Mol. Cells, Vol. 23, No. 1, pp. 94-99

Communication

Molecules and Cells

PMA Activates Stat3 in the Jak/Stat Pathway and Induces SOCS5 in Rat Brain Astrocytes

Mi-Na Hwang, Kwang Soo Kim[1], Yo-Woo Choi, Ilo Jou[1], and Sungpil Yoon*

Research Institute, National Cancer Center, Goyang 411-764, Korea;

[1] Department of Pharmacology, Ajou University School of Medicine, Suwon 442-721, Korea.

(Received August 24, 2006; Accepted December 7, 2006)

Suppressors of cytokine signaling (SOCS) family members are negative feedback regulators of the Jak/Stat pathway, which is an essential inflammatory signaling pathway. We investigated expression of eight members of the SOCS family in rat astrocytes, using two inflammatory stimulants, PMA and IFN-γ. Only a few SOCS genes were induced by both stimulants, and we detected an increase in SOCS5 protein with PMA. PMA activated the Jnk, Erk, p38, and Jak/Stat signal pathways. In addition, it increased the level of activated-Stat3 resulting from tyrosine phosphorylation. A gel-shift assay showed that a protein in nuclear extracts from PMA-treated cells was able to bind to Stat binding elements. These results suggest that activated Stat3 binds to SOCS promoters and leads to their transcriptional induction.

Keywords: Astrocyte; IFN-γ; Jak/Stat; PMA; SOCS; Stat3.

Introduction

Glial cells are the most abundant cells in the brain, and participate in the immune response to brain injury. Astrocytes, a component of the glial cells, are activated and restore the integrity of brain tissue in response to pathological stimuli or extracellular signals; this process results in a variety of inflammatory effects. Since long-term inflammation can cause damage to the brain and neurodegeneration, activated glial cells can both benefit and harm brain activity (Hwang *et al*., 2005; Ridet *et al*., 1997; Streit *et al*., 1999). Therefore, it is important to understand which genes are up- or down-regulated and how long they are expressed in activated glial cells, as well as to identify the transcription factors and/or signals that control their expression.

Suppressors of cytokine signaling (SOCS) family members are negative feedback regulators of the Jak/Stat pathway, which is an essential inflammatory signaling pathway (Igaz *et al*., 2001; Kishimoto *et al*., 1994). Phosphorylation of Jak leads to the phosphorylation of "signal transducers and activators of transcription" (Stats). The phosphorylated Stats are released from the receptor complexes, form dimers, and translocate to the nucleus where they bind to promoters in target genes and regulate transcription including that of SOCS genes (Igaz *et al*., 2001; Kishimoto *et al*., 1994; Wen *et al*., 1995). The SOCS family consists of at least eight members, namely cytokine-induced SH2 protein (CIS) and SOCS1-7. SOCS proteins contain a central SH2 domain, a conserved C-terminus referred to as the SOCS box, and unique N-termini (Hilton *et al*., 1998). Once SOCS proteins are made they inhibit Jak/Stat signaling by repressing Jak activity or the binding of Stats to the cytoplasmic domains of receptors (Endo *et al*., 1997). Thus, SOCS members play an active positive role in anti-inflammation of the brain.

SOCS family proteins are induced via Jak/Stat pathways by cytokines, growth factors and several immunomodulators (Starr *et al*., 1999). Interferon-γ (IFN-γ), a prominent pro-inflammatory cytokine, induces expression of SOCS family proteins as well as expression of inflammatory mediators (Frohman *et al*., 1989; Vanguri, 1995). The signal transduction pathways of IFN-γ in the brain involve the expression of SOCS via Jak/Stat pathways (Darnell *et al*., 1994). Phorbol 12-myristate 13-acetate (PMA) activates protein kinase C (PKC) and causes inflammatory

* To whom correspondence should be addressed.
Tel: 82-31-920-2461; Fax: 82-31-920-2002
E-mail: yoons@ncc.re.kr

Abbreviations: EMSA, electrophoretic mobility shift assay; IFN-γ, interferon-γ; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; RT; reverse transcription; SOCS, suppressors of cytokine signaling; Stat, signal transducer and activator of transcription.

activation in the brain (Arai *et al*., 2003; Vitkovic *et al*., 2005), and there is relationship between PKC activation and the Jak/Stat signaling pathways (Aeder *et al*., 2004; Cohen *et al*., 2005).

