtained from sham and septic rats.** SHAM: sham operated rat; CLP18, 18hr after cecal ligation and puncture operation (late sepsis). Lane 1: cytoplasm extract fraction; 2: nuclear fraction; 3: DNA-binding protein fraction. M: molecular weight marker (97.4 Kd and 67 Kd, from top to bottom).

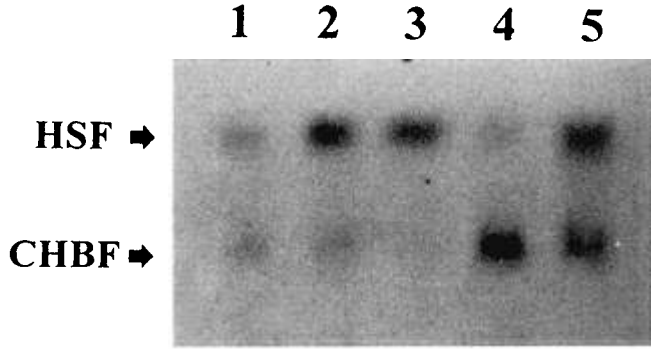

FIG. 4. **Gel mobility-shift DNA-binding assay of heat inducible HSE-binding factor and constitutive HSE-binding factor.** Arrow with HSF indicates the heat inducible HSE-binding factor and arrow with CHBF indicates the constitutive HSE-binding factor. Lane 1: intact rat; 2: 0 h after heat shock treatment; 3: 24 h after heat shock treatment; 4: sham operated rat; 5: 18hr after cecal ligation and puncture operation (late sepsis).

h after heat treatment (lane 3, Fig. 4). The HSF1 of the sham-treated group was similar to that of the intact rats, which showed insignificant generation of HSE-IHBF complex, while CHBF was quite apparent. During the late stage of sepsis, the inducible integration of HSF1 was induced to HSE, similar to the heat-shocked situation. However, CHBF did not dissociate from HSE, as shown in lane 5 of Fig. 4.

Detection of hsp72 mRNA

A fragment with 360 bp in length was amplified by 22 reaction cycles from *hsp72* mRNA, and a fragment of 420 bp from beta-actin mRNA. As shown in Fig. 5, it revealed a scanty level of RT-PCR product in the intact normal control group, but the amount was increased 6-fold in the 2 h post-heat-shock group, indicating a successful transcription of the *hsp72* gene. Although the RT-PCR product was also detectable in the sham-treated group and CLP-operated group, no difference was noted compared to the intact control group.

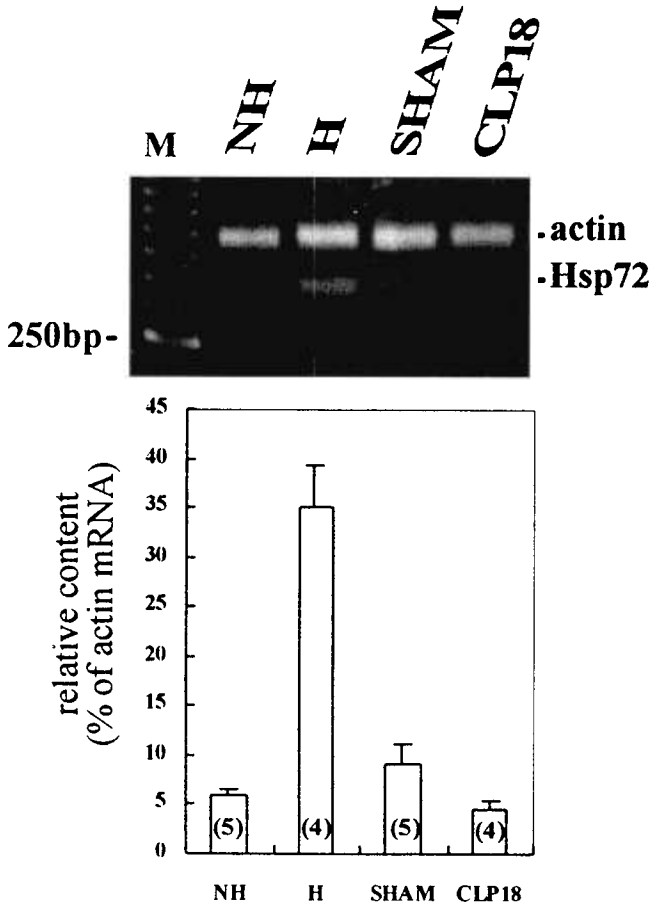

FIG. 5. **Detection and quantitative analysis of *hsp72* mRNA by reverse transcription-polymerase chain reaction (RT-PCR).** The products of RT-PCR of *hsp72* mRNA is indicated by Hsp72 (360 bp), while actin mRNA was amplified simultaneously and acted as a reference marker (420 bp) (upper panel). Values of relative intensities of *hsp72* mRNA to actin mRNA are shown at the lower panel. Vertical bars indicate S.E.M. Number of experiment is shown in parentheses in each column. NH: intact rat; H: 2 h after heat shock treatment; SHAM, sham operated rat; CLP18: 18hr after cecal ligation and puncture operation (late sepsis).

