Transcriptional Activation of the cAMP-responsive Modulator Promoter in Human T Cells Is Regulated by Protein Phosphatase 2A-mediated Dephosphorylation of SP-1 and Reflects Disease Activity in Patients with Systemic Lupus Erythematosus

Received for publication, July 26, 2010, and in revised form, November 1, 2010. Published, JBC Papers in Press, November 19, 2010, DOI 10.1074/jbc.M110.166785

Yuang-Taung Juang‡1, Thomas Rauen‡1, Ying Wang‡, Kunihiro Ichinose§, Konrad Benedykt, Klaus Tenbrock¶, and George C. Tsokos‡2

From the ‡Division of Rheumatology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02115, the §Department of Cellular Injury, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910, and the ¶Department of Pediatrics, Rheinisch-Westfaelische Technische Hochschule University of Aachen, 52074 Aachen, Germany

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with numerous abnormalities recorded at the cellular, molecular, and genetic level. Expression of the basic leucine zipper transcription factor cAMP-responsive element modulator (CREM) was reported to be abnormally increased in T cells from SLE patients. CREMα suppresses IL-2 and T cell receptor ζ chain gene transcription by direct binding to the respective promoters. Here, we show that increased CREM expression is the result of enhanced promoter activity. DNA binding analyses reveal direct binding of transcription factor specificity protein-1 (SP-1) to the CREM promoter resulting in binding analyses reveal direct binding of transcription factor specificity protein-1 (SP-1) to the CREM promoter resulting in enhanced transcriptional activity and increased CREM expression. Protein phosphatase 2A is known to activate SP-1 through dephosphorylation at its serine residue 59. Our results show that nuclei from SLE T cells contain lower levels of Ser59-phosphorylated SP-1 protein and a stronger SP-1 binding to the CREM promoter. We conclude that protein phosphatase 2A accounts for enhanced SP-1 dephosphorylation at Ser59 in SLE T cells. More importantly, CREM promoter activity mirrors reliably disease activity in SLE patients, underscoring its potential role as a biomarker for the prediction of flares in SLE patients.

Systemic lupus erythematosus (SLE)3 is a chronic inflammatory disease characterized by an autoimmune process affecting every organ, including joints, kidneys, skin, and the central nervous system (1). T cells have been demonstrated to be important in the pathogenesis of SLE (2). Functional abnormalities in SLE T cells are largely underwritten by altered gene transcription, and recent studies have revealed a vast number of transcription factors in SLE T cells displaying changes in their expression levels and function. Among them, the α-isofrom of the transcriptional regulator cAMP-responsive element modulator (CREM) has emerged as a potent regulator of target genes that are crucially involved in T cell physiology, e.g. interleukin (IL)-2, T cell receptor ζ chain, and transcription factor c-fos (3–5).

The CREM protein family constitutes a group of transcription factors that belong to the superfamily of basic domain/leucine zipper domain proteins such as cAMP-responsive element binding protein (CREB) and activating transcription factors. All of them share high structural similarities and possess the ability to bind specifically cis-regulatory DNA sequences as homo- or heterodimers. The target motifs are palindromic DNA sequences denoted cAMP response elements (CRE, TGACGTCA) as well as the 5′-TGAC half-sites of this palindrome, both of which are highly conserved within numerous promoters of eukaryotic genes (6). Increased intracellular cAMP levels stimulate a multitude of kinases, e.g. calmodulin kinase IV, that subsequently phosphorylate and thereby activate CREM (7).

Previous studies from our group have provided evidence that CREMα expression is increased in T cells from SLE patients (8). We have shown that CREMα binds to the −180 site of the IL-2 promoter in SLE T cells and thereby suppresses IL-2 production in these cells. Decreased IL-2 levels render SLE patients more susceptible to infections (9). In turn, suppression of CREMα expression in SLE T cells by introducing a plasmid encoding antisense CREMα into these cells sufficiently restores their IL-2 content and normalizes effector cell function (10). Transcriptional down-regulation of T cell receptor ζ chain by CREMα binding to the corresponding promoter contributes to the structural abnormalities of the CD3 complex in SLE T cells. Because CREMα also represses transcription of the AP-1 family member c-fos, it limits the activity of this transcription enhancer family and thereby indirectly contributes to the defective IL-2 production in SLE T cells (2).