In this study we investigated the expression of eight members of the SOCS family in rat astrocytes in response to two inflammatory stimulants, PMA and IFN-γ. We also studied which signaling pathway was activated or responsible for the SOCS expression. We detected the induction of a number of the SOCS genes and an increase in SOCS5 protein after PMA treatment. We found that the Jnk, Erk, p38, Jak signal pathways were activated by PMA and that PMA treatment led to the phosphorylation of tyrosine residues only on Stat3, not on other Stats. A gel-shift assay showed that a protein in nuclear extracts from PMA-treated cells was able to bind to Stat binding elements. These results suggest that Stat3 activation may be sufficient for binding to SOCS promoters and for inducing SOCS transcription. Therefore, we suggest that PMA-induced SOCS expression is activated by Stat3.

Materials and Methods

Reagents Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma (USA). Interferon-γ (IFN-γ) was from Peprotech (Rocky Mountain, NJ). Antibodies against the phosphorylated forms of Stat1 (tyrosine-), Stat3 (tyrosine- and serine-), Stat5, and Stat6 were from Cell Signaling Technology (USA). Antibodies against the phosphorylated forms of Jnk1, Jak1, and c-Src were from Biosource (USA). Antibodies against phosphorylated-p38, p38, phosphorylated-Erk1/2, Erk1/2, Stat5, Jak1, and Jnk1 were from Santa Cruz Biotechnology (USA). Antibodies against SOCS5 and phospho-serine-Stat1 were from Abcam (UK). Anti-SOCS1 antibody was from Zymed (USA), and antibodies against Stat1, Stat3, Stat6, and GAPDH were from Stressgen (Canada).

Cell culture Primary astrocytes were cultured from 1- to 3-day-old Sprague-Dawley rats, as described previously (Zhou *et al*., 1998). Briefly, the cortices were triturated into single cells in MEM containing 10% FBS and plated into 75 cm^2 T-flasks (0.5 gemisphere/flask) for 2–3 weeks. Microglia were detached from the flasks by mild shaking and the astrocytes remaining in the flask were harvested with 0.1% trypsin, plated in dishes and cultured in MEM supplemented with 10% FBS.

RT-PCR Total RNA was extracted using RNAzol B (TELTEST, USA). First-stranded cDNA was synthesized from 2 μg of total RNA using a reverse transcription kit (Promega, USA). To amplify double-stranded cDNA, PCR was performed in a 25 μl reaction mixture containing 5 μl of the reverse transcribed cDNA. The sequences of the PCR primers used are described in Table 1. The GAPDH gene was amplified to normalize the results. After the PCR reactions, 10 μl samples were electrophoresed on 1.5% agarose gels.

Table 1. Primer sequences for RT-PCR.

Name	Sequences
INOS Yang *et al*., 2004	F-TCACTGGGACAGCACAGAAT
	R-TGTGTCTGCAGATGTGCTGA
CIS	F-CGACTCTAGCTTCCGACTGG
	R-TCAGAGCTGGAACGGGTACT
SOCS1 Choi *et al*., 2005	F-ACACTCACTTCCGCACCTTC
	R-AGCAGCTCGAAAAGGCAGTC
SOCS2	F-AAGACGTCAGCTGGACCGAC
	R-TCTTGTTGGTAAAGGCAGTCCC
SOCS3 Choi *et al*., 2005	F-ACCAGCGCCACTTCTTCACG
	R-GTGGAGCATCATACTGATCC
SOCS4	F-TGTTCGTCCATTGAGTTGGA
	R-GAAGCACTGTTGGCAGTTAT
SOCS5	F-GCCTTACAGCTGGGACTGAG
	R-GGCTTTGACTGCTTGCTGTA
SOCS6	F-TCTTCTCCCATGGAGGTGTC
	R-TACCACCCCTGTTTTGCAAG
SOCS7	F-GAGCCTCAGCTTTCGATCAC
	R-TTGGAAATGAGCTGCGCTTC
GAPDH Choi *et al*., 2005	F-TCCCTCAAGATTGTCAGCAA
	R-AGATCCACAACGGATACATT

Western blot analysis For Western blot analysis, total proteins were extracted by the TCA method (Reid and Schatz, 1982). Briefly, cells in 60 mm dishes were washed three times with 5 ml PBS buffer. To each plate was added 500 μl of 20% TCA and the cells were dislodged by scraping and transferred to Eppendorf tubes. Proteins were pelleted by centrifugation for 5 min at 3000 rpm and resuspended in Tris-HCl (pH 8.0) buffer. Protein concentrations were estimated by the dye-binding method. The proteins were dissolved in SDS-PAGE loading buffer (Invitrogen), and subjected to Western blot analysis.