DISCUSSION

Two kinds of *hsp* genes have been reported, constitutive and inducible. Inducible *hsp* genes are expressed in response to a wide range of environmental and pathophysiological stresses (7). The products of inducible heat shock genes, the Hsps family, are documented as protective proteins. Among the family, Hsp72, a member of the Hsp70 category, is the most inducible one in mammalian cells. The induction of *hsp* gene activation and expression is autoregulated (10). The expression is believed to be mediated by the heat shock factors (HSFs), which are constitutively expressed in the cytoplasm of eukaryocytes (20, 21). In humans, two kinds of HSF genes have been cloned, HSF1 and HSF2 (22). HSF1 is responsible for mediating the cellular stress response of induction of inducible *hsp* gene expression. During heat shock, the HSF1 monomer oligomerizes in cytoplasm, to be prerequisite for DNA binding activity, and translocated to the nucleus in minute (23). The trimeric or polymeric HSF1 binds to heat shock element (HSE), a conserved sequence found upstream of all genes encoding Hsp (24). In most cases of stress, HSF-HSE complex formation promotes the expression of the *hsp* gene and induces the synthesis of Hsps.

Considering the adverse metabolic changes during sepsis, including the release of mediators, it is reasonable to propose that Hsp72 synthesis should be promoted during septic conditions. In earlier studies, as well as in the present study, however, we found no accumulation of Hsp72 upon sepsis in either lymphocytes or major vital organs, including the liver, heart, brain, and kidneys (16). Furthermore, the capability of Hsps induction of lymphocytes from septic rats, *in vitro,* is nonetheless maintained, by providing a sufficient inducer, e.g., heat at 42°C. It is inferred that the process of the *hsp* gene expression is inactivated or blocked by certain substances prevailing during sepsis. The inert response of Hsps induction may be due to suppression of HSF synthesis; failure in activation of HSF; failure in HSE-HSF binding; failure in transcription or failure in translation. Our present results show the presence of HSF in cytosolic as well as nuclear fraction of lymphocytes from heat-shocked and CLP-induced septic rats, indicating that HSF was synthesized and activated during the late septic stage. In addition, utilizing a mobility-shift assay, we have confirmed that HSE-HSF binding indeed occurred in septic lymphocytes. The accumulation of mRNA of the *hsp* gene or Hsp72 synthesis was however not increased. These data provide clues that a two-step or multi-step process in expression of the *hsp* gene existed during sepsis, and that the protein synthesis was unsuccessful despite the presence of heat shock transcription factor-DNA binding activity.

Similar phenomena have been documented by other investigators who have found that binding of HSF to HSE had no direct correlation to the transcription of Hsp70 (25–27). Morimoto and colleagues revealed that salicylate or indomethacin induced, *in vitro*, HSF1-DNA binding activity that was transcriptionally inert, indicating that HSF-DNA binding was necessary but insufficient for transcriptional activation (28). Recently, Bruce et al. showed that oxidative injury rapidly activated the heat shock transcription factor, but failed to in-

crease levels of Hsps in NIH-373 cell (29). These observations suggest that some mechanism(s) or factor(s) other than HSF-HSE binding are required for the regulation of *hsp* gene expression. The dynamic distribution of HSF shows that change of HSF1 from the CLP-induced sepsis group was similar to that of the 0 h after-heat-shock group. These results indicate that the HSF1 of lymphocytes obtained from CLP-induced sepsis is activated and translocated into the nucleus; however it is only in part bound to DNA. We predict that it is possible a critical step influencing Hsp expression is associated with the process of HSF-DNA binding.