---

3 The abbreviations used are: SLE, systemic lupus erythematosus; CRE, cAMP-responsive element; CREB, CRE-binding protein; CREM, CRE modulator; ICER, inducible cAMP early repressor; PP2A, protein phosphatase 2A; SP-1, specificity protein 1.

‡ This work was supported, in whole or in part, by National Institutes of Health Grants AI42269 and AI68787 (to G. C. T.). This work was also supported by Deutsche Forschungsgemeinschaft Grant RA1927-1/1 (to T. R.) and Internationales Zentrum für Klinische Forschung Münster Grant FG5/H9256 (to K. T.).

§ Both authors contributed equally to this work.

1 To whom correspondence should be addressed: Division of Rheumatology, Dept. of Medicine, Beth Israel Deaconess Medical Center (CL5-937), 330 Brookline Ave., Boston, MA 02115. Tel.: 617-735-4161; Fax: 617-735-4170; E-mail: gtsokos@bidmc.harvard.edu.

2 The abbreviations used are: SLE, systemic lupus erythematosus; CRE, cAMP-responsive element; CREB, CRE-binding protein; CREM, CRE modulator; ICER, inducible cAMP early repressor; PP2A, protein phosphatase 2A; SP-1, specificity protein 1.
CREM Promoter Regulation in SLE T Cells

The CREM gene was initially isolated almost 20 years ago and comprises multiple exons (11). Since that time, a large number of different CREM isoforms has been identified, and the composition of the individual exons in these isoforms determines the functional propensities, e.g. whether they act as transcriptional activators or repressors (12). In contrast to CREB, expression and activity of the various CREM isoforms are finely regulated, not only post-transcriptionally, but also at the level of gene transcription. The CREM gene contains at least two distinct functional promoters. The inducible cAMP early repressor (ICER) isoforms of the CREM family are transcriptionally controlled through the intronic ICER promoter (11). ICER inducibility is highly cell type-specific, and its kinetics is characteristic of the early response genes. So, for example, ICER expression peaks rapidly upon stimulation with corticotrophin-releasing hormone in pituitary gland cells (13). In contrast, the CREM promoter located at the 5’-end of the CREM gene regulates transcription of the other CREM isoforms and was initially thought to be constitutively activated as expression of most CREM isoforms is ubiquitous and not inducible. However, because we found an increased amount of the transcriptional repressor CREMe mRNA in T cells from patients with SLE compared with healthy individuals (8) we asked whether elevated CREMe mRNA levels may represent the result of enhanced transcriptional activation through the CREM promoter in these cells and what key factors may be involved in CREM gene transcription.

EXPERIMENTAL PROCEDURES

Study Subjects—All SLE patients included in our studies were diagnosed according to the American College of Rheumatology classification criteria (14) and were recruited from the Division of Rheumatology at Beth Israel Deaconess Medical Center, Boston, MA, and the Department of Medicine at the Uniformed Services University of the Health Sciences, Bethesda, MD. All 12 SLE patients included in the CREM promoter assessment (as depicted in Fig. 2) were females at a median age of 36.5 years (range 22–62 years), four were of black ethnicity, and two were African-Americans. Six patients with rheumatoid arthritis or other autoimmune diseases as well as healthy individuals were chosen as controls. The study protocol was approved by the institutional review boards of all involved institutions, and written informed consent was obtained from all participating subjects.

T Cell Purification—Peripheral venous blood was collected in heparin-lithium tubes, and T cells were purified as described before (8).

Expression Plasmids—Luciferase reporter plasmids driven by serially truncated CREM promoters were generated using pGL3-Basic vector (Promega, Madison, WI). The sequences for the PCR primers used to amplify different regions of CREM promoters linked to restriction sites of KpnI (forward primers) and NheI (reverse primer) were as follows: CREM-400 forward, 5’-ttttgtaccacccctgagaagctgacaa-3’; CREM-500 forward, 5’-ttttgtacccgaagaattggagcaagcagtctgggactc-3’; CREM-800 forward, 5’-ttttgtacccgaagaattggagcaagcagtctgggactc-3’; CREM-1000 forward, 5’-ttttgtacccgaagaattggagcaagcagtctgggactc-3’; CREM-reverse, 5’-ttttgtacccgaagaattggagcaagcagtctgggactc-3’. Amplified PCR fragments were cloned into pGL3-Basic vector. Site-directed mutagenesis at the SP-1 binding site denoted −468 (CREM-500mut) was generated using a targeting primer with the following sequence: 5’-gcagagagcagatgctgag-3’. Expression vectors for PP2A and PP2A mutant were kindly provided by B.A. Hemmings (Basel, Switzerland (15)). The following plasmids were purchased: pcDNA3 (Invitrogen), pORF9-hSP1 (Invivogen, San Diego, CA). All plasmid preparations were performed using the Maxiprep kit (Qiagen, Valencia, CA).