Electrophoretic mobility shift assay (EMSA) The crude nuclear proteins in the cell extracts were assayed by EMSA as described previously (Min *et al*., 2003). Briefly, 20 μl mixtures containing 2 μg of nuclear extract in a reaction buffer consisting of 8.5 mM EDTA, 8.5 mM EGTA, 8% glycerol, 0.1 mM $ZnSO_4$, 50 μg/ml poly (dI-dC), 1 mM DTT, 0.3 mg/ml bovine serum albumin (BSA), 6 mM $MgCl_2$, and γ-^{32}P-radio-labeled oligonucleotide probe (3×10^4 cpm), with or without a 20-fold excess of unlabeled probe were incubated for 30 min on ice. DNA-protein complexes were separated on 6% polyacrylamide gels in Tris/glycine buffer, and the dried gels were exposed to X-ray film. The following double-stranded oligonucleotides were used in these studies; proximal Stat binding element, 5′-CAGTTCC-AGGAATCGGGGGGC-3′, 21bp (Auernhammer *et al*., 1999; Ehlting *et al*., 2005); GAS/ISRE, 5′-AAGTACTTTCAGTTT-CATATTACTCTA-3′, 27 bp (Santa Cruz Biotechnology, sc-

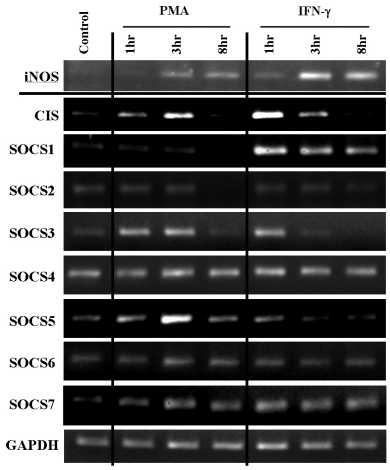

Fig. 1. PMA and IFN-γ induce members of the SOCS family in a short period of time. Primary astrocytes were serum-starved for 24 h and stimulated with 1 μM PMA or IFN-γ (10 U/ml) for the indicated times. Total RNA was isolated and RT-PCR was performed to measure the mRNA levels of iNOS, SOCS members, and GAPDH. The primers for PCR are shown in Table 1.

2537). The 5′-end labeled probes were prepared with 40 μCi (γ-^{32}P) ATP, using T4 polynucleotide kinase (Promega, USA) and purified on Sephadex G-25 Quick Spin Columns (Roche Molecular Biochemicals).

Results

Inflammatory stimulation by PMA and IFN-γ induces certain members of the SOCS family PMA and IFN-γ are inflammatory stimulants (Arai *et al*., 2003; Darnell *et al*., 1994; Vitkovic *et al*., 2005). We observed that both stimulants induced iNOS expression, a representative product of inflammation in immune cells. Expression of iNOS peaked at three hours and continued for eight hours in response to both treatments (Fig. 1). To examine the induction of all the members of the SOCS family we first performed a computer search for cDNA sequences of rat SOCS genes in PubMed but could not identify sequences for SOCS4, SOCS5, SOC6. Using mouse SOCS cDNA sequences we identified the corresponding rat genomic loci and were able to design RT-primers for SOCS4, SOCS5, and SOCS6. The primer sequences for SOCS1 and SOCS3 have been previously reported (Choi *et al*., 2005), and the primers for CIS, SOCS2, and SOCS7 were newly designed from their cDNA sequences in PubMed (Table 1). The primer pairs generated the expected sizes of PCR products, and we found that IFN-γ induced CIS, SOCS1, and SOCS3 within a short time of stimulation (Fig. 1). However, we did not detect any induction of: SOCS2, SOCS4, SOCS6, or SOCS7. PMA induced CIS and SOCS3 (but not SOCS1) in a manner similar to IFN-γ stimulation. The induction by PMA was maintained for three hours, whereas expression of CIS, SOCS1, and SOCS3 peaked at one hour with IFN-γ. We also detected induction of the SOCS5 gene by PMA.

