Transcriptional Regulation of the Group IIA Secretory Phospholipase A2 Gene by C/EBPδ in Rat liver and its Relationship to Hepatic Gluconeogenesis during Sepsis

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Abstract

Background: The present study was undertaken to test hypothesis that altered transcription of secretory Phospholipase A2 (sPLA2) gene in rat liver is regulated by CCAAT/enhancer binding protein δ (C/EBPδ), and to assess its relationship to hepatic gluconeogenesis during the progression of sepsis.

Methods: Sepsis was induced by Cecal Ligation and Puncture (CLP). Experiments were divided into three groups, control, early sepsis (9 h after CLP), and late sepsis (18 h after CLP).

Results: DNA mobility and super shift assays reveal that C/EBP complexes in the liver consisted of at least three isoforms: C/EBPα, C/EBPβ, and C/EBPδ; and various C/EBP isoforms were capable of interacting with each other. Hepatocyte transfection experiments demonstrate that under normal conditions, binding of C/EBPδ to sPLA2 gene enhanced sPLA2 promoter activity and the binding resulted in an increase in hepatic gluconeogenesis. Under pathological conditions such as sepsis, binding of C/EBPδ to sPLA2 promoter increased during early and late phases of sepsis, and the increases in C/EBPδ binding correlated with increases in sPLA2 mRNA abundance and sPLA2 protein levels. Under otherwise the identical experimental conditions, hepatic gluconeogenesis was reduced during early and late phases of sepsis and the sepsis-induced reductions in liver gluconeogenesis were aggravated by binding of C/EBPδ to sPLA2 gene.

Conclusions: These results link C/EBPδ binding to altered sPLA2 promoter, and to hepatic gluconeogenesis under normal and pathological conditions. It is suggested that C/EBPδ-sPLA2-hepatic gluconeogenesis may function as a signalling axis affecting glucose homeostasis during the progression of sepsis.

Keywords: Secretory Phospholipase A2 (sPLA2); CCAAT/enhancer binding protein δ (C/EBPδ); Transcriptional regulation; Liver gluconeogenesis; Sepsis

Introduction

Phospholipase A2 (PLA2), an enzyme protein that catalyzes the hydrolysis of phospholipids at the sn-2 position to generate lysophospholipids and free fatty acids, has been implicated to play a critical role in the pathogenesis of inflammatory disorders including shock and sepsis [1-10]. In sepsis patients, plasma PLA2 activity was increased and the increased circulating PLA2 correlated positively with severity of organ dysfunction and the eventual mortality [1-4]. In human volunteers, endotoxin challenge activated serum PLA2, and elicited many features of sepsis syndrome [5,6]. In laboratory animals, secretory PLA2 (sPLA2) and cytosolic PLA2 (cPLA2) activities were increased in plasma and various organs including liver, heart, lung, spleen, thymus, and aorta following endotoxin administration [6-9] and the increased plasma sPLA2 was proportional to the decrease in the mean arterial blood pressure [6]. The notion that PLA2 plays an important role in the pathogenesis of sepsis and septic shock is further supported by recent finding that treatment of sepsis animals with antisense oligonucleotides targeting sPLA2, and cPLA2, in conjunction with antibiotics, decreased sPLA2 and cPLA2 protein expression in major organs, and the decreased tissue PLA2 protein expression in multiple organs was accompanied by an absolute reduction of 30.8% in 35-day mortality, in rats with sepsis [10].

Further studies on the underlying mechanism have revealed that the sepsis-induced over expression of sPLA2 was regulated transcriptionally. In the rat model in which the animals exhibited a biphasic feature that closely resembling the clinical sepsis syndrome [11-13], the sPLA2 activities were activated in the liver during early and late phases of sepsis [14,15] and in the heart during late phase of sepsis [16]. The activated sPLA2 activities were found to correlate with concomitant increases in the steady-state level of sPLA2 mRNA, the rate of transcription of sPLA2 gene transcript, and sPLA2 protein expression [15,16]. These findings indicate that sPLA2 activity/expression was over expressed during sepsis and the sepsis-induced over expression was regulated at the transcriptional level [15,16].