Luciferase Assays—Three million T cells were transfected with a total of 3 μg of the indicated plasmids and 10 ng of Renilla control vector by an Amaxa transfection system (Amaxa, Cologne, Germany). Whenever an effector:reporter co-transfection was performed, the molar ratio between the two was 3:1. Five hours after transfection, cells were collected and lysed, and luciferase activity was quantified using the Dual Luciferase Assay system (Promega) and a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). Transfection efficiency was quantified by measurement of Renilla enzyme activity. Each effector:reporter transfection experiment was repeated at least three times, and values in the bar diagrams are given as mean ± S.E.

mRNA Extraction, RT-PCR, and Real-time Quantitative RT-PCR—Total RNA was isolated from three million T cells using the RNasy Mini kit (Qiagen). Residual genomic DNA contamination was removed by using DNase I (Qiagen). Sequences for PCR primers were as follows: CREM forward, 5’-tcggaacttgccgacgcga-3’; CREM reverse, 5’-ctgactaagacgaggattg-3’; 18 S rRNA forward: 5’-actaaccagggaaacctca-3’; 18 S rRNA reverse, 5’-aaccagacactgcctgcac-3’. Real-time PCR amplification of CREM was carried out using the same primers on a LightCycler 480 (Roche Applied Science), and threshold cycles (Ct) values were used to calculate relative mRNA expression by the ΔΔCt relative quantification method against 18 S rRNA expression.

Preparation of Nuclear Protein Extracts—Five million T cells from individual subjects were collected and used for preparation of nuclear protein extracts as described before (16).

Electrophoretic Mobility Shift Assays (EMSAs)—Double-stranded DNA probes harboring the −468 SP-1 binding site within the CREM-500 promoter (5’-agagccaggacagcccttcgagccacgagcagac-3’) were radiolabeled using a T4-polynucleotide kinase and [γ-32P]ATP. Binding reactions were performed at room temperature for 20 min in binding buffer (20 mM HEPES (pH 7.4), 1 mM MgCl2, 10 μg ZnSO4, 20 mM KCl, 15% Ficoll) containing 2–3 μg of nuclear protein and 1 μg of either poly(dl)poly(dC) or poly(dG)poly(dC) in a total volume of 20 μl. Electrophoresis and autoradiography were performed as described previously (16). Band intensities of protein-DNA complexes were quantified by OptiQuant™ software. For supershift assays, either 4 μg of monoclonal anti-SP-1 (Santa Cruz Biotechnology, Santa Cruz, CA) or an unrelated IgG were added to the binding reaction. Sequences for competition oligonucleotides were as follows: competitor
CREM Promoter Regulation in SLE T Cells

![Chromatin Immunoprecipitation (ChIP) Assay](image)

**FIGURE 1.** Characterization of the promoter region that is responsible for the increased expression of CREM in T cells from SLE patients. A, the human CREM gene contains two promoters. The 5′-promoter is responsible for the transcription of CREM, gene and the 3′-promoter is for the transcription of ICER gene, which encodes only part of CREM gene, mainly the DNA binding domains. B, a region spanning ~500 bp from the transcription initiation site mediates the increased promoter activity in SLE T cells. Serially truncated CREM promoters were cloned into the luciferase reporter construct as depicted by the schematic. The constructs were then transfected by nucleofector reagents into human primary T cells from either normal individuals or SLE patients. To control for the transfection efficiency, the luciferase activity shown was normalized with the activity of the control Renilla gene. n.s., not significant.

Results

CREM-500 Promoter Activity Reflects SLE Disease Activity—We generated luciferase reporter constructs containing serially truncated human CREM promoters ranging ~400, 500, 800, or 1,000 bp from the transcription initiation site (as schematically depicted in Fig. 1B). T cells obtained from at least three pairs of SLE patients and healthy control individuals were transfected with these reporters. Luciferase activity for each reporter was calculated relative to the basal empty reporter activity (Fig. 1B). Activity of the CREM promoter spanning ~500 bp, denoted CREM-500, was significantly elevated when introduced into SLE T cells compared with T cells from healthy individuals. (3.08 ± 1.90 versus 0.87 ± 0.33, p < 0.05). The activity of other CREM promoter constructs was much lower than that of the CREM-500 reporter, in both normal and SLE T cells. Furthermore, there were no significant differences in these constructs between normal and SLE T cells. We concluded that the CREM-500 promoter contains the crucial cis-regulatory elements mediating transcriptional CREM up-regulation in SLE T cells.

