Arch Pharm Res Vol 27, No 4, 396-401, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Regulation of c-Fos and c-Jun Gene Expression by Lipopolysaccharide and Cytokines in Primary Cultured Astrocytes: Effect of PKA and PKC Pathways

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(Received October 22, 2003)

The effects of lipopolysaccharide (LPS) and several cytokines on the c-fos and c-jun mRNA expression were examined in primary cultured astrocytes. Either LPS (500 ng/mL) or interferon-γ (IFN-γ; 5 ng/mL) alone increased the level of c-fos mRNA (1 h). However, tumor necrosis factor-α (TNF-α; 10 ng/mL) or interleukin-1β (IL-1β; 5 ng/mL) alone showed no significant induction of the level of c-fos mRNA. TNF-α showed a potentiating effect in the regulation of LPS-induced c-fos mRNA expression, whereas LPS showed an inhibitory action against IFN-γ-induced c-fos mRNA expression. LPS, but not TNF-α, IL-1β and IFN-γ, increased the level of c-jun mRNA (1 h). TNF-α and IFN-γ showed an inhibitory action against LPS-induced c-jun mRNA expression. Both phorbol 12-myristate 13-acetate (PMA; 2.5 mM) and forskolin (FSK; 5 mM) increased the c-fos and c-jun mRNA expressions. In addition, the level of c-fos mRNA was expressed in an antagonistic manner when LPS was combined with PMA. When LPS was co-treated with either PMA or FSK, it showed an additive interaction for the induction of c-jun mRNA expression. Our results suggest that LPS and cytokines may be actively involved in the regulation of c-fos and c-jun mRNA expressions in primary cultured astrocytes. Moreover, both the PKA and PKC pathways may regulate the LPS-induced c-fos and c-jun mRNA expressions in different ways.

Key word: Lipopolysaccharide, Cytokines, PMA, Forskolin, c-Fos, c-Jun, Astrocytes

INTRODUCTION

Lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, induces several genes, which are associated with inflammation processes (Morrison and Ryan, 1987). In addition, LPS induces TNF-α mRNA expression in macrophages in a synergistic manner, when combined with interferon-γ (IFN-γ) (Koerner *et al.*, 1987). Cross-talk between the signal transduction pathways upstream of the activation of the transcription factors contributes to the generation of these synergistic actions (Cheshire and Baldwin 1997; Cheshire *et al.*, 1999; Robinson and Cobb, 1997). Cooperative activity of proinflammatory agents profoundly influences the immune response to the infections and the efficiency of cellular clearance mechanism (Paludan, 2000).

Several lines of evidence have demonstrated that LPS is involved in the regulation of c-fos and c-jun mRNA expressions. For example, LPS alone increased the c-jun, but not the c-fos, mRNA level. However, LPS showed a potentiating effect for c-fos mRNA expression when C6 rat glioma cells were co-treated with LPS and cycloheximide (Kim *et al.*, 2000). However, LPS increases both the c-fos and c-jun mRNA levels in primary cortical glial culture, mesangial cells and the murine macrophage J774.2 cell line (Granger *et al.*, 2000; Simi *et al.*, 2000; Tengku-Muhammad *et al.*, 2000). In addition, several cytokines, such as TNF-α (Ahmad *et al.*, 1998; Ryuto *et al.*, 1996), IFN-γ (Granger *et al.*, 2000; Rubio, 1997) and interleukin-1β (IL-1β) (Barnea *et al.*, 2000; Goldring *et al.*, 1994), are known to be involved in the regulation of c-fos or c-jun mRNA expressions in various types of cells.

Both c-fos and c-jun belong to gene families whose

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products share general structural features with other transcription factors, which together constitute a superfamily, termed basic-zipper proteins. These proteins form homo- and heterodimers by way of a leucine-zipper structure (Landschulz *et al.*, 1988). For example, Fos-Jun heterodimers bind to the activator protein-1 (AP-1) consensus sites (-TGACTCA-) (Rajaram and Kerppola, 1997). Both protein kinase A and C (PKA and PKC) activators are involved in the regulation of immediate early gene, such as c-fos and c-jun, expressions. For example, forskolin (FSK), a PKA activator, increases the c-fos mRNA level, but causes down-regulation of the c-jun mRNA levels in C6 rat glioma cells (Lee *et al.*, 2001). In contrast, phorbol 12-myristate 13-acetate (PMA), a protein kinase C activator, increases both the c-fos and c-jun mRNA levels in C6 rat glioma cells (Lee *et al.*, 2001).