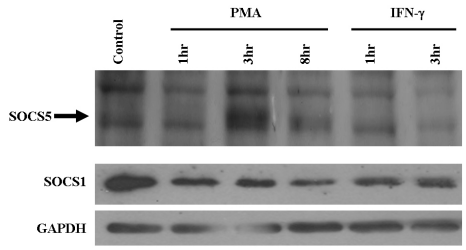

Fig. 2. PMA increases SOCS5 protein. Primary astrocytes were serum-starved for 24 h and stimulated with 1 μM PMA or IFN-γ (10 U/ml) for the indicated times. Cells extracts were fractionated by SDS-PAGE and Western-blot analysis was performed using antibodies against SOCS1, SOCS5, and GAPDH.

We investigated protein levels using antibodies against CIS, SOCS1, SOCS3, and SOCS5 (Fig. 1). We were only able to detect expression of SOCS1 and SOCS5 proteins (Fig. 2) and we observed no increase in SOCS1 protein in response to either treatment (Fig. 2) suggesting that SOCS1 may be regulated by translational control. Expression of SOCS5 (64 kDa) was detected three hours after PMA treatment, the same time that its mRNA increased (Fig. 2). The 64 kDa band was visible between two non-specific bands (see arrow in Fig. 2A).

PMA activates the Jnk, Erk, p38 and Jak signal pathways, but not Src phosphorylation We investigated the effect of PMA on the intensity and duration of phosphorylation of Jak1, Jnk1, Erk, and p38 since these kinases have been shown to be associated with SOCS expression and to mediate inflammation in astrocytes. We found that PMA activated Jak1, Jnk1, Erk1/2 and p38 with maximum levels at one hour (Figs. 3A and 3B). We did not detect any phosphorylation of Jnk1 or p38 in cells treated with IFN-γ (Figs. 3A and 3B), and Erk1/2 was only slightly activated. Previously, it was reported that PMA induced serine/threonine phosphorylation of c-Src in glioblastoma cells (Amos *et al*., 2005), but we did not detect any phosphorylation of c-Src by PMA or IFN-γ (Fig. 3A).

PMA phosphorylate only tyrosine residues of Stat3 among the Stat proteins Since Jak1 was activated by PMA and IFN-γ (Fig 3B), we examined whether they in turn phosphorylated any Stat proteins. IFN-γ signaling led to tyrosine-phosphorylation of Stat1, Stat5, and Stat6 whereas we found that PMA only induced tyrosine-phosphorylation of Stat3. This increased by one hour and then declined rapidly (Fig. 3C). We did not detect any serine phosphorylation of Stat1 or Stat3 (Fig. 3C).

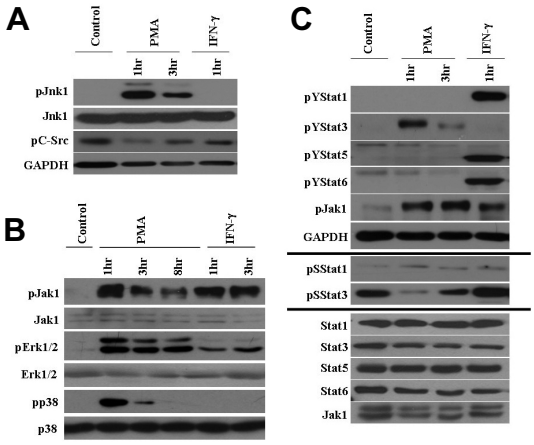

Fig. 3. A–B. PMA activates Jnk1, Erk, Jak1, and p38. Primary astrocytes were serum-starved for 24 h and stimulated with 1 μM PMA or IFN-γ (10 U/ml) for the indicated times. Cells extracts were fractionated by SDS-PAGE and Western-blot analysis was performed using antibodies against phosphorylated-Jnk1, c-Src, -Jak1, -Erk1/2, and -p38, and un-phosphorylated Jak1, Jnk1, Erk1/2, and p38. Anti-GAPDH antibody was used as a loading control. **C.** PMA phosphorylates only the tyrosine residue of Stat3. Western-blot analysis was performed with the samples indicated in Figs. 3A and 3B using antibodies against tyrosine phosphorylated (pY) Stat1, Stat3, Stat5, Stat6, and Jak1, serine phosphorylated (pS) Stat1, and Stat3, and GAPDH. The membranes were stripped out and re-probed with antibodies against Stat1, Stat3, Stat5, Stat6, and Jak1.