CREM-500 Promoter Activity Reflects SLE Disease Activity—We next increased the number of SLE patients and healthy controls to 12 individuals/group and included six patients with rheumatoid arthritis and other autoimmune diseases (Fig. 2A). We purified T cells from these patients and transfected them with the CREM-500 reporter plasmid. The relative CREM-500 promoter activity level was significantly elevated in SLE T cells compared with T cells from healthy controls (3.54 ± 2.23 versus 1.51 ± 1.31, respectively, p < 0.05). In T cells from patients with other autoimmune diseases CREM-500 promoter activity was enhanced as well (2.16 ± 1.66); however, this was not significant compared...
Statistical correlation is given as

which was mutated at the predicted

petition assays by inclusion of two oligonucleotides, one of

specifically that SP-1 binds to this site we extended our com-

sion of the irrelevant competitor but completely abrogated by

the intensity of one particular band (indicated by an

arrow)

expected, inclusion of the first one did not significantly alter

band intensity whereas the latter one decreased band intensi-

ity in a concentration-dependent manner (Fig. 3C, lanes

6–9). Taken together, these DNA-binding analyses indicate

that endogenous SP-1 protein specifically interacts with the

−468 site within the CREM promoter and participates in the

complexes observed with nuclear protein extracts from hu-

man T cells.

We next determined the trans-regulatory capacities of SP-1

on the CREM promoter using the wild-type CREM-500 lucif-

erase reporter and a CREM-500 plasmid containing a −468 site

mutation (Fig. 3D). Human T cells were transiently co-

transfected with the respective promoter constructs and ei-

ther pCDNA3 vector or an expression plasmid encoding SP-1

and subsequently assayed for luciferase activity. Notably, SP-1

significantly trans-activated the CREM-500 promoter (2.00 ±

0.24; p < 0.05), most likely through its binding to the −468

site as mutation at this site renders the CREM-500 reporter

not inducible by SP-1 (1.13 ± 0.31; p = 0.69).

CREM Gene Transcription in SLE T Cells Is Up-regulated

through Enhanced SP-1 Binding to the −468 Site of the CREM

Promoter—After we identified SP-1 as a transcriptional regu-

lator at the −468 site within the CREM promoter we next

wanted to clarify whether there is a difference in the binding

affinity of endogenous SP-1 protein to this sequence between

nucleoprotein from SLE patients and normal individuals.

Therefore, we obtained T cell nucleoprotein lysates from 11

SLE patients and 8 controls and performed EMSAs using the

CREM-500 (−468) oligonucleotide as a probe (Fig. 4A). In-

deed, using SLE T cell nucleoprotein resulted in a significantly

stronger band intensity of the specific protein

complexes observed with nuclear protein extracts from hu-

man T cells. ChIP analyses were performed in normal and SLE T cell

samples (n = 2) using a polyclonal SP-1 antibody. Results from

these experiments confirmed the EMSA data and indicate in-

creased SP-1 binding to the CREM promoter in SLE T cells in

vivo (a representative result of the ChIP is shown in Fig. 4B).

SP-1 Activation Is Mediated through Dephosphorylation by

Phosphatase PP2A—SP-1 function is highly dependent on its

phosphorylation status, and its serine 59 has been identified

as a major target for phosphatase PP2A during cell cycle

interphase (18). Because we already proved that SP-1 by itself

trans-activates CREM-500 promoter (see Fig. 3D), we next

asked whether addition of PP2A to this reaction can further

increase CREM-500 activation. Human T cells were trans-

fected with the wild-type CREM-500 promoter construct or its

mutant CREM-500mut, as well as the SP-1-encoding plas-

mid with and without overexpression of PP2A (Fig. 5A). In-

clusion of PP2A enhanced trans-activational capacity of SP-1

on the CREM-500 promoter (1.60 ± 0.26; p < 0.05). As ex-

pected, there was no activation of the CREM-500mut reporter

(0.69 ± 0.16; p = 0.13). This transcriptional effect was con-

firmed at the mRNA level using T cells transfected with either

SP-1, PP2A, or both expression plasmids (Fig. 5B). Co-expres-

sion of SP-1 and its activating phosphatase PP2A yielded a

robust 40.5-fold increase of CREM mRNA as quantified by

real-time PCR (Fig. 5B, bar diagram). Specificity of PP2A ef-

fect was further substantiated by using a PP2A construct un-
able to dephosphorylate substrates due to a site-directed mutation within its active enzymatic domain (15). This defective PP2A protein was not able to increase SP-1 effect on the wild-type CREM-500 promoter (Fig. 5C).