The role of LPS in the regulation of c-fos and c-jun mRNA expressions in primary cultured astrocytes has not been well characterized. In addition, the presumptive modulatory roles of various kinds of proinflammatory cytokines in the regulation of LPS-induced c-fos or c-jun mRNA expressions are, as yet, unknown. Furthermore, the modulatory roles of PKA and PKC activators in the regulation of LPS-induced c-fos and c-jun mRNA expressions have not been well characterized. Thus, in the present study, the effects of various kinds of cytokines, FSK or PMA were examined on the c-fos and c-jun mRNA expressions induced by LPS in primary cultured astrocytes.

MATERIALS AND METHODS

Primary astrocyte-enriched cultures and chemicals

Primary astrocyte-enriched cultures were prepared from the whole cortex of 1-day-old Sprague-Dawley rats. The cortex was rapidly dissected in ice-cold calcium/magnesium free Hank's balanced salt solution (HBSS) (Gibco, Grand Island, NY) at pH 7.4. The tissue was then minced, incubated in HBSS containing trypsin (2 mg/mL) for 20 min at 37°C and washed twice in plating medium; Dulbecco's modified Eagle's medium (DMEM) (Gibco), containing 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2.2 g/L sodium bicarbonate, 0.6% (w/v) D-glucose and 20 mg/mL gentamicin, and then disrupted by trituration through a Pasteur pipette. The cells were introduced to 25 cm^2 culture flasks (Falcon, Franklin, NJ). The cultures were incubated at 37°C in 5% CO_2, and after 1 day the medium was completely changed to the culture medium: DMEM containing 5% heat-inactivated FBS, 2.2 g/L sodium bicarbonate, 0.6% (w/v) D-glucose and 20 mg/mL gentamicin. Half the culture medium was exchanged with fresh medium twice a week. After 14-15 days the cells were shaken on an orbital shaker for at least one day to remove the microglia. The cells then formed a dense monolayer composed of mostly type I astrocytes (96%), which were positive for glial fibrillary acidic protein (GFAP) (Sigma, St. Louis, MO), but rarely positive for OX42 (≈3%) (Serotech, NY), and negative for the A2B5 (Boehringer Mannheim, Mannheim, Germany) and neurofilament 68 (Sigma) antigens (Vilijn *et al.*, 1988). The cells were incubated with new culture medium for 24 h prior to incubation with the chemicals. The LPS and FSK were purchased from Sigma chemicals, the PMA from RBI (Natick, MA) and the TNF-α, IL-1β, and IFN-γ were purchased from R&D systems (Woongbee Meditech, Seoul, Korea).

Isolation of total RNA

Total cellular RNA was extracted from primary cultured astrocytes using rapid guanidine thiocyanate-water saturated phenol/chloroform extraction, and subsequently precipitated with acidic sodium acetate (Chomczynski and Sacchi, 1987). The total cellular RNA in the aqueous phase was precipitated with cold isopropyl alcohol and the pellet washed with 70% ethyl alcohol. Isolated RNA pellets were dissolved in 30-40 mL of 0.1% diethyl pyrocarbonate (DEPC) water and 400 X diluted RNA solution, and subjected to spectrophotometric analysis at 260 and 280 nm.

Preparation of DIG-labeled cRNA probes

The cRNA probes for c-fos (Curran *et al.*, 1987), c-jun (Bohman *et al.*, 1987) and cyclophilin (Danielson *et al.*, 1988) were synthesized *in vitro* from a linearized expression vector, which contained the SP6 or T7 viral promoters. One microgram of the linearized plasmid was mixed with an RNA labeling mixture containing ATP, CTP, GTP, and Dig-labeled-UTP, transcription buffer, and SP6 or T7 RNA polymerase. After incubation at 37°C for 2 h, the mixture was co-incubated with DNase I (RNase free) at 37°C for 15 min, precipitated in ethanol containing lithium chloride at 70°C for 30 min, and washed with 70% chilled ethanol (Boehringer Mannheim, Germany).