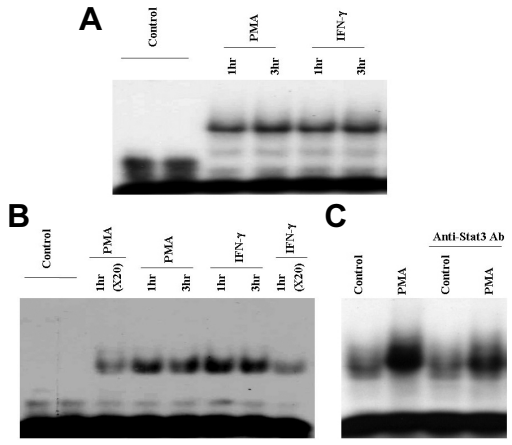

Fig. 4. Nuclear extracts of PMA or IFN-γ stimulated cells contain proteins binding to the proximal Stat or GAS/ISRE elements. **A.** Primary astrocytes were stimulated with 1 μM PMA or IFN-γ (10 U/ml) for one hour and three hours (marked above the gel). The control lane was not treated with any stimulants. After preparation of nuclear extracts**,** the oligonucleotide binding activity of proximal Stat elements was measured by EMSA. **B.** The nuclear extracts used in (A) were tested for binding to the GAS/ISRE element. As a control a twenty-fold excess of non-radiolabeled probe was mixed with the radio-labeled probes in PMA (marked as 1 h and 20X) and IFN-γ (marked as 1 h and 20X) lanes. **C.** Primary astrocytes were stimulated with 1 μM PMA for three hours (marker as PMA). The control lanes were not treated with any stimulants. Nuclear extracts (PMA or Control) were tested for binding to the GAS/ISRE element. The EMSA was performed in the presence (marked as Anti-Stat3 Ab) or absence of 1 μg of the anti-Stat3 antibody.

PMA and IFN-γ increase binding to proximal Stat and GAS/ISRE elements Phosphorylated Stat dimers bind functional GAS/ISRE elements and proximal Stat binding elements in several inflammation-related genes. Therefore, we investigated the effects of PMA and IFN-γ on binding to these elements. Rat brain primary astrocytes were treated with PMA or IFN-γ for one or three hours. Nuclear extracts were then prepared and assayed for proximal Stat or GAS/ISRE binding activity by EMSAs. We found that extracts from PMA- or IFN-γ-stimulated cells shifted the Stat bands (Figs. 4A and 4B). Binding to the GAS element by extracts of either PMA or IFN-γ stimulated cells peaked at three hours, whereas binding to the proximal Stat binding element reached its highest level by one hour (Figs. 4A and 4B). Addition of anti-Stat3 antibody reduced the shifted band formed by PMA-treated extracts (Fig. 4C). This suggests that the anti-Stat3 antibody removed Stat3, leaving the binding activity for the GAS/ISRE elements. We conclude that the shifted band formed by the PMA-treated extracts includes Stat3.

Discussion

We have investigated which SOCS genes are regulated in the transition from resting to activated glial cells. We investigated the expression of eight members of the SOCS family in primary astrocyte cells using two inflammatory stimulants, PMA and IFN-γ. Both stimulants induced iNOS expression indicating that the astrocytes in the presence of PMA or IFN-γ were in a state of inflammation for at least eight hours. IFN-γ induced stronger iNOS expression, and may cause greater inflammation (Fig. 1). Previously, it was shown that IFN-γ activates the Jak/Stat pathway and up-regulates CIS, SOCS1, and SOCS3 (Choi *et al*., 2005; Yang *et al*., 2004). We also observed IFN-γ induction of these compounds for a short time after stimulation (Fig. 1). However, we did not detect any induction of SOCS2, SOCS4, SOCS6, or SOCS7. PMA induced CIS and SOCS3 (but not SOCS1) in a manner similar to IFN-γ. The induction by PMA was maintained for three hours, whereas expression of CIS, SOCS1, and SOCS3 peaked at one hour with IFN-γ. These findings suggest that the time of induction of SOCS genes is dependent on the inflammatory stimulant. We also found induction of the SOCS5 gene by PMA. Expression of SOCS5 with IFN-γ stimulation was also evident at one hour, but the

