Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 329 (2005) 125–131
BBRC
www.elsevier.com/locate/ybbrc

# Anti-inflammatory roles of retinoic acid in rat brain astrocytes: Suppression of interferon-γ-induced JAK/STAT phosphorylation☆

Woo-Hyuck Choi[a], Kyung-Ae Ji[a], Sae-Bom Jeon[a,b], Myung-Soon Yang[a,b], Ho Kim[c], Kyoung-jin Min[d], Minho Shong[c], Ilo Jou[d], Eun-Hye Joe[a,b,d,*]

[a] *Neuroscience Graduate Program, Ajou University School of Medicine, Suwon 442-721, Republic of Korea*
[b] *Brain Disease Research Center, Ajou University School of Medicine, Suwon 442-721, Republic of Korea*
[c] *Department of Internal Medicine, Chungnam National University, Taejon, Republic of Korea*
[d] *Department of Pharmacology, Ajou University School of Medicine, Suwon 442-721, Republic of Korea*

Received 7 January 2005

**Abstract**

The anti-inflammatory effect of retinoic acid (RA) has been investigated for several decades. However, the underlying mechanisms responsible for this effect are largely unknown. In this study, we demonstrate that 9-*cis*-RA (cRA) and all-*trans*-RA (tRA) inhibit interferon-γ (IFN-γ)-induced inflammatory responses in astrocytes. In primary cultured rat brain astrocytes and C6 astroglioma cells, both cRA and tRA decreased IFN-γ-induced expression of interferon regulatory factor-1. Both RA isoforms also reduced IFN-γ-induced activation of signal transducers and activators of transcription (STAT)1, STAT3, Janus kinase (JAK)1, and JAK2. This inhibitory effect was significant when cells were pre-treated with RA prior to IFN-γ. Furthermore, the effect of pre-treated RA was abolished in the presence of cycloheximide, indicating a requirement for de novo protein synthesis. Suppressors of cytokine signaling (SOCS), which are negative regulators of the JAK/STAT pathway, may be candidate mediators of the anti-inflammatory function of RA. Both cRA and tRA induced SOCS3 mRNA expression. These results suggest that RA induces an anti-inflammatory effect by suppressing the activation of the JAK/STAT pathway in IFN-γ-treated astrocytes. SOCS3 may be at least one of the mechanisms that mediate the anti-inflammatory roles of RA.


*Keywords:* Retinoic acid; JAK/STAT; SOCS

Brain inflammation aggravates brain injury and is thus a risk factor of neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease [1]. Astrocytes, the most abundant cells in the central nervous system, participate in brain inflammation. In response to brain injury, astrocytes produce several inflammatory mediators, such as cytokines, cell adhesion molecules, and extracellular matrix proteins [2,3].

Interferon-γ (IFN-γ) plays predominant immunomodulatory roles in astrocytes. Exposure of astrocytes to IFN-γ leads to increased expression of various inflammation-associated molecules, including interferon

☆ *Abbreviations:* cRA, 9-*cis*-retinoic acid; tRA, all-*trans*-retinoic acid; SOCS, suppressors of cytokine signaling; IRF-1, interferon regulatory factor-1; JAK, Janus kinase; STAT, signal transducers and activators of transcription; RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid response element; iNOS, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-α; ERK, extracellular signal regulated kinase; JNK/SAPK, c-jun N-terminal kinase/stress-activated protein kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB.

* Corresponding author. Fax: +82 31 219 5069.
*E-mail address:* ehjoe@ajou.ac.kr (E.-H. Joe).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.01.110

regulatory factor-1 (IRF-1), intracellular adhesion molecule-1 (ICAM-1), and macrophage chemoattractant protein 1 (MCP-1) [4–6]. IFN-γ activates two Janus kinases (JAK), specifically, JAK1 and JAK2, via phosphorylation. In turn, phosphorylated JAK1 and JAK2 stimulate the phosphorylation of tyrosine residues in the cytoplasmic tail of IFN-γ receptors, which provide the docking sites for signal transducers and activators of transcription (STAT). Following recruitment to the receptor, STAT proteins are phosphorylated on both tyrosine and serine residues, released from the receptor complex, and form homodimers that translocate to the nucleus where they regulate the transcription of IFN-γ-responsive genes [7,8].