Recently, a new concept in regulation of *hsp* gene expression focusing on the constitutive HSE-binding factor (CHBF) partly explains the mystery. In 1993, Liu et al. pointed out the "dual control" of heat shock response, in which a constitutive heat-shock element-binding factor is involved (30). The formation of HSE-HSF1 DNA-protein complex is a general step in the activation of heat shock protein genes, but the activation may be controlled by other DNA-binding proteins. Several investigators have observed that constitutive HSE-binding factor (CHBF), a molecule different from HSF1 in molecule weight, exists in mammalian cells at 37°C (30–32). CHBF is suggested to be involved in the regulation of heat shock response and appears to be a negative regulator of *hsp70* gene transcription. Liu et al. reported that heat shock treatment, at 45°C dissociated CHBF from HSE, and that the dissociation appeared to correlate with the transcription of the *hsp70* gene in Rat-1 cells (30). They also showed that dissociation of HSE-CHBF complex by a high dosage of arsenite, cadmium, or salicylate induced significant Hsp70 synthesis. In this study, we also found CHBF was present in intact rats by gel mobility-shift assay. Following the heat shock treatment, the HSF1 bound to HSE first, and then CHBF dissociated from HSE later, coincident with the induction of Hsp72 synthesis. In sepsis, DNA-binding of HSF1 was detected; however, HSE-CHBF complex did not dissociate at all, leading to no accumulation of *hsp* mRNA and Hsp72. These data afford the evidence that dissociation of CHBF-HSE complex may play a decisive role in regulating the *hsp70* gene expression during sepsis.

Our findings are of clinical significance, since we present fundamental evidence of induction failure of Hsp72 synthesis in a disease state—sepsis. These observations provide certain insights into the molecular pathogenesis during sepsis; denoting the necessity for searching for an innovative management of the disease by increasing the host self-protective defense capability. In fact, several *in vitro* studies have been undertaken in this direction. Nishimura and Dwyer have documented that addition of 1, 10-phenanthroline (a potent intracellular iron chelator) to aspirin-cocultured rat astrocytes induces synthesis of Hsp68 (26). Amici et al. have revealed that high doses of aspirin enhance Hsp70 synthesis, whereas only HSE-HSF binding is detected at lower doses (32). These studies have provided the fundamental data for exploring the therapeutical potential of Hsps.

In conclusion, multi-step regulation of Hsp synthesis was revealed in CLP-induced sepsis. HSF-HSE binding occurring in late sepsis is not sufficient for successful induction of Hsp synthesis, which in turn may be due to the failure of dissociation of CHBF from HSE. Further investigation to identify the factors responsible for interfering with the dissociation of CHBF-DNA binding during sepsis may provide better understanding of the pathogenesis of the disease entity.

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REFERENCES

1. Parrillo JE: Pathogenetic mechanisms of septic shock. *N Engl J Med* 328:1471–1477, 1993.
2. Wichterman KA, Chaudry IH, Baue AE: Studies of peripheral glucose uptake during sepsis. *Arch Surg* 114:740–745, 1979.
3. Hwang TL, Lau YT, Huang SF, Chen MF, Liu MS: Changes of alpha-1-adrenergic receptors in human liver during intraabdominal sepsis. *Hepatology* 20:638–642, 1994.
4. Ziegler EJ, Fisher CJ, Jr, Sprung CL, Straube RC, Sadoff JC, Foulke GE, Wortel CH, Fink MP, Dellinger RP, Teng NN: Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. *N Engl J Med* 324:429–436, 1991.
5. Bone RC, Fisher CJ Jr., Clemmer TP, Slotman GJ, Metz CA, Balk RA: Sepsis syndrome: a valid clinical entity. *Crit Care Med* 17:389–393, 1989.
6. Voelly R, Bromley P, Kocker HP: Structural similarities between heat shock proteins form different eukaryotes in induction of heat shock proteins. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982.
7. Morimoto RI, Sarge KD, Abravaya K: Transcriptional regulation of heat shock genes: A paradigm for inducible genomic responses. *J Biol Chem* 267:21987–21990, 1992.
8. Chen HW, Yang SL, Jing HH, Tsai YF, Tsai LY, Chen SS, Yang RC: Synthesis of Hsp72 induced by exercise in high temperature. *Chinese J Physiol* 38:241–246, 1995.
9. Locke M, Noble EG, Tanguay RM, Feild MR, Ianuzzo SE, Ianuzzo CD: Activation of heat-shock transcription factor in rat heart after heat shock and exercise. *Am J Physiol* 268:C1387–C1394, 1995.
10. Chopp M, Chen H, Ho KL, Dereski MO, Brown E, Hetzel FW, Welch KM: Transient hyperthermia protects against subsequent forebrain ischemic cell damage in the rat. *Neurology* 39:1396–1398, 1989.
11. Barbe MF, Tytell M, Gower DJ, Welch WJ: Hyperthermia protects against light damage in the rat retina. *Science* 241:1817–1820, 1988.
12. Yang, RC, Yang SL, Chen HW, Lai SL, Chen SS, Chiang CS: Previous heat shock treatment attenuates bicuculline-induced convulsion in rats. *Exp Brain Res* 108:18–22,1996.
13. Hotchkiss R, Nunnally I, Lindquist S, Taulien J, Perdrizet G, Karl I: Hyperthermia protects mice against the lethal effects of endotoxin. *Am J Physiol* 265:R1447–R1457, 1993.
14. Ribeiro SP, Villar J, Downey GP, Edelson JD, Slutsky AS: Sodium arsenite induces heat shock protein-72 kilodalton expression in the lungs and protects rats against sepsis. *Crit Care Med* 22:922–929, 1994.
15. Villar J, Ribeiro SP, Mullen JB, Kuliszewski M, Post M, Slutsky AS: Induction of the heat shock response reduces mortality rate and organ damage in a sepsis-induced acute lung injury model. *Crit Care Med* 22: 914–921, 1994.
16. Yang RC, Wang CI, Chen HW, Chou FP, Lue SI, Hwang KP: Heat shock treatment decreases the mortality of sepsis in rats. *Kaohsiung J Med Sci* 14:664–672,1998
17. Buchman TG: Manipulation of stress gene expression: A novel therapy for the treatment of sepsis? *Crit Care Med* 22:901–903, 1994.