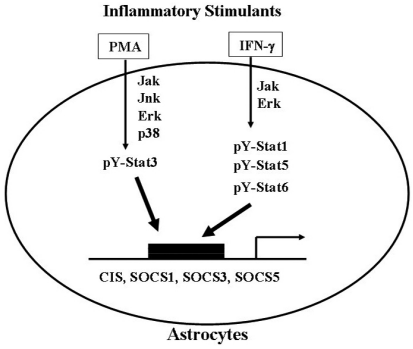

Fig. 5. Current results and models are summarized in this schematic diagram. The two inflammatory stimulants, PMA and IFN-γ, use different mechanisms to regulate the expression of SOCS family genes in rat astrocytes. PMA activates the Jnk, Erk, p38, and Jak signal pathways, whereas IFN-γ activates the Jak and Erk pathways only. PMA increases activated Stat3 by tyrosine phosphorylation, whereas IFN-γ activates Stat1, Stat5, and Stat6. Both stimulants activate CIS and SOCS3. PMA also induces SOCS5 (but not SOCS1).

amount was minimal. It is possible that expression of SOCS5 occurs very early after IFN-γ stimulation. However, we concluded that SOCS5 was not induced by IFN-γ, when compared the induction times of CIS, SOCS1 and SOCS3.

In our Western blot analysis we did not detect any proteins in the size range of CIS or SOCS3. However, SOCS proteins have been found to be too unstable for detection by Western blot analysis. We were only able to detect expression of SOCS1 and SOCS5 (Fig. 2). Since there was no induction of SOCS1 mRNA by PMA, detection of the corresponding protein was not to be expected (Fig. 2). Previously it was reported that SOCS1 translation is regulated by a 5′-untranslated region (Chen *et al*., 2002), and this might explain why we did not observe any increased protein. The expression of SOCS5 (64 kDa) was detected at three hours after PMA treatment and disappeared by eight hours, which matched the degradation time for the mRNA (Fig. 1). As mentioned above, the SOCS1 gene could be regulated by translational control. Therefore, induction of SOCS5 by PMA may be regulated at the transcriptional level; this suggests that SOCS5 was induced by PMA and translated without any regulation. It has been shown previously that induction of CIS, SOCS1, SOCS2 and SOCS3 occurs during inflammation of the brain. This study is the first demonstration of the induction of SOCS5 protein in astrocytes in an inflammatory state.

We also studied the role of the Jnk, Erk, p38 signal pathways in SOCS expression. The Jnk, Erk, p38, Jak signal pathways were activated by PMA whereas Erk1/2 activation was only slightly increased by IFN-γ. In addition, we did not detect any phosphorylation of Jnk1 or p38 in cells treated with IFN-γ. These results suggest that PMA and IFN-γ activate different signal pathways, although induction of the SOCS genes was similar (Fig. 1).