Suppressors of cytokine signaling (SOCS) family proteins negatively regulate JAK and STAT pathways that mediate the action of cytokines [9–14]. SOCS family proteins contain a central SH2 domain, a conserved SOCS box in the C-terminus, and a unique N-terminus. To date, CIS and SOCS1–SOCS7 have been identified. SOCS proteins directly interact with the JAK family and inhibit their catalytic activity or interact with phosphorylated tyrosine residues in the cytoplasmic domains of cytokine receptors [12–14]. In particular, SOCS1 and SOCS3 play significant roles in the regulation of inflammation [15,16]. In keratinocytes, SOCS1 and SOCS3 inhibit the activation of STAT1 and STAT3, and reduce IFN-γ-induced expression of ICAM-1, HLA-DR, IFN-γ-inducible protein-10 (IP-10), and MCP-1 [15]. Stronger STAT3 activation and more severe colitis are detected in SOCS mutant transgenic mice, compared to wild-type animals [16]. Thus, SOCS1 and SOCS3 specifically inhibit IFN-γ-induced activation and/or activity of JAK1 and JAK2, and consequently STAT activation [12–14].

All-*trans*-retinoic acid (tRA) and 9-*cis*-retinoic acid (cRA) are natural derivatives of vitamin A. The anti-inflammatory effect of retinoic acid was reported several decades ago. Retinoid inhibits the production of superoxide anion radicals by human polymorphonuclear leukocytes [17]. Oral administration of cRA reduces inflammation in rats with experimentally induced arthritis and iodide ointment-induced skin inflammation [18,19]. In macrophages and mesangial cells, RA reduces iNOS expression [20,21]. However, despite considerable research on the anti-inflammatory activity of retinoic acid, the specific mechanism by which RA exerts this effect remains to be determined. In this study, we propose that inhibition of the JAK/STAT pathway via expression of SOCS3 is at least one mechanism that mediates the anti-inflammatory effect of RA in astrocytes.

## Materials and methods

*Reagents.* IFN-γ was purchased from Calbiochem (LA, CA). 9-*cis*-Retinoic acid and all-*trans*-retinoic acid were from Biomol (Plymouth Meeting, PA). Minimal essential medium (MEM) was from Gibco (Grand Island, NY). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Hyclone (Logan, UT). Antibodies against STAT1, STAT3, and phosphorylated STAT1 and 3 were purchased from Cell Signaling Technology (Beverly, MA). Antibody against phosphorylated JAK1 was from Calbiochem (San Diego, CA), while the antibody specific for phosphorylated JAK2 was from UBI (Charlottesville, VA). Antibody against SOCS3 was from Santa Cruz Biotechnology (Santa Cruz, CA). Peroxidase-conjugated secondary antibodies were acquired from Vector Lab. (Burlingame, CA), and the enhanced chemiluminescence (ECL) system was obtained from Sigma. PCR primers were from Bioneer (Seoul, Korea). RNAzol B and reverse transcriptase from avian myeloblastosis virus were purchased from TEL-TEST (Friendswood, TX) and Takara (Japan), respectively.

*Preparation of cells.* Primary astrocytes were cultured from 1- to 3-day-old Sprague–Dawley rats, as described previously [22]. Cortices were triturated into single cells in MEM (Gibco) containing 10% fetal bovine serum (Hyclone) and plated into 75 $cm^2$ T-flasks (0.5 hemisphere/flask) for 10–14 days. To prepare pure astrocytes, microglia were removed from T-flasks by mild shaking. Cells remaining in flasks after the removal of microglia were harvested with 0.1% trypsin and plated onto dishes or plates. C6 rat astroglioma cells were obtained from the American Type Culture Collection (ATCC, CCL-107). Cells were grown in DMEM supplemented with 5%(v/v) FBS.