Non-isotope Northern blot hybridization analysis

Ten micrograms of total RNA were denatured, subjected to electrophoresis on 1% agarose-formaldehyde gels (Kopchik *et al.*, 1981) and transferred to nylon hybond-N hybridization membrane sheets (Amersham, Buckinghamshire, England). After UV cross-linking, the membranes were prehybridized at 68°C for at least 1 h in prehybridization buffer (5 X SSC, 50% formamide, 0.02% SDS, 0.1% sodium N-lauroyl sarcosine, and 2% blocking reagent). The Dig-labeled c-fos and c-jun probes were added to prehybridization buffer containing 50% forma-

mide. The membranes were incubated overnight at 68°C in a shaking water bath, and washed twice for 15 min per wash in 2 X wash solution (2X SSC, 0.1% SDS) at room temperature. Then, the membranes were washed twice, for 15 min per wash, in 0.1 X washing solution (0.1 X SSC, 0.1% SDS) at 68°C. After equilibrating the membranes in buffer I (100 mM maleic acid [pH 7.5] and 150 mM NaCl,) for 1 min, they were gently agitated in buffer II (1% blocking reagent in buffer I) for 30-60 min. The membranes were hybridized with the diluted anti-DIG-alkaline phosphatase [1:10,000 (75 mU/mL)] in buffer II for 30 min. After washing the membrane twice, for 15 min per wash, in 0.3% Tween 20 (in buffer I), the membranes were equilibrated in buffer III (100 mM Tris-HCl [pH 9.5], 100 mM NaCl and 50 mM $MgCl_2$) for 2 min. Diluted CSPD® (Boehringer Mannheim, Germany) (1:100 diluted in buffer III) was spread over the surface of membrane, and after incubation at 37°C for 15-20 min, were exposed to Hyperfilm-ECL (Amersham, Buckinghamshire, England) for detection of the chemi-luminescent signal. For rehybridization, the membranes were washed for 20 min at room temperature in sterile millipore water, then overnight at 65°C in 50 mM Tris-HCl (pH 8.0), 50% dimethylformamide and 1% SDS to remove the hybridized probe, and rehybridized to a Dig-labeled rat cyclophilin cRNA probe, a gene encoding peptidyl-prolyl (propyl?) *cis-trans* isomerase, which is constitutively expressed in most mammalian tissues, with the exception of skeletal muscle (Takahashi *et al.*, 1989).

Statistical analysis

The expressions of c-fos and c-jun mRNA induced by the chemicals were quantified with Bio-profiil Bio-1D application (Vilber-Lourmat, France), and expressed as percentages of the control c-fos and c-jun mRNA levels. One-way analysis of variance (ANOVA), with the Newman-Keuls multiple comparison test, was used to compare the relative abundance of mRNA between the groups. *P* values less than 0.05 were considered significant.

RESULTS

c-Fos and c-jun mRNA expressions by LPS and cytokines

The concentrations of LPS (500 ng/mL), TNF-α (10 ng/mL), IL-1β (5 ng/mL) and IFN-γ (5 ng/mL) were chosen on the basis of preliminary studies and previous publications (Lee *et al.*, 2003). As shown in Fig. 1A, LPS or IFN-γ alone increased the c-fos mRNA levels (1 h). However, neither TNF-α nor IL-1β alone showed significant inductions of c-fos mRNA expression. In the interaction study, TNF-α showed a potentiating effect for the up-regulation of LPS-induced c-fos mRNA expression, whereas LPS showed an inhibitory action against IFN-γ-induced c-fos mRNA expression. In addition, IL-1β did not