Advances in the studies of molecular biology of sPLA2 have indicated that sPLA2 gene in the liver contains four distinct regulatory elements in the promoter region: A (-35 to -6), B (-125 to - 86), C (-209 to -176), and D (-247 to -211) [17-19]. Element C binds positive regulatory factors and element D binds a negative regulatory factor. Element C contains CCAAT/enhancer binding protein δ (C/EBPδ)

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binding sites with high affinity [19]. Since C/EBPδ has been reported to function as a positive regulator for sPLA2 gene transcription [19,20] and the C/EBPδ isoform expression has been reported to increase in multiple tissues following endotoxin administration [21-23], the present study was undertaken to test our hypothesis that the altered transcription of sPLA2 gene is regulated by C/EBPδ in the liver, and to assess its relationship to hepatic glucose homeostasis, during the progression of sepsis. A network figure of regulation signalling pathway of factors mentioned above was illustrated in Figure 1.

Materials and Methods

Materials

Expression plasmids including C/EBPα(5649 bp), C/EBPδ (5254 bp), and C/EBPβ (4529 bp), cloned individually into BamHI-HindIII sites of plasmid pMEX, were obtained from Dr. Peter F. Johnson, National Cancer Institute, National Institutes of Health. The P2 (-1614 to +20) promoter for sPLA2 gene constructed into XbaI-HindIII sites of native pUC-SH-CAT plasmid, was obtained from Dr. Jean Luc Olivier, Universite Pierre et Marie Curie, France. Rat group II PLA2, cDNA cloned into Smal and EcoRI sites of plasmid pGEM3Z was a gift of Dr. Jun Ishizaki, Shionogi, Japan. The pSV-β-galactosidase (β-gal) reporter vector, pCAT (CAT=chloramphenicol acetyltransferase) basic vector, pcAT enhancer vector, and pGL3 basic vector, were purchased from Promega. C/EBPα, C/EBPβ, C/EBPδ, and CREB (cAMP response element binding protein) antibodies were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit polyclonal antibody specific to sPLA2, IIA and mouse monoclonal antibody against β-actin were products of Abcan (Cambridge, MA) and Sigma-Aldrich (St. Louis, MO), respectively.

Rat sepsis model

All animal experiments were performed with the approval of the Animal Care Committee of Saint Louis University School of Medicine, and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats weighing from 270 to 300 g were used. They were divided into three groups: control, early sepsis and late sepsis. Number of animals included in each group was 6. All animals were fasted overnight with free access to water. Sepsis was induced by Cecal Ligation and Puncture (CLP) as described by Wichterman et al. [24] with minor modification [15,25]. Under isoflurane anesthesia, a laparotomy was performed and the cecum was ligated and punctured twice with an18-gauge needle. The cecum was then returned to the peritoneal cavity and the abdomen was closed in two layers. Control rats were sham-operated (a laparotomy was performed and the cecum was manipulated but neither ligated nor punctured) and time-matched. The values obtained at three time points (0, 9, and 18 h) for sham-operated controls were virtually identical. All animals were resuscitated subcutaneously with 4 mL/100 g body wt of normal saline at the completion of surgery and also at 7 h post-surgery. Early and late sepsis refers to those animals sacrificed at 9 and 18 h, respectively, after CLP. Previous experiments show that septic rats were in hyperdynamic/hyperglycemic state (characterized by increases in body temperature, heart rate and cardiac output, and with elevated blood glucose, lactate, epinephrine and norepinephrine concentrations) during early sepsis while they were in hypodynamic/hypoglycemic state (characterized by decreases in body temperature, heart rate, cardiac output, mean arterial blood pressure, blood glucose level, and with increased blood lactate, epinephrine and norepinephrine concentrations) during late sepsis [25]. The mortality rates were 0% for control, 9% for early sepsis, and 20% for late sepsis. Only those animals that survived at each designated time point were included in the experiments.

Determination of sPLA2 protein level by Western blot analysis

Western blot analysis was performed according to the method of Ausubel et al. [26] with modification as previously described by us [15]. Samples of liver homogenate were denatured and subjected to Sodium Dodecyl Sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (10-20% polyacrylamide gradient gel). Proteins separated by SDS-PAGE were transferred to polyvinylidene fluoride membranes (Bio-Rad) and nonspecific binding sites were blocked with 10% nonfat dry milk in Tris-Buffered Saline (TBS). Blots were washed three times with TBS (TBS containing 0.5% Tween 20) followed by incubation with specific antibodies against sPLA2, IIA and β-actin for 2.5 and 1 h, respectively, at room temperature, β-actin was used as an internal standard. Subsequently, the blots were washed and incubated with immunoglobulin, peroxidase-linked species-specific secondary antibodies (Amersham Life Science) for 1 h at room temperature. Blots were developed using an Enhanced Chemiluminescent (ECL) detection reagent (Amersham Life Science) and finally exposed to Hyperfilm-ECL (Amersham Life Science). Protein bands on the film were scanned and quantified, and the relative densities were normalized for β-actin expression.