We have previously reported that the PP2A protein amount is increased in T cells from lupus patients to a degree that reflects the respective SLE disease activity status (19). PP2A is known to activate specifically SP-1 through dephosphorylation at its serine residue 59. Accordingly, we set out to determine the phosphorylation status of SP-1 protein at Ser59 in SLE T cells versus normal T cells (Fig. 5C). We analyzed nucleoprotein lysates obtained from eight lupus T cell and five normal T cell samples by Western blotting using antibodies against either Ser59-phosphorylated SP-1 or total SP-1 protein. Fig. 5D shows representative results from two normal and two lupus T cell analyses. Densitometry of the respective band intensities of all samples demonstrated a significantly lower ratio of Ser(P)59 SP-1 over total SP-1 protein in T cells from lupus patients compared with normal T cells (0.30 ± 0.18, n = 8 versus 0.76 ± 0.32, n = 5; p < 0.05). This supports the hypothesis that increased PP2A amounts in SLE T cells mediate Ser59 dephosphorylation and thus functional activation of SP-1.

DISCUSSION

In this paper we present evidence that increased expression of the transcriptional repressor CREMα in T cells from SLE...
CREM Promoter Regulation in SLE T Cells

We reported previously on CREMα overexpression in SLE T cells (8). Increased CREMα levels account for suppressed expression of a multitude of T cell–related genes, including IL-2 and T cell receptor ζ chain (3, 4). IL-2 has well been demonstrated to be important in 1) the generation and function of T regulatory cells, 2) activation-induced cell death, and 3) the generation of cytotoxic cells that are necessary for the defense against infections (20).

We found that increased transcription activity, as assayed by CREM promoter activity, is responsible for the increased CREM expression in SLE patients. Notably, our analyses displayed a statistically strong correlation between the CREM promoter activity levels and SLE disease activity indices, which are a marker of disease activity capturing both clinical and laboratory manifestations in SLE patients. This result indicates that modulating CREM expression may be beneficial for the control of autoimmune processes and inflammation in SLE patients. Furthermore, CREM promoter activity represents a new candidate biomarker for lupus disease activity. Longitudinal studies need to be conducted to define the temporal relationship between CREM promoter activity and clinical activity. In contrast to most biomarker studies that assay candidate molecules on either mRNA or protein expression level, to the best of our knowledge, this study is the first one to utilize the promoter activity of a gene as read-out system. Replacement of the luciferase gene by a GFP-encoding sequence should allow for an easier assessment of CREM promoter activity.

Our current study points to protein phosphatase PP2A as a key player in the CREMα up-regulation in SLE T cells. We demonstrated previously that expression levels of PP2A are increased in SLE T cells. In addition, higher PP2A expression levels are present in patients with higher disease activity. This finding complements our previous results in which we showed that PP2A mediates dephosphorylation of p-CREB, which contributes to the defective production of IL-2 in SLE T cells (19). Both CREB and CREM are members of the basic domain/leucine zipper domain proteins transcription factor family, although their transcriptional effects on IL-2 regulation are antithetic. Thus, PP2A employs two distinct mechanisms to suppress IL-2 expression in SLE patients: activating a suppressor (CREMα) and concurrently suppressing an activator (p-CREB). Similar mechanisms may also take place in the abnormal expression of other genes in SLE T cells as >3000 genes encode the CRE site on their promoters (5).
Transcription factor SP-1 is ubiquitously expressed in numerous cell types, and its binding site is present in the promoter of most genes. A closer analysis of the core region of the human CREM promoter revealed at least six sites highly homologous to the consensus SP-1 binding site. Interestingly, our studies indicate that SP-1 binding to one of these sites (at position -468 relative to the transcription initiation site) is responsible for transcriptional activation of the CREM promoter and thus for increased CREM expression in primary human T cells. At this point, we cannot exclude that other SP-1 sites may also become functional for CREM expression in a different context such as different cell development stages.