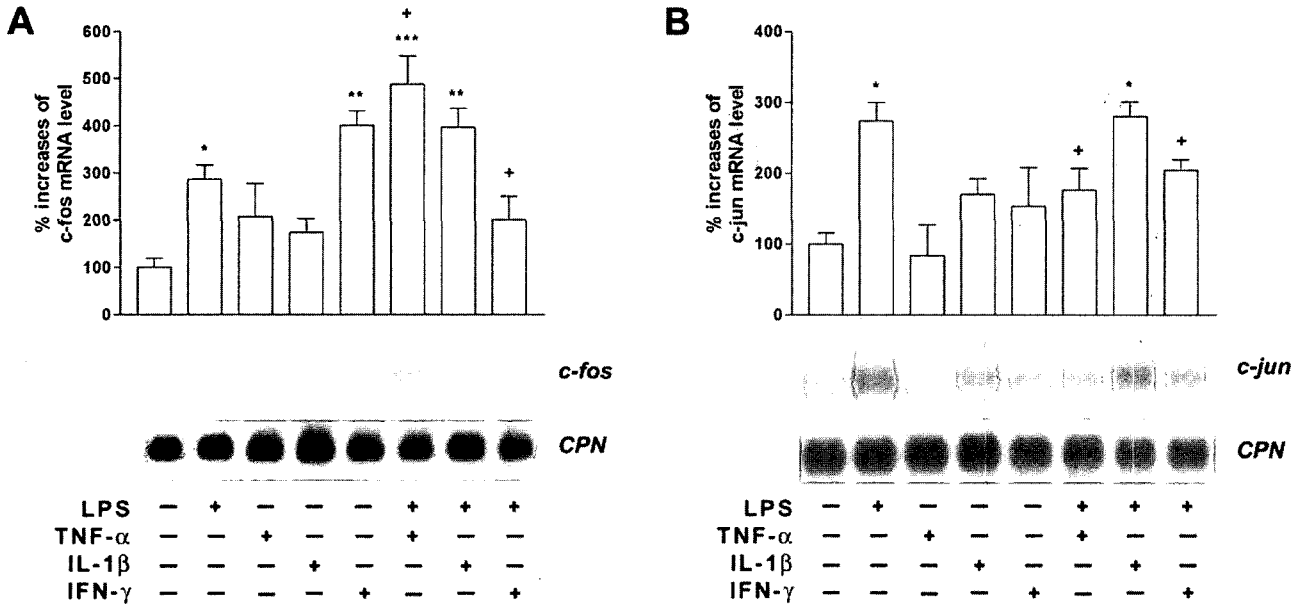

Fig. 1. The effects of lipopolysaccharide (LPS) and cytokines on c-fos (A) and c-jun (B) mRNA expressions in primary cultured astrocytes. Northern blot analysis was performed at 1 h in the cells treated with the LPS and cytokines. The final concentrations were as follows: LPS, 500 ng/mL; TNF-α, 10 ng/mL; IL-1β, 5 ng/mL, and IFN-γ, 5 ng/mL. Cyclophilin (CPN) was used as an internal loading control. The vertical bars in graphs indicate the S.E.M. (**P*<0.05, ***P*<0.01, and ****P*<0.001 compared to the control group; +*P*<0.05 compared to the LPS-treated group; n=3 independent experiments).