Determination of the steady-state level of sPLA2 mRNA by Northern blot analysis

Northern blot analysis was performed according to the method of Sambrook et al. [27] with modification as previously described by us [15]. Total cellular RNAs were extracted from liver tissues with acid guanidinium thiocyanate-phenol-chloroform mixture using a RNA isolation kit (bulletin 1, TEL-TEST “B”). Poly(A)+ mRNAs were isolated from total RNA by column chromatography on oligo(dT)-cellulose type 7. RNA and poly(A)+ mRNA concentrations were determined by absorbance at 260 nm, and the purities were assayed by the 260/280 nm ratio. Samples containing poly(A)+ mRNA were denatured, size fractionated, and then transferred to nylon membranes (Micron Separations). The membranes were ultraviolet cross-linked, baked, and incubated with specific probe used was a 750 bp EcoRI-PstI fragment purified from the rat group IIA cDNA. After hybridization, the membranes were washed thoroughly and the sPLA2 hybridization signals were scanned and quantified.

Figure 1: A network figure of regulation signaling pathway of factors mentioned in the introduction section. Minus (−) signs indicate inhibition.
DNA mobility shift assay (DMSA) and supershift assay

Nuclear extracts used for DMSA and supershift assay were prepared by the method of Roy et al. [28]. Liver tissues were minced, homogenized, and centrifuged (1,000 g x 8 min). The resultant pellets were lysed, homogenized, and centrifuged (12,000 g x 8 min) again. The final supernatant was dialyzed for 1 h against a buffer (50 mM KCl, 4 mM MgCl₂, 20 mM K₃PO₄, 1 mM β-mercaptoethanol), stored at -70°C, and then used for DMSA and supershift assay. DMSA and supershift assay were performed according to the method of Gao et al. [29]. For DMSA, the reaction mixture in a final volume of 20 μl contained 1 ng of 32P-labeled probe, 10 μg of nuclear extract, 20 mM Tris-HCl, pH 7.9, 1.5 % glycerol, 1 mM DTT, 0.5 mM PMSF, and 2 μg of poly(dI-dC). In competition experiments, radioactive probe and competitor were mixed prior to the addition of nuclear extract. Reactions were allowed to proceed for 20 min at 25°C, and DNA bindings were subsequently analyzed by electrophoresis (10 % polyacrylamide gel). The 32P-labeled oligonucleotide used was 5'-TGC AGA TTG CGC AAT CTG CA-3' and the 32P labeling was achieved by using T4 kinase and 50 μCi of [γ-32P] ATP. For supershift assay, the experiments were carried out in the same manner as for DMSA except that appropriate antibodies against C/EBPα, C/EBPβ, and C/EBPδ, were added to the binding reactions. For supershift analysis of the interaction between various C/EBP isoforms and CREB, CREB antibody and CREB oligonucleotide probe were used.

Measurement of sPLA2 promoter activity (hepatocyte culture, transfection experiment, CAT assay, and β-gal assay)

sPLA₂, promoter activity was determined based on the activity ratio of chloramphenicol acetyltransferase (CAT) to β-galactosidase (β-gal) in hepatocytes upon transfection with various promoter-reporter plasmids [29]. Hepatocytes used for transfection experiments were isolated according to a collagenase perfusion protocol [30]. Freshly isolated hepatocytes were washed and plated onto polylysine-coated culture dishes in attachment medium (GIBCO). After 3 h, the medium was changed to DMEM containing 5 % fetal calf serum, 1×10⁶ M dexamethasone, 10 ng/ml EGF, 5 μg/ml insulin, 2.5 μg/ml fungizone, 50 μg/ml gentamycin, 67 μg/ml penicillin, and 100 μg/ml streptomycin. The cells were then transfected with various expression plasmids harboring sPLA₂, P2 promoter, CAT, β-gal, C/EBPα, C/EMPβ, or C/EBPδ coding regions. Transfections were performed using Lipofection (GIBCO/BRL) as a transfecting reagent and were allowed to proceed for 6 h. After transfection, the cells were incubated in a reduced serum medium for 4 h and then changed to normal growth medium for 60 h. At the end of transfection experiments, the cell were harvested, lysed, and then assayed for CAT and β-gal activities [19,29]. For sPLA₂ promoter activity assay parallel plates were transfected with TK-CAT basic and TK-CAT control plasmids to serve as negative and positive controls, respectively. For β-gal assay, parallel plates were transfected with pSV basic and pSV control plasmids to serve as negative and positive controls, respectively. All values of CAT activities in hepatocyte extract were normalized to β-gal activities in the same extract.