*Reverse transcription and polymerase chain reaction (RT-PCR).* Total RNA was extracted using RNAzol B, and the corresponding cDNA was prepared using reverse transcriptase originating from Avian Myeloblastosis Virus, according to the manufacturer's instructions. The following PCR primers were employed: (R) 5′-AGCAGC TCGAAAAGGCAGTC-3′, (F) 5′-ACACTCACTTCCGCACCTTC-3′ for SOCS1; (R) 5′-GTGGAGCATCATACTGATCC-3′, (F) 5′-AC CAGCGCCACTTCTTCACG-3′ for SOCS3; and (F) 5′-TCCCTCAA GATTGTCAGCAA-3′, (R) 5′-AGATCCACAACGGATACATT-3′ for GAPDH. PCR products were separated by electrophoresis on a 1.5% agarose gel and detected under UV light.

*Western blot analysis.* Cells were washed twice with cold phosphate-buffered saline and lysed in ice-cold modified RIPA buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM $Na_3VO_4$, and 1 mM NaF) containing protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 100 μg/ml leupeptin, 10 μg/ml pepstatin, 1 μg/ml aprotinin, and 2 mM EDTA). Cell lysates were centrifuged for 20 min at 13,000*g* at 4 °C, and the supernatant was collected. Proteins were separated by SDS–PAGE and transferred to nitrocellulose membrane. The membrane was incubated with primary antibodies, followed by peroxidase-conjugated secondary antibodies, and visualized using an enhanced chemiluminescence system.

## Results

### *Retinoic acid reduces IFN-γ-induced IRF-1 protein expression*

Previous studies have shown that retinoic acid (RA) reduces IFN-γ-induced expression of iNOS and TNF-α in macrophages and mesangial cells [20,21]. In view of this finding, we propose that RA may affect several molecules induced by IFN-γ. Interferon regulatory factor (IRF) family proteins mediate the inflammatory effect of IFN-γ [23]. Accordingly, we examined the effect of RA on IFN-γ-induced IRF-1 protein expression. In Western blot analysis, IRF-1 protein synthesis was detected within 1 h of treatment with IFN-γ (10 U/ml) in

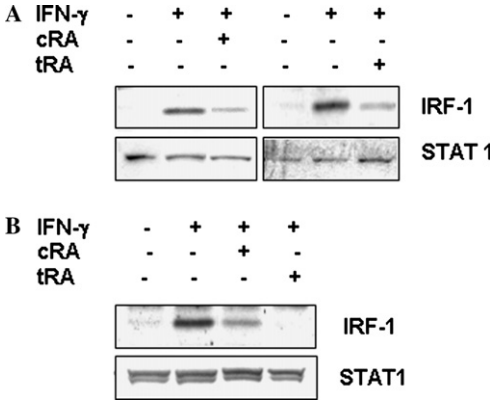


Fig. 1. Retinoic acid reduces IFN-γ-induced IRF-1 expression. Primary cultured rat brain astrocytes (A) and C6 cells (B) were treated with 9-*cis*-retinoic acid (cRA, 20 μM) or all-*trans*-retinoic acid (tRA, 20 μM) 4 h prior to IFN-γ (10 U/ml). After 1 h, cell lysates were separated by SDS–PAGE and Western blot analysis was performed using anti-IRF-1 antibody, as described in Materials and methods. The membrane was stripped and analyzed sequentially with anti-STAT1 antibodies as a loading control. Data are representative of three independent experiments.

both primary cultured rat astrocytes and C6 rat astroglioma cells (Fig. 1). Primary cultured astrocytes (Fig. 1A) and C6 astroglioma cells (Fig. 1B) pre-treated with 9-*cis*-RA (cRA) or all-*trans*-RA (tRA) 4 h prior to IFN-γ displayed decreased IRF-1 expression.