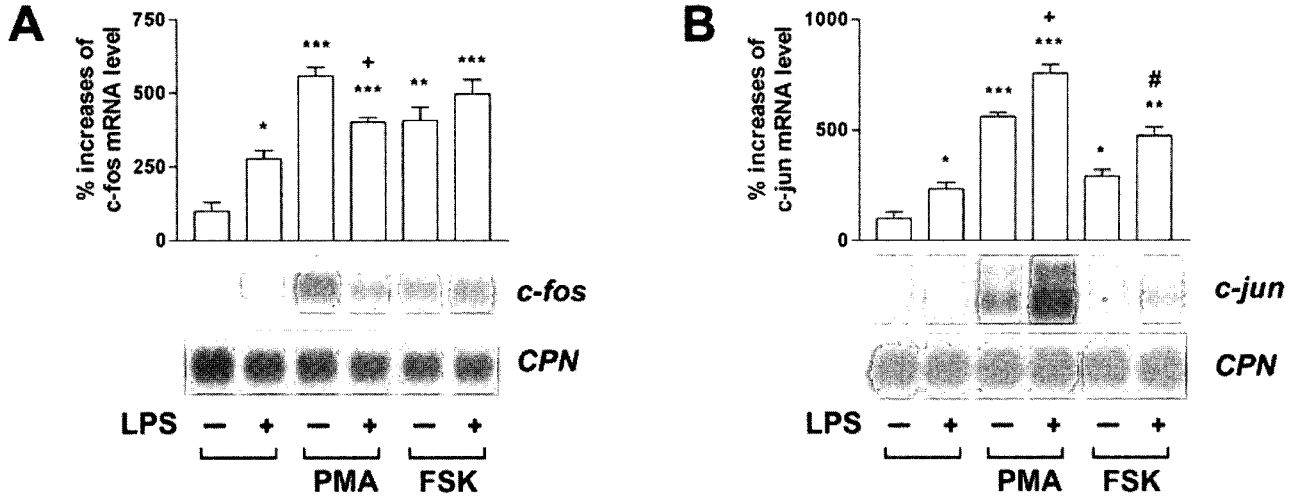

Fig. 2. The effects of lipopolysaccharide (LPS), phorbol 12-myristate 13-acetate (PMA) and forskolin (FSK) on the c-fos (A) and c-jun (B) mRNA expressions in primary cultured astrocytes. Northern blot analysis was performed at 1 h in the cells treated with the control medium, LPS (500 ng/mL) alone, PMA (2.5 μM) alone, FSK (5 μM) alone and LPS plus either PMA or FSK. Cyclophilin (CPN) was used as an internal loading control. The vertical bars in graphs indicate the S.E.M. (*P<0.05, **P<0.01, and ***P<0.001 compared to the control group; ^{+}P<0.05 compared to the PMA-treated group, $^{\#}P$<0.05 compared to the FSK-treated group; n=3 independent experiments).

affect LPS-induced c-fos mRNA expression.

As shown in Fig. 1B, LPS, but not TNF-α, increased the c-jun mRNA level (1 h). Although IL-1β or IFN-γ alone appear to increase the c-jun mRNA level to some extent, its increase was not statistically significant. TNF-α and IFN-γ showed inhibitory actions against the LPS-induced c-jun mRNA expression. However, IL-1β did not affect the LPS-induced c-jun mRNA expression.

Effect of PMA and FSK on c-fos and c-jun mRNA expression induced by LPS

The concentrations of PMA (2.5 μM) and FSK (5 μM) were chosen on the basis of preliminary studies and previous publications (Won and Suh, 2000). As shown in Fig. 2A, PMA or FSK alone increased the c-fos mRNA levels. The c-Fos mRNA level was also attenuated when PMA and LPS were combined, whereas FSK did not alter the LPS-induced c-fos mRNA expression.

As shown in Fig. 2B, PMA or FSK alone increased the c-jun mRNA level. PMA or FSK showed an additive interaction for the regulation of c-jun mRNA expression, when co-treated with LPS.

DISCUSSION

It was found in the present study that various kinds of cytokines and LPS were actively involved in the regulation of immediate early genes, such as c-fos and c-jun, in primary cultured astrocytes. The c-fos mRNA expression was also found to be increased in response to LPS or IFN-γ stimulation in primary cultured astrocytes. This result was similar to the previous studies where the upregulation of c-fos mRNA level was found in LPS- or IFN-γ-treated human monocytes, primary cortical glial culture, mesangial cells and the murine macrophage J774.2 cell line (Dokter *et al.*, 1993; Granger *et al.*, 2000; Rubio, 1997; Simi *et al.*, 2000). In C6 rat glioma cells, however, the c-fos mRNA expression was not up-regulated by LPS in a different kinetic way (Kim *et al.*, 2000). In the present study it was found that TNF-α or IL-1β alone did not affect the c-fos mRNA expressions in primary cultured astrocytes. This finding was not in line with several previous studies, where the c-fos mRNA level was increased by TNF-α or IL-1β (Ahmad *et al.*, 1998; Barnea *et al.*, 2001; Goldring *et al.*, 1994; Ryuto *et al.*, 1996). Taken together, differential mechanisms may be involved in the regulation of the c-fos mRNA expressions by LPS and cytokines in tissue- or cell-specific ways. However, it has not been excluded that the concentration used in the present study was insufficient for the induction of c-fos mRNA expression.