Determination of gluconeogenesis

Gluconeogenesis was determined based on the ability of hepatocyte to produce glucose from alanine [31,32]. Following the completion of transfection experiment, hepatocytes (4×10⁶ cells) were incubated in 1 ml of Krebs-Ringer bicarbonate buffer saturated with 95% O₂-5% CO₂. The incubation was proceeded in the presence or absence of 20 mM alanine for 50 min at 37°C under 95% O₂-5% CO₂. At the end of incubation, hepatocytes were deproteinized with barium hydroxide and zinc sulfate solution. The mixture was centrifuged at 14,000 g for 2 min. The resultant supernatant was neutralized and its glucose content was subsequently assayed based on colorimetric glucose oxidase procedure [32]. Values obtained in the presence were subtracted for those in the absence of alanine, and used as gluconeogenesis activities.

Statistical analysis

Results were presented as mean ± SEM. Number of experiments was 6 for each group. Statistical analysis of the data was performed using one-way analysis of variance with a post hoc analysis using Student-Newman-Keuls tests. All calculations were performed using the standard statistical software SPSS 14.0 (Armonk, NY). A p value of less than 0.05 was accepted as statistically significant.

Results

Figure 2 shows DMSA and supershift analysis of C/EBP isoforms and their interaction with each other and with CREB in the control rat liver. Hepatic nuclear extracts prepared from control rat liver bound 32P-labeled C/EBP probe, forming C/EBP: DNA complex bands (lanes 3, 5, 7, and 9). These C/EBP complex bands were almost completely displaceable by nonlabeled C/EBP probe (comparison between lanes 3 and 1), and furthermore, they were supershifted by antibodies specific to C/EBPα (lane 4), C/EBPβ (lane 7), and C/EBPδ (lane 9). These results demonstrate that C/EBP complex was composed of various isoforms including C/EBPα, C/EBPβ, and C/EBPδ. In addition to reacting with antibodies specific to C/EBPα, C/EBPβ, and C/EBPδ isoforms, the C/EBP complex band was supershifted by antibody specific to CREB (comparison between lanes 3 and 5) and displaceable by nonlabeled CREB oligonucleotide (comparison between lanes 3 and 2). These findings indicate that C/EBP complex in the liver was composed of at least three isoforms: C/EBPα, C/EBPβ, and C/EBPδ; and furthermore, various C/EBP isoforms were capable of interacting with each other and with CREB.

Figure 3 depicts binding of C/EBPδ to sPLA₂ promoter (Figures 3A and 3B) and its relationship to changes in steady-state level of sPLA₂.
its heterodimeric complex with C/EBPα or C/EBPβ isoform.

plasmid enhanced sPLA2 promoter activity by 39% (p<0.01), while during late phase in sPLA2 protein level during the progression of sepsis (Figure 3D). These results demonstrate that binding of C/EBPβ to sPLA2 promoter in the liver was increased during the progression of sepsis, and the sepsis-induced increases in C/EBPβ binding to sPLA2 promoter were correlated with concomitant increases in sPLA2 gene transcript and protein level.

Figure 4 shows interaction between various subtypes of C/EBP expression plasmids and sPLA2 promoter activities in hepatocytes isolated from control rats. Co-transfection of control hepatocytes with sPLA2 promoter-reporter vector and C/EBPβ expression plasmid enhanced sPLA2 promoter activity by 39% (p<0.01), while co-transfection with sPLA2 promoter-reporter vector and C/EBPα expression plasmid, or with sPLA2 promoter-reporter vector and C/EBPδ expression plasmid, had no effect. The C/EBPδ-induced enhancement in sPLA2 promoter activity was further increased from 39 to 84% when C/EBPδ and C/EBPα expression plasmids were co-transfected. Similarly, the C/EBPδ-induced enhancement in sPLA2 promoter activity was further potentiated from 39 to 107% when C/EBPδ and C/EBPα expression plasmids were co-transfected. These results demonstrate that in the control liver, C/EBPδ in its monomeric form functions as an activator for sPLA2 promoter, while C/EBPα or C/EBPβ in its monomeric form had no effect. In addition, the activation of PLA2 promoter by monomeric C/EBPδ was further potentiated by its heterodimeric complex with C/EBPα or C/EBPβ isoform.