18. Dong LW, Yang J, Tong LJ, Hsu HK, Liu MS: Group II phospholipase A2 gene expression is transcriptionally regulated in rat liver during sepsis. *Am J Physiol* 273:G706–G712, 1997.
19. Ovelgonne H, Bitorina M, van Wijk R: Stressor-specific activation of heat shock genes in H35 rat hepatoma cell. *Toxicol Appl Pharmacol* 135:100–109, 1995.
20. Sarge KD, Murphy SP, Morimoto RI: Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA binding activity, and nuclear localization and can occur in the absence of stress. *Mol Cell Biol* 13:1392–1407, 1993.
21. Baler R, Dahl G, Voellmy R: Activation of human heat shock genes is accompanied by oligomerization, modification and rapid translocation of heat shock transcription factor HSF1. *Mol Cell Biol* 13:2486–2496, 1993.
22. Nakai A, Morimoto RI: Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway. *Mol Cell Biol* 13:1983–1997, 1993.
23. Westwood JT, Wu C: Activation of Drosophila heat shock factor: conformational change associated with a monomer-to-trimer transition. *Mol Cell Biol* 13:3481–3486, 1993.
24. Kingston RE, Schuetz TJ, Larin Z: Heat-inducible human factor that binds to a human hsp70 promoter. *Mol Cell Biol* 7:1530–1534, 1987.
25. Cotto JJ. Kline M, Morimoto RI: Activation of heat shock factor1 DNA binding precedes stress- induced serine phosphorylation: evidence for a multistep pathway of regulation. *J Biol Chem* 271:3355–3358, 1996.
26. Nishimura RN, Dwyer BE: Pharmacological induction of heat shock protein68 sythesis in cultured rat astrocytes. *J Biol Chem* 270:29967–29970, 1995.
27. Hensold JO, Hunt CR, Calderwood SK, Housman DE, Kingston RE: DNA binding of heat shock factor to the heat shock element is insufficient for transcriptional activation in murine erythroleukemia cells. *Mol Cell Biol* 10:1600–1608, 1990.
28. Lee BS, Chen J, Angelidis C, Jurivich DA, Morimoto RI: Pharmacological modulation of heat shock factor 1 by anti-inflammatory drugs results in protection against stress-induced cellular damage. *Proc Natl Acad Sci USA* 92:7207–7211, 1995.
29. Bruce JL, Price BD, Coleman CN, Calderwood SK: Oxidative injury rapidly activates the heat shock transcription factor but fails to increase levels of heat shock proteins. *Cancer Res* 53:12–15, 1993.
30. Liu RY, Kim D, Yang SH, Li GC: Dual control of the heat shock response: involvement of a constitutive heat shock element binding factor. *Proc Natl Acad Sci USA* 90:3078–3082, 1993.
31. Kim D, Ouyang H, Li GC: Heat shock protein (hsp) 70 accelerates the recovery of heat-shocked mammalian cells through its modulation of heat shock transcription factor HSF1. *Proc Natl Acad Sci USA* 92:2126–2130, 1995.
32. Amici C, Rossi A, Santoro MG: Aspirin enhances thermotolerance in human erythroleukemic cells: an effect associated with the modulation of the heat shock response. *Cancer Res* 55:4452–4457, 1995.