In the present study, it was also found that the c-jun mRNA expression was increased by LPS stimulation in primary cultured astrocytes. In other cells, similar results were reported. For example, upregulation of the c-jun mRNA level was found in LPS-treated primary cortical glial cultures (Simi *et al.*, 2000), C6 glioma cells (Kim *et al.*, 2000), murine macrophage J774.2 cell line (Tengku-Muhammad *et al.*, 2000), human myelomonocytic cells (Bertani *et al.*, 1989), human monocytes (Dokter *et al.*, 1993) and murine hippocampal cultures (Friedman *et al.*, 1992). Additionally, in the present study it was found that TNF-α, IL-1β or IFN-γ alone did not significantly affect the c-jun mRNA expressions in primary cultured astrocytes.

This finding was not in line with several previous studies, where the c-jun mRNA level was increased by TNF-α in human myelomonocytic cells (Bertani *et al.*, 1989), human fibroblasts (Conca *et al.*, 1989) and macrophage (Brenner *et al.*, 1989), by IL-1β in murine hippocampal cultures (Friedman *et al.*, 1992), and by IFN-γ in cultured mouse astrocytes (Rubio, 1997). Although it has not ruled out that the concentration of cytokines in the present study was insufficient for the induction of c-jun mRNA expression, this discrepancy was explained by the differential mechanisms that might potentially be involved in the regulation of the c-fos mRNA expressions by LPS and cytokines in cell- or tissue-specific ways.

In addition, TNF-α showed a potentiating effect on the LPS-induced c-fos mRNA expression, but an inhibitory action against the LPS-induced c-jun mRNA level. Furthermore, IFN-γ attenuated both the LPS-induced c-fos and c-jun mRNA expressions. Although the exact underlying mechanisms, or the significance of TNF-α and IFN-γ in the regulation of LPS-induced c-fos and c-jun mRNA expressions, are currently unknown, it is suggested that multiple cytokines may act in a different way during inflammation to regulate the c-fos and c-jun mRNA expressions.

Previously, we have reported that PMA increased both the c-fos and c-jun mRNA expressions in both primary cultured astrocytes (Won *et al.*, 1989) and C6 rat glioma cells (Lee *et al.*, 2001). FSK increased the c-fos mRNA level, whereas the c-jun mRNA expression was down-regulated by FSK in C6 rat glioma cells (Lee *et al.*, 2001). In the present study, FSK alone was demonstrated to increase both the c-fos and c-jun mRNA levels in primary astrocytes. Thus, it can be easily speculated that different signaling pathways may be involved in the c-fos and/or c-jun mRNA expressions between glioma cells and primary astrocytes. It was also found that LPS attenuated the PMA-, but not the FSK-, induced c-fos mRNA level. Although LPS alone was able to increase the c-fos mRNA expression, LPS appears to inhibit the c-fos mRNA expression stimulated by the PKC pathway. Furthermore, in the present study it was also found that LPS has an additive effect on both the PMA- and FSK-induced c-jun mRNA expressions. It is suggested that both the PKA and PKC pathways may positively regulate the LPS-induced c-jun mRNA expression in primary rat astrocytes.

According to several reports, alterations of the c-fos gene have been observed during brain injury, CNS trauma, infectious disease and ischemia (Arenander and de Vellis, 1994; Sheng and Greenberg, 1990; Yu *et al.*, 1995). Among the mechanisms of several diseases, which are related with the expressions of the c-fos and c-jun genes, the present study suggests the expressions of c-fos and c-jun genes in astrocytes may be modulated by certain endotoxins, such as LPS, and various cytokines.

ACKNOWLEDGEMENT

This work was supported by a Research Grant from Hallym University, South Korea.

REFERENCES

Ahmad, M., Theofanidis, P., and Medford, R.M., Role of activating protein-1 in the regulation of the vascular cell adhesion molecule-1 gene expression by tumor necrosis factor-alpha. *J. Biol. Chem.*, 273, 4616-4621 (1998).

Arenander, A., de Vellis, and J., Early response gene induction in astrocytes as a mechanism for encoding and integrating neuronal signals. *Prog. Brain Res.*, 94, 177-188 (1994).