### *Retinoic acid inhibits IFN-γ-induced JAK and STAT activation*

Since IFN-γ induces IRF-1 expression via activation of STAT family transcription factors [24], we examined whether RA affected STAT activation. We investigated the tyrosine phosphorylation of STAT1 and STAT3 (markers of activation) in Western blots using antibodies against phospho-tyrosine-specific STAT1 (pSTAT1) and STAT3 (pSTAT3). IFN-γ-induced phosphorylation of STAT1 and STAT3 was significantly reduced when primary cultured astrocytes and C6 cells were pre-treated with RA compounds 4 h prior to IFN-γ (pre). However, co-treated RA compounds (co) exerted less effect on the phosphorylation of STAT1 and STAT3 in primary cultured astrocytes and no effect in C6 cells (Figs. 2A–D). RA compounds did not reduce the expression levels of STAT1 and STAT3 (Figs. 2A and C). We also found that tRA suppressed IFN-γ-induced phosphorylation of STAT1 during the whole experimental time-period from 15 to 60 min (Fig. 2E). In a dose–response experiment, an obvious inhibitory effect was observed at a minimum concentration of 10 μM tRA (Fig. 2F). These results indicate that both cRA and tRA suppressed IFN-γ-induced Tyr-phosphorylation of STATs without changing the expression level of STATs. Furthermore, pre-treated RA compounds exerted a stronger effect on the phosphorylation level of STATs than co-treated ones.

Since JAK1 and JAK2 are required for IFN-γ-induced Tyr-phosphorylation of STAT [7,8], we examined the effect of RA on IFN-γ-stimulated Tyr-phosphorylation of JAK1 and JAK2. In primary cultured astrocytes and C6 cells, both cRA and tRA significantly reduced IFN-γ-induced Tyr-phosphorylation of JAK1 (pJAK1) and JAK2 (pJAK2) when cells were pre-treated with RA while the expression level of JAK1 and JAK2 remained unchanged (Figs. 3A and B). As shown in STAT phosphorylation, RA compounds suppressed IFN-γ-induced Tyr-phosphorylation of JAK during the experimental time-period from 15 to 60 min (data not shown). Taken together, these results suggest that RA compounds suppressed IFN-γ signaling at the level of JAK without changing the expression of JAK and STAT.

### *De novo protein synthesis is involved in the inhibition of IFN-γ signaling by pre-treatment with RA*

Since pre-treatment with RA suppressed IFN-γ-induced activation of STAT1 and STAT3 in primary astrocytes and C6 cells, we determined the time-period required for inhibition. Cells were pre-treated with 20 μM cRA for 1–4 h, followed by IFN-γ (10 U/ml) for 30 min. The inhibitory effect of cRA on STAT1 phosphorylation was evident upon treatment with RA more than 3 h prior to IFN-γ (Fig. 4A). In view of the data, we propose that de novo protein synthesis is involved in the inhibition of IFN-γ signaling by cRA. As expected, the protein synthesis inhibitor, cycloheximide (CHX, 100–300 ng/ml), abolished the inhibitory effect of cRA in a dose-dependent manner, while CHX alone had little effect on STAT1 phosphorylation (Fig. 4B). These results suggest that de novo protein synthesis is required for the inhibition of IFN-γ signaling by RA treatment.

### *Retinoic acid induces SOCS3 mRNA expression*

We propose that SOCS family proteins are involved in the anti-inflammatory effect of RA. In this study, we focus on SOCS1 and SOCS3, which inhibit the important mediators of the inflammatory function of IFN-γ, STAT1, and STAT3 [12–14]. Primary cultured astrocytes and C6 cells were treated with 20 μM cRA or 20 μM tRA, and SOCS1 and SOCS3 mRNA levels were assayed using RT-PCR (Fig. 5). In both primary cultured astrocytes and C6 cells, SOCS3 mRNA expression increased within 30 min, which was sustained for 2–3 h in response to both cRA and tRA (Figs. 5A and B). However, SOCS1 transcription was not significant in either primary cultured astrocytes or C6 cells (Figs. 5A and B). RA directly induced SOCS3 expression without