Figure 5 depicts changes in gluconeogenesis following co-transfection of sPLA2 promoter-reporter vector and various subtypes of C/EBP expression plasmids in hepatocytes isolated from control rats. It is noteworthy that the experimental protocols for Figure 5 were identical to those for Figure 4 except that the end-point measurements were different: namely, gluconeogenesis for Figure 5 versus sPLA2 mRNA abundance and sPLA2 protein expression. Although the C/EBPδ-induced enhancement in hepatic gluconeogenesis remained elevated, no further potentiation was observed when C/EBPδ was complexed with C/EBPα or C/EBPβ expression plasmid.

Figure 6 shows changes in gluconeogenesis following co-transfection of sPLA2 promoter-reporter vector and C/EBPδ expression plasmid in hepatocytes isolated from control and septic rats. When control and septic hepatocytes were transfected with sPLA2 promoter-reporter vector, glucose production was reduced by 16.7% (p<0.01) and 34.8% (p<0.01) during early and late phases, respectively, of sepsis (comparison of empty columns in Figure 6A). When control and septic hepatocytes were co-transfected with sPLA2 promoter-reporter vector and C/EBPδ expression plasmid, glucose production was reduced by 16.7% (p<0.01) and 34.8% (p<0.01) during early and late phases, respectively, of sepsis (comparison of shaded columns in Figure 6A). When data presented in Figure 6A were re-plotted as shown in Figure 6B, it is apparent that C/EBPδ binding to sPLA2 promoter plays a significant role in aggravating the reduction in hepatic gluconeogenesis during the progression of sepsis. These results together with those presented...
Table 1 depicts yields and viabilities of hepatocytes isolated from control, early sepsis, and late sepsis rats during various steps of co-transfection experiment. Yields of hepatocytes (9.1-9.3×10⁶ cells/g wet wt.) were virtually identical among control, early sepsis, and late sepsis groups. Viabilities of hepatocytes were indifferent among three experimental groups (control, early sepsis, and late sepsis) at any given time points during the co-transfection procedure, although they were decreased from 95.2-96.1 % before the transfection (3 h post-isolation) to 48.7-50.2 % after the transfection (9 h post-transfection), and remained at 44.3-46.9 % prior to lysis (79 h post-isolation). These data indicate that changes observed in sPLA₂ promoter activity (Figure 3) and gluconeogenesis (Figure 6) during the progression of sepsis was not experimental artifacts due to hepatocyte yield and viability.

Figure 7 shows uptake efficiencies of transfecting multiple vectors in hepatocytes isolated from control, early sepsis, and late sepsis animals. Transfection efficiencies were negligible in all three experimental groups (control, early sepsis, and late sepsis) when hepatocytes were transfected with pSV-β-gal basic vector. The transfection efficiencies were increased by approximately 40-fold in all experimental groups when hepatocytes were co-transfected with two (β-gal reporter + sPLA₂ promoter-reporter), three (β-gal reporter + sPLA₂ promoter-reporter + C/EBPα expression plasmid), and four (β-gal reporter + sPLA₂ promoter-reporter + C/EBPα expression plasmid + C/EBPδ expression plasmid) vectors. There were no differences in uptake efficiencies among control, early sepsis, and late sepsis groups when hepatocytes were transfected with one, two, three, or four transfecting molecules. These results reinforce the notion that the sepsis-induced alterations in sPLA₂ promoter activity (Figure 3) and hepatic glucose production (Figure 6) were not experimental artifacts due to transfection (uptake) efficiency.

**Discussion**

In this study, the DNA mobility shift and supershift assays have revealed that C/EBP complexes in rat liver consisted of at least three isoforms: C/EBPα, C/EBPβ, and C/EBPδ (Figure 2), and various C/EBP isoforms were capable of interacting with each other and with CREB (Figures 2 and 4). Subsequent hepatocyte culture and co-transfection experiments demonstrated that under physiological (control) conditions, binding of C/EBPδ to sPLA₂ gene in the liver enhanced sPLA₂ promoter activity (Figure 4) and the binding had a functional significance in regulating liver glucose metabolism, i.e., stimulating hepatic gluconeogenesis (Figure 5). Further experiments revealed that under pathological conditions such as sepsis, binding of C/EBPδ to sPLA₂ promoter increased consecutively during early and late phases of sepsis, and the increases in C/EBPδ binding to sPLA₂ gene correlated with concomitant increases in sPLA₂ mRNA abundance and sPLA₂ binding to the altered sPLA₂ promoter activity, and consequently impairing hepatic glucose homeostasis during the progression of sepsis. Based on these data, it is suggested that C/EBPδ-sPLA₂ gene transcription-hepatic gluconeogenesis may function as a signaling axis contributing to the formation of hypoglycemia during the progression of sepsis.
protein level (Figure 3). Under otherwise the identical experimental conditions, hepatic gluconeogenesis was successively reduced during early and late phases of sepsis and the sepsis-induced reductions in hepatic glucose production were aggravated by the binding of C/EBPβ to sPLA2 promoter (Figure 6). These results, to our knowledge, provide the first experimental evidence linking C/EBPβ binding to sPLA2 promoter, and to the altered hepatic glucose homeostasis under normal as well as the pathological conditions. Furthermore, the results suggest that C/EBPβ-sPLA2-hepatic gluconeogenesis may function as a signaling axis affecting glucose homeostasis during the progression of sepsis.