Barnea, A., Roberts, J., Keller, P., and Word, R.A., Interleukin-1beta induces expression of neuropeptide Y in primary astrocyte cultures in a cytokine-specific manner: induction in human but not rat astrocytes. *Brain Res.*, 896, 137-145 (2001).

Bertani, A., Polentarutti, N., Sica, A., Rambaldi, A., Mantovani, A., Colotta, and F., Expression of c-jun protooncogene in human myelomonocytic cells. *Blood*, 74, 1811-1816 (1989).

Bohman, D., Bos, T.J., Admon, A., Nishimura, T., Vogt, P.K., Tjian, and R., Human protooncogene c-jun encodes a DNA-binding protein with structural and functional properties of transcription factor AP-1. *Science*, 238, 1386-1392 (1987).

Brenner, D.A., O'Hara, M., Angel, P., Chojkier, M., and Karin, M., Prolonged activation of jun and collagenase genes by tumour necrosis factor-alpha. *Nature*, 337, 661-663 (1989).

Cheshire, J.L. and Baldwin, A.S. Jr., Synergistic activation of NF-kappaB by tumor necrosis factor alpha and gamma interferon *via* enhanced I kappaB alpha degradation and de novo I kappaB beta degradation. *Mol. Cell Biol.*, 17, 6746-6754 (1997).

Cheshire, J.L., Williams, B.R., and Baldwin, A.S. Jr., Involvement of double-stranded RNA-activated protein kinase in the synergistic activation of nuclear factor-kappaB by tumor necrosis factor-alpha and gamma-interferon in preneuronal cells. *J. Biol. Chem.*, 274, 4801-4806 (1999).

Chomczynski, P. and Sacchi, N., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, 162, 156-159 (1987).

Conca, W., Kaplan, P.B., and Krane, S.M., Increases in levels of procollagenase messenger RNA in cultured fibroblasts induced by human recombinant interleukin 1 beta or serum follow c-jun expression and are dependent on new protein synthesis. *J. Clin. Invest.*, 83, 1753-1757 (1989).

Curran, T., Gordon, M.B., Rubino, K.L., and Sambucetti, L.C., Isolation and characterization of c-fos (rat) cDNA and analysis of post-translational modification *in vitro*. *Oncogene*, 2, 79-84 (1987).

Danielson, P.E., Forss-Petter, S., Brow, M.A., Calavetta, L., Douglass, J., Milner, R.J., and Sutchliffe, J.G., p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. *DNA*, 7, 261-267 (1988).

Dokter, W.H., Esselink, M.T., Halie, M.R., and Vellenga, E., Interleukin-4 inhibits the lipopolysaccharide-induced expression of c-jun and c-fos messenger RNA and activator protein-1 binding activity in human monocytes. *Blood*, 81, 337-343 (1993).

Friedman, W.J., Altiok, N., Fredholm, B.B., and Persson, H., Mechanisms of nerve growth factor mRNA regulation by interleukin-1 beta in hippocampal cultures: role of second messengers. *J. Neurosci. Res.*, 33, 37-46 (1992).

Goldring, M.B., Birkhead, J.R., Suen, L.F., Yamin, R., Mizuno, S., Glowacki, J., Arbiser, J.L., and Apperley, J.F., Interleukin-1 beta-modulated gene expression in immortalized human chondrocytes. *J. Clin. Invest.*, 94, 2307-2316 (1994).

Granger, R.L., Hughes, T.R., Ramji, and D.P., Gene, stimulus and cell-type specific regulation of activator protein-1 in mesangial cells by lipopolysaccharide and cytokines. *Biochim. Biophys. Acta*, 1492, 100-107 (2000).

Kim, Y.H., Choi, M.R., Song, D.K., Huh, S.O., Jang, C.G., and Suh, H.W., Regulation of c-fos gene expression by lipopolysaccharide and cycloheximide in C6 rat glioma cells. *Brain Res.*, 28, 227-230 (2000).

Koerner, T.J., Adams, D.O., and Hamilton, T.A., Regulation of tumor necrosis factor (TNF) expression: interferon-gamma enhances the accumulation of mRNA for TNF induced by lipopolysaccharide in murine peritoneal macrophages. *Cell Immunol.*, 109, 437-443 (1987).