Jak/Stat signaling pathways are the major pathways that positively regulate the expression of SOCS genes. It has been shown that phosphorylation of Stats is dependent on the activation of Jaks, and activated Stats are responsible for induction of the SOCS genes. We investigated which Stat members were responsible for induction of the SOCS genes and found that PMA only activated tyrosine-phosphorylation of Stat3. This suggests that Stat3 activation by PMA might be the major Jak/Stat pathway involved. When we compare the transcriptional induction time (three hours) of SOCS genes by PMA (Fig. 1) and of one hour for Stat3 (Fig. 3C), the results suggest that the Stat3-induced SOCS genes degrade the positive transcriptional activators (Stat3) by feedback inhibition. Stat1 and Stat3 have redundant functions in gene regulation. Hence activation of Stat3 by PMA may be analogous to the SOCS gene induction of activated Stat1 by IFN-γ. Previously, it was showed that phosphorylation of Stat3 in primary microglia occurred 15 minutes after IFN-γ stimulation (Jeon *et al*., 2005). However, in astrocytes, we did not detect any phosphorylation of Stat3 by IFN-γ at one hour (Fig. 3C). It appears that the absence of Stat3 activation by IFN-γ, in contrast to the activation of Stat1, Stat5 and Stat6, is related to the cell type-specificity of astrocytes. Our results suggest that activation of Stat3 by PMA may be sufficient for SOCS expression in the Jak/Stat pathway. Although Jak1, a kinase for Stats, was activated by both PMA and IFN-γ, there appear to be differences in the activation patterns of the different Stat members (Fig. 3C). As shown in Figs. 3A–3B, there was also a large difference in the levels of activation of the Jnk, p38, and Erk signal pathways by PMA and IFN-γ. This may result in activation of different Stats. The GAS/ISRE element is responsible for binding of phosphorylated Stat dimers in the promoter region of several inflammation-related genes. It has been shown that the proximal Stat binding element in the SOCS3 promoter is responsible for Stat activity (Chen *et al*., 2002; Jeon *et al*., 2005). Therefore, we studied the effects of PMA and IFN-γ on binding to the proximal Stat and GAS/ISRE elements by EMSA. We found that PMA and IFN-γ stimulation shifted a band containing the Stat binding site (Figs. 4A and 4B). The shifted band was reduced upon addition of the anti-Stat3 antibody, suggesting that activated Stat3 was bound to the GAS/ISRE elements and was present in the shifted band. Considering that PMA only activated Stat3 (Fig. 3C), we may conclude that PMA-activated Stat3 is involved in the major Jak/Stat pathway and is sufficient for activating the SOCS genes. These results seem to be related to the cell type specificity of astrocytes. Thus, we propose that Stat3 plays a major role in SOCS induction by PMA in astrocytes. In addition, the activation of Stat3 by PMA may have a similar role to

the SOCS gene induction of activated Stat1, Stat5, or Stat6 by IFN-γ. Our current results and model are summarized in Fig. 5.

Acknowledgment This work was supported by research grants from the National Cancer Center (0510042-1 and -2), South Korea.

References

Aeder, S. E., Martin, P. M., Soh, J. W., and Hussaini, I. M. (2004) PKC-eta mediates glioblastoma cell proliferation through the Akt and mTOR signaling pathways. *Oncogene* **23**, 9062–9069.

Amos, S., Martin, P. M., Polar, G. A., Parsons, S. J., and Hussaini, I. M. (2005) Phorbol 12-myristate 13-acetate induces epidermal growth factor receptor transactivation via protein kinase Cdelta/c-Src pathways in glioblastoma cells. *J. Biol. Chem.* **280**, 7729–7738.

Arai, K., Lee, S. R., and Lo, E. H. (2003) Essential role for ERK mitogen-activated protein kinase in matrix metalloproteinase-9 regulation in rat cortical astrocytes. *Glia* **43**, 254–264.

Auernhammer, C. J., Bousquet, C., and Melmed, S. (1999) Autoregulation of pituitary corticotroph SOCS-3 expression: characterization of the murine SOCS-3 promoter. *Proc. Natl. Acad. Sci. USA* **96**, 6964–6969.

Chen, X. P., Losman, J. A., Cowan, S., Donahue, E., Fay, S., *et al*. (2002) Pim serine/threonine kinases regulate the stability of Socs-1 protein. *Proc. Natl. Acad. Sci. USA* **99**, 2175–2180.

Choi, W. H., Ji, K. A., Jeon, S. B., Yang, M. S., Kim, H., *et al*. (2005) Anti-inflanmmatory roles of retinoic acid in rat brain astrocytes: Suppression of interferon-gamma-induced JAK/STAT phosphorylation. *Biochem. Biophys. Res. Commun.* **329**, 125–131.

Cohen, S., Dovrat, S., Sarid, R., Huberman, E., and Salzberg, S. (2005) JAK-STAT signaling involved in phorbol 12-myristate 13-acetate-and dimethyl sulfoxide-induced 2′-5′ oligoadenylate synthetase expression in human HL-60 Leukemia cells. *Leuk. Res.* **29**, 923–931.

Darnell, J. E. Jr., Kerr, I. M., and Stark, G. R. (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264**, 1415–1421.