Alteration in hepatic glucose homeostasis in one of the key metabolic features during the progression of sepsis. The altered hepatic glucose metabolism is characterized by a rapid depletion of hepatic glycogen content, an impaired glycogen synthesis, an accelerated glycogenolysis, and a depressed gluconeogenesis [11,33]. The ultimate result of these metabolic alterations is the development of hyperglycemia during the initial phase of sepsis followed by a transition from hyper- to hypo-glycemia during late phase of sepsis [11,33]. Regulation of liver glucose metabolism is a complicated process that includes numerous hormonal regulatory factors such as catecholamines [α1 adrenergic receptor (α1AR) and β2 adrenergic receptor (β2AR) agonists], glucagon, vasopressin, angiotensin, and insulin. α1AR agonist, vasopressin, and angiotensin stimulate gluconeogenesis and glycogenolysis while α1AR agonist, vasopressin, and angiotensin stimulate glucogenolysis and glycogenolysis while they inhibit glycolysis via changes in intracellular Ca2+/calmodulin-linked protein kinases and phosphorylation of a number of protein substrates [34,35]. β2AR agonist and glucagon enhance gluconeogenic and glucolytic fluxes through activation of cAMP-dependent protein kinases and interaction with membrane receptors. Insulin, in contrast, opposes the actions of the above-mentioned hormones via phosphorylation of various protein substrates [34,35]. Our findings that binding of C/EBPβ to sPLA2 enhances sPLA2 promoter activity, activates sPLA2 gene transcription, increases sPLA2 protein expression, and finally depressing hepatic glucose production, may have a physiological significance in contributing to the understanding of the altered hepatic glucose metabolism during sepsis, because it provides an additional facet that hepatic glucose dyshomeostasis can be regulated via a non-hormonal route, i.e., C/EBPβ-sPLA2-hepatic gluconeogenesis signaling axis.

C/EBPβ is a member of the C/EBP family of transcription factors and it has been implicated to play an important role in the inflammatory responses such as sepsis and endotoxemia [22,23,36]. C/EBPβ mRNA and protein are expressed in normal tissues at a low level but are rapidly and drastically induced in many tissues by bacterial lipopolysaccharide or numerous proinflammatory mediators [21-23,36-40]. C/EBPβ expression was increased in human umbilical vein endothelial cells and in vitro incubation of cells with lipopolysaccharide [39]. C/EBPβ-DNA binding activity and C/EBPβ protein expression were upregulated in skeletal muscles following induction of sepsis in rats [40]. C/EBPβ mRNA levels were increased in multiple organs including kidney, spleen, brain, heart, intestine, lung, testes, and fat in mice after treatment with lipopolysaccharide [21]. C/EBPβ mRNA abundance and protein level were augmented in liver, lung, and kidney tissues in mice upon priming and challenge with endotoxin, and furthermore, the C/EBPβ-deficiency mice decreased endotoxin-induced systemic inflammation and partly protected against mortality [22,33]. These observations together with those presented in current study demonstrate that C/EBPβ is an important transcription factor contributing to the pathogenesis of sepsis.

C/EBPβ-binding motifs have been identified in the regulatory regions of various proinflammatory genes including those encoding sPLA2, IL-6, IL-8, IL-1β, TNF-α, inducible nitric oxide synthase, etc. [19,20,36-38]. Of particular relevance is the induction of sPLA2 during the development of sepsis [6,14-16] and the therapeutic implication on the improvement of clinical outcome upon neutralization of the over-transcribed sPLA2 gene [10]. Treatment of septic rats with antisense oligonucleotides targeting sPLA2, and cPLA2, in conjunction with antibiotics, reduced target protein expression in multiple organs including liver, heart, and kidney, resulting in an absolute reduction of 30.8 % in 35-day mortality [10]. Since the sepsis-induced activation of sPLA2 is considered to be solely secondary to the transcriptional activation of its gene [15-17] and that C/EBPβ has been identified to function as a positive regulator for sPLA2 gene transcription [19,20], it is possible that activation of C/EBPβ would translate into increases in sPLA2 mRNA abundance and protein expression. This possibility is confirmed by present findings that sepsis-induced increases in C/EBPβ binding to sPLA2 promoter correlated positively with concomitant increases in sPLA2 gene transcription and protein expression during the progression of sepsis. Of particular significance is that the activated C/EBPβ-sPLA2 signaling resulted in a deranged hepatic function by reducing its ability to produce glucose. These findings thus open a therapeutic option by targeting C/EBPβ, in addition to sPLA2, for the treatment of sepsis.