Kopchik, T.T., Cullen, R., and Stacey, D.W., Rapid analysis of small nucleic acid samples by gel electrophoresis. *Anal. Biochem.*, 115, 419-423 (1981).

Landschulz, W.H., Johnson, P.F., and McKnight, S.L., The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science*, 240, 1759-1764 (1988).

Lee, J.K., Choi, S.S., Won, J.S., and Suh, H.W., The Regulation of inducible nitric oxide synthase gene expression induced by lipopolysaccharide plus tumor necrosis factor-a in C6 cells: the involvements of AP-1 and NFκB. *Life Sci.*, 73, 595-609 (2003).

Lee, J.K., Won, J.S., Choi, M.R., Kim, Y.H., and Suh, H.W., Differential effects of forskolin and phorbol 12-myristate-13-acetate on the c-fos and c-jun mRNA expression in rat C6 glioma cells. *Mol. Cells*, 12, 11-16 (2001).

Morrison, D.C. and Ryan, J.L., Endotoxins and disease mechanisms. *Annu. Rev. Med.*, 38, 417-432 (1987).

Paludan, S.R., Synergistic action of pro-inflammatory agents: cellular and molecular aspects. *J. Leukoc. Biol.*, 67, 18-25 (2000).

Rajaram, N. and Kerppola, T.K., DNA bending by Fos-Jun and the orientation of heterodimer binding depend on the sequence of the AP-1 site. *EMBO J.*, 16, 2917-2925 (1997).

Robinson, M.J. and Cobb, M.H., Mitogen-activated protein kinase pathways. *Curr. Opin. Cell Biol.*, 9, 180-186 (1997).

Rubio, N., Interferon-gamma induces the expression of immediate early genes c-fos and c-jun in astrocytes. *Immunology*, 91, 560-564 (1997).

Ryuto, M., Ono, M., Izumi, H., Yoshida, S., Weich, H.A., Kohno, K., and Kuwano, M., Induction of vascular endothelial growth factor by tumor necrosis factor alpha in human glioma cells. Possible roles of SP-1. *J. Biol. Chem.*, 271, 28220-28228 (1996).

Sheng, M. and Greenberg, M.E., The regulation and function of c-fos and other immediate early genes in the nerve system. *Neuron*, 4, 477-485 (1990).

Simi, A., Ingelman-Sundberg, M., and Tindberg, N., Neuroprotective agent chlomethiazole attenuates c-fos, c-jun, and AP-1 activation through inhibition of p38 MAP kinase. *J. Cereb. Blood Flow Metab.*, 20, 1077-1088 (2000).

Takahashi, N., Hayano, T., and Suzuki, M., Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature*, 337, 473-475 (1989).

Tengku-Muhammad, T.S., Hughes, T.R., Foka, P., Cryer, A., and Ramji, D.P., Cytokine-mediated differential regulation of macrophage activator protein-1 genes. *Cytokine*, 12, 720-726 (2000).

Vilijn, M.H., Vaysse, P.J., Zukin, R.S., and Kessler, J.A., Expression of preproenkephlalin mRNA by cultured astrocytes and neurons. *Proc. Natl. Acad. Sci. U.S.A.*, 85, 6551-6553 (1988).

Won, J.S., Song, D.K., Kim, Y.H., Huh, S.O., and Suh, H.W., The stimulation of rat astrocytes with phorbol-12-myristate-13-acetate increases the proenkephalin mRNA: involvement of proto-oncogenes. *Brain Res. Mol. Brain Res.*, 54, 288-297 (1998).

Won, J.S. and Suh, H.W., The differential molecular mechanisms underlying proenkephalin mRNA expression induced by forskolin and phorbol-12-myristic-13-acetate in primary cultured astrocytes. *Brain Res. Mol. Brain Res.*, 84, 41-51 (2000).

Yu, A.C., Lee, Y.L., Fu, W.Y., and Eng, L.F., Gene expression in astrocytes during and after ischemia. *Prog. Brain Res.*, 105, 245-253 (1995).