SP-1 function is highly regulated through a number of post-translational modifications, e.g., phosphorylation occurring at multiple serine and threonine residues; however, only serine 59 is specifically dephosphorylated by PP2A (18). This finding corroborates with our previous results that expression of PP2A was increased in SLE T cells and the current finding that nuclear SP-1 protein phosphorylated at serine 59 is decreased in SLE T cells whereas cellular amounts of total SP-1 protein are comparable between T cells from lupus patients and healthy individuals. It was shown previously that dephosphorylation of SP-1 increased its affinity to DNA whereby this enhanced its transcriptional activity. In addition to phosphorylation, O-linked glycosylation has also been reported to regulate the SP-1 activity. Whether O-linked glycosylation is also involved in the PP2A-mediated SP-1 effects on the human CREM promoter deserves further investigation as reciprocal phosphorylation and O-linked glycosylation are common mechanisms regulating the activity of a number of intracellular proteins.

Dysregulated function of T cells is central to the disordered immune system in SLE patients. The presented characterization of PP2A > SP-1 > CREM signaling pathway (Fig. 6) unravels another key component of lupus T cell abnormalities and extends our understanding of the pathogenic potential of SLE T cells.

REFERENCES
1. Rahman, A. and Isenberg, D. A. (2008) N. Engl. J. Med. 358, 929–939
2. Tenbrock, K., Juang, Y. T., Kyttarlis, V. C., and Tsokos, G. C. (2007) Rheumatology 46, 1525–1530
3. Solomou, E. E., Juang, Y. T., Gourley, M. F., Kammer, G. M., and Tsokos, G. C. (2001) J. Immunol. 166, 4216–4222
4. Tenbrock, K., Kyttarlis, V. C., Ahlmann, M., Ehrchen, J. M., Tolnay, M., Melkonyan, H., Mawrin, C., Roth, J., Sorg, C., Juang, Y. T., and Tsokos, G. C. (2005) J. Immunol. 175, 5975–5980
5. Kyttarlis, V. C., Juang, Y. T., Tenbrock, K., Weinstein, A., and Tsokos, G. C. (2004) J. Immunol. 173, 3557–3563
6. Sassone-Corsi, P. (1995) Annu. Rev. Cell Dev. Biol. 11, 355–377
7. Juang, Y. T., Wang, Y., Solomou, E. E., Li, Y., Mawrin, C., Tenbrock, K., Kyttarlis, V. C., and Tsokos, G. C. (2005) J. Clin. Invest. 115, 996–1005
8. Kyttarlis, V. C., Wang, Y., Juang, Y. T., Weinstein, A., and Tsokos, G. C. (2006) Lupus 15, 840–844
9. Iliopoulos, A. G., and Tsokos, G. C. (1996) Semin. Arthritis Rheum. 25, 318–336
10. Tenbrock, K., Juang, Y. T., Gourley, M. F., Nambiar, M. P., and Tsokos, G. C. (2002) J. Immunol. 169, 4147–4152
11. Foulkes, N. S., Borrelli, E., and Sassone-Corsi, P. (1991) Cell 64, 739–749
12. Laoide, B. M., Foulkes, N. S., Schlotter, F., and Sassone-Corsi, P. (1993) EMBO J. 12, 1179–1191
13. Della Fazia, M. A., Servillo, G., Foulkes, N. S., and Sassone-Corsi, P. (1998) FEBS Lett. 434, 33–36
14. Hochberg, M. C. (1997) Arthritis Rheum. 40, 1725
15. Evans, D. R., Myles, T., Hofsteenge, J., and Hemmings, B. A. (1999) J. Biol. Chem. 274, 24038–24046
16. Solomou, E. E., Juang, Y. T., and Tsokos, G. C. (2001) J. Immunol. 166, 5665–5674
17. Moulton, V. R., and Tsokos, G. C. (2010) J. Biol. Chem. 285, 12490–12496
18. Vicart, A., Lafeyvre, T., Imbert, J., Fernandez, A., and Kahn-Perlès, B. (2006) J. Mol. Biol. 364, 897–908
19. Katsiari, C. G., Kyttarlis, V. C., Juang, Y. T., and Tsokos, G. C. (2005) J. Clin. Invest. 115, 3193–3204
20. Lieberman, L. A., and Tsokos, G. C. (2010) J. Biomed. Biotechnol., in press