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References

1. Guidet B, Plot O, Masliah J, Barakett V, Maury E, et al. (1996) Secretory non-pancreatic phospholipase A2 in severe sepsis: relation to endotoxin, cytokines and thromboxane B2. Infection 24: 103-108.
2. Vadis P, Pruzanski W, Stefanski E, Sterbny B, Mustard R, et al. (1988) Pathogenesis of hypotension in septic shock: correlation of circulating phospholipase A2 levels with circulatory collapse. Crit Care Med 16: 1-7.
3. Nakos G, Kitsiouli E, Hatzidaki E, Koulouras V, Touqui L, et al. (2005) Phospholipases A2 and platelet-activating-factor acetylhydrolase in patients with acute respiratory distress syndrome. Crit Care Med 33: 772-779.
4. Uusitalo-Seppälä R, Peuraurovi H, Koskinen P, Vahlberg T, Rintala EM (2012) Role of plasma bactericidal/permeability-increasing protein, group IIA phospholipase A2, C-reactive protein, and white blood cell count in the early detection of severe sepsis in the emergency department. Scand J Infect Dis 44: 697-704.
5. Pruzanski W, Stefanski E, Wilmore DW, Martich GD, Hoffman AGD, et al.
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(1990) Sequential activation of TNF-phospholipase A2 axis following i.v. endotoxin challenge in human volunteers. Faseb J 4: A1714.

6. Vadás P, Przuñzawski W (1993) Induction of group II phospholipase A2 expression and pathogenesis of the sepsis syndrome. Circ Shock 39: 160-167.

7. Nakano T, Arita H (1990) Enhanced expression of group II phospholipase A2 gene in the tissues of endotoxin shock rats and its suppression by glucocorticoids. FEBS Lett 273: 23-26.

8. Liu MS, Kang GF, Ghosh S (1988) Activation of phospholipases A1 and A2 in heart, liver, and blood during endotoxin shock. J Surg Res 45: 472-480.

9. Liu MS, Takeda H (1982) Endotoxin-induced stimulation on phospholipase A2 activities in dog hearts. Biochem Med 29: 62-69.

10. Liu MS, Liu CH, Wu G, Zhou Y (2012) Antisense inhibition of secretory and cytosolic phospholipase A2 reduces the mortality in rats with sepsis. Crit Care Med 40: 2132-2140.

11. Cerra FB (1989) Multiple organ failure syndrome. In: Multiple Organ Failure. Vadas P, Pruzanski

12. Lausevic Z, Vukovic G, Stojimirovic B, Trbojevic-Stankovic

13. Iskander KN, Osuchowski MF, Steams-Kurosawa DJ, Kurosawa S, Stepien D, et al. (2013) Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. Physiol Rev 93: 1247-1288.

14. Tong LJ, Dong LW, Liu MS (1998) GTP-binding protein mediated phospholipase A2 activation in rat liver during the progression of sepsis. Mol Cell Biochem 180: 55-61.

15. Dong LW, Yang J, Tong LJ, Hsu HK, Liu MS (1997) Group II phospholipase A2 gene expression is transcriptionally regulated in rat liver during sepsis. Am J Physiol 273: G706-712.

16. Liu MS, Yang RC, Hsu C, Chen YH, Liu CH, et al. (2013) Changes in group II phospholipase A2 gene expression in rat heart during sepsis. J Surg Res 181: 272-278.

17. Lauasevic Z, Vukovic G, Stojimirovic B, Trbojevic-Stankovic J, Resanovic V, et al. (2010) Kinetics of C-reactive protein, interleukin-6 and -10, and phospholipase A2-II in severely traumatized septic patients. Vojnosanit Preg 67: 893-897.

18. Olivier JL, Fan Q, Salvat C, Ziarl M, Kong L, et al. (1994) Positive and negative hepatic regulation of the human type II phospholipase A2 gene. Biochemistry 33: 7134-7145.

19. Fan Q, Paradon M, Salvat C, Bereziat G, Olivier JL (1997) C/EBP factor suppression of inhibition of type II secreted phospholipase A2 promoter in HepG2 cells: possible role of single-strand binding proteins. Mol Cell Biol 17: 4238-4247.