Ehlting, C., Haussinger, D., and Bode, J. G. (2005) Sp3 is involved in the regulation of SOCS3 gene expression. *Biochem. J.* **387**, 737–745.

Endo, T. A., Masuhara, M., Yokouchi, M., Suzuki, R., Sakamoto, H., *et al*. (1997) A new protein containing an SH2 domain that inhibits JAK kinases. *Nature* **387**, 921–924.

Frohman, E. M., Frohman, T. C., Dustin, M. L., Vayuvegula, B., Choi, B., *et al*. (1989) The induction of intercellular adhesion molecule 1 (ICAM-1) expression on human fetal astrocytes by interferon-gamma, tumor necrosis factor alpha, lymphotoxin, and interleukin-1: relevance to interacerabral antigen presentation. *J. Neuroimmunol.* **23**, 117–124.

Hilton, D. J., Richardson, R. T., Alexander, W. S., Viney, E. M., Willson, T. A., *et al*. (1998) Twenty proteins containing a C-terminal SOCS box form five structural classes. *Proc. Natl. Acad. Sci. USA* **95**, 114–119.

Hwang, J. I., Shin, K. J., Oh, Y. S., Choi, J. W., Lee, Z. W., *et al*. (2005) Phospholipase C-β3 mediates the thrombin-induced Ca^{2+} response in glial cells. *Mol. Cells* **19**, 375–381.

Igaz, P., Toth, S., and Falus, A. (2001) Biological and clinical significance of the JAK-STAT pathway; lessons from knockout mice. *Inflamm. Res.* **50**, 435–441.

Jeon, S. B., Ji, K. A., You, H. J., Kim, J. H., Jou, I., *et al*. (2005) Nordihydroguariaretic acid inhibits IFN-gamma-induced STAT tyrosine phosphorylation in rat brain astrocytes. *Biochem. Biophys. Res. Commun.* **328**, 595–600.

Kishimoto, T., Taga, T., and Akira S. (1994) Cytokine signal transduction. *Cell* **76**, 253–262.

Min, K., Jou, I., and Joe, E. (2003) Plasminogen-induced IL-1β and TNF-α production in microglia is regulated by reactive oxygen species. *Biochem. Biophys. Res. Commun.* **312**, 969–974.

Reid, G. A. and Schatz, G. (1982) Import of proteins into mitochondria. Extramitochondrial pools and post-translational import of mitochondrial protein precursors *in vivo*. *J. Biol. Chem.* **257**, 13062–13067.

Ridet, J. L., Malhotra, S. K., Privat, A., and Gage, F. H. (1997) Reactive astrocytes: cellular and molecular cues to biological function. *Trends Neurosci.* **20**, 570–577.

Starr, R. and Hilton, D. J. (1999) Negative regulation of the JAK/STAT pathway. *Bioessays* **21**, 47–52.

Streit, W. J., Walter, S. A., and Pennell, N. A. (1999) Reactive microgliosis. *Prog. Neurobiol.* **57**, 563–581.

Vanguri, P. (1995) Interferon-gamma-inducible genes in primary glial cells of the central nervous system: comparisons of astrocytes with microglia and Lewis with brown Norway rats. *J. Neuroimmunol.* **56**, 35–43.

Vitkovic, L., Aloyo, V. J., Maeda, S., Benzil, D. L., Bressler, J. P., *et al*. (2005) Identification of myristorylated alanine-rich C kinase substrate (MARCKS) in astrocytes. *Front. Biosci.* **10**, 160–165.

Wen, Z., Zhong, Z., and Darnell, J. E. Jr. (1995) Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell* **82**, 241–250.

Yang, M. S., Lee, J., Ji, K. A., Min, K. J., Lee, M. A., *et al*. (2004) Thrombin induces suppressor of cytokine signaling 3 expression in brain microglia via protein kinase Cdelta activation. *Biochem. Biophys. Res. Commun.* **317**, 811–816.

Zhou, Z. H., Chaturvedi, P., Han, Y. L., Aras, S., Li, Y. S., *et al*. (1998) IFN-gamma induction of the human monocyte chemoattractant protein (hMCP)-1 gene in astrocytoma cells: functional interaction between an IFN-gamma-activated site and a GC-rich element. *J. Immunol.* **160**, 3908–3916.