20. Poli V, Mancini FP, Cortese R (1990) IL-6DBP, a nuclear protein involved in interleukin-6 signal transduction, defines a new family of leucine zipper proteins related to C/EBP. Cell 63: 643-653.

21. Alam T, An MR, Papaconstantinou J (1992) Differential expression of three C/EBP isoforms in multiple tissues during the acute phase response. J Biol Chem 267: 5021-5024.

22. Slobostra SH, Groot AP, Obdeijn MH, Reitsma PH, ten Cate H, et al. (2007) Gene expression profiling identifies C/EBPdelta as a candidate regulator of endotoxin-induced disseminated intravascular coagulation. Am J Respir Crit Care Med 176: 602-609.

23. Yan C, Johnson PF, Tang H, Ye Y, Wu M, et al. (2013) CCAAT/enhancer-binding protein delta is a critical mediator of lipopolysaccharide-induced acute lung injury. Am J Pathol 182: 420-430.

24. Wichertman KA, Baue AE, Chaudry IH (1990) Sepsis and septic shock--a review of laboratory models and a proposal. J Surg Res 29: 189-201.

25. Tang C, Liu MS (1996) Initial externalization followed by internalization of beta-adrenergic receptors in rat heart during sepsis. Am J Physiol 270: R254-263.

26. Ausubel FM, Brents R, Kingston RE, Moore DD, Seidman JD, et al. (1994) Immunoblotting and immunodetection. Curr Proto Mol Biol 2: 10.8.14.

27. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning. (2ndedn), Cold Spring Harbor Laboratory.

28. Roy RJ, Gosselin P, Guérin SL (1991) A short protocol for micro-purification of nuclear proteins from whole animal tissue. Biotechniques 11: 770-777.

29. Gao B, Jiang L, Kunos G (1996) Transcriptional regulation of alpha(1b) adrenergic receptors (alpha(1b)AR) by nuclear factor 1 (NF1): a decline in the concentration of NF1 correlates with the downregulation of alpha(1b)AR gene expression in regenerating liver. Mol Cell Biol 16: 5997-6008.

30. Preiksaitis HG, Kan WH, Kunos G (1982) Decreased alpha 1-adrenoceptor responsiveness and density in liver cells of thyroidectomized rats. J Biol Chem 257: 4321-4327.

31. Burelle Y, Filipi C, Péronnet F, Leverve X (2000) Mechanisms of increased gluconeogenesis from alanine in rat isolated hepatocytes after endurance training. Am J Physiol Endocrinol Metab 278: E35-42.

32. Raabo E, Terkildsen TC (1960) On the enzymatic determination of blood glucose. Scand J Clin Lab Invest 12: 402-407.

33. Baue AE (1994) Multiple organ failure, multiple organ dysfunction syndrome, and the systemic inflammatory response syndrome-where do we stand? Shock 2: 385-397.

34. Exton JH (1987) Mechanisms of hormonal regulation of hepatic glucose metabolism. Diabetes Metab Rev 3: 163-183.

35. Piksis SJ, Claus TH, el-Maghrabl MR (1988) The role of cyclic AMP in rapid and long-term regulation of gluconeogenesis and glycolysis. Adv Second Messenger Phosphoprotein Res 22: 175-191.

36. Poli V (1998) The role of C/EBP isoforms in the control of inflammatory and native immune functions. J Biol Chem 273: 29279-29282.

37. Natsuka S, Akira S, Nishio Y, Hashimoto S, Sugita T, et al. (1992) Macrophage differentiation-specific expression of NF-IL6, a transcription factor for interleukin-6. Blood 79: 460-466.

38. Hu HM, Tian Q, Baer M, Spooner CJ, Williams SC, et al. (2000) The C/EBP bZIP domain can mediate lipopolysaccharide induction of the proinflammatory cytokines interleukin-6 and monocyte chemotactant protein-1. J Biol Chem 275: 16373-16381.

39. Beck GC, Rafat N, Brinkkoetter P, Hanusch C, Schulte J, et al. (2006) Heterogeneity in lipopolysaccharide responsiveness of endothelial cells identified by gene expression profiling: role of transcription factors. Clin Exp Immunol 143: 523-533.

40. Penner G, Gang G, Sun X, Wray C, Hasseilgen PO (2002) C/EBP DNA-binding activity is upregulated by a glucocorticoid-dependent mechanism in septic muscle. Am J Physiol Regul Integr Comp Physiol 282: R439-444.