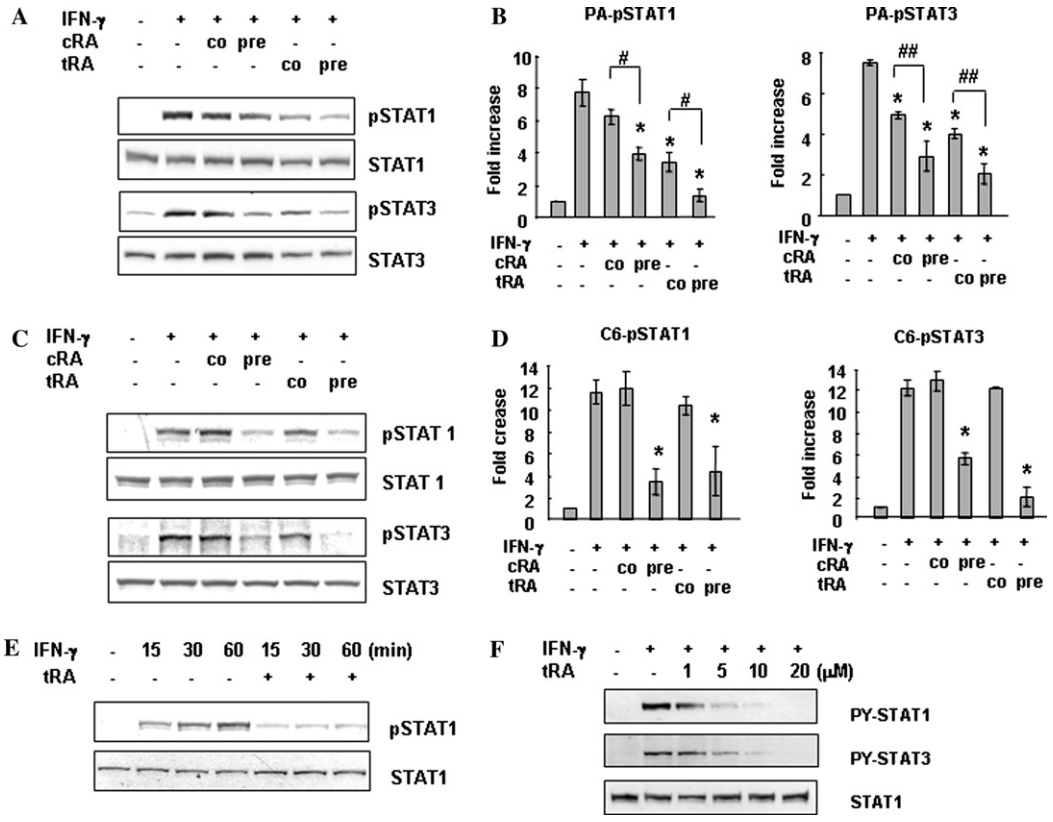


Fig. 2. Retinoic acid reduces IFN-γ-induced activation of STAT1 and STAT3. Primary cultured astrocytes (A) and C6 cells (C) were treated with cRA (20 μM) or tRA (20 μM), together with (co) or 4 h prior to (pre) IFN-γ (10 U/ml). Thirty minutes later, activation of STAT1 and STAT3 was measured by Western blot, using antibodies specific for phospho-Tyr-STAT1 (pSTAT1) and phospho-Tyr-STAT3 (pSTAT3), respectively. Membranes were stripped and analyzed sequentially with STAT1 and STAT3. (B,D) The intensities of the bands of pSTAT1 and pSTAT3 were measured using Image Gauge and normalized against those of unphosphorylated counterbands. Values are means ± SEM of three independent experiments. $^{*}p < 0.01$ as compared with intensities of the bands of IFN-γ-induced pSTATs in the absence of RA. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ as comparing intensities of co- and pre-treated RA. (E) Primary cultured astrocytes were treated with tRA (20 μM) for 4 h. Then cells were treated with IFN-γ (10 U/ml) for indicated times. Level of pSTAT1 was detected by Western blot. Membranes were sequentially analyzed with STAT1 antibody. (F) Primary astrocytes were treated with the indicated amount of tRA for 4 h. Then cells were treated with IFN-γ (10 U/ml) for 30 min. Levels of pSTAT1 and STAT1 were detected by Western blot. Data are representative of more than three independent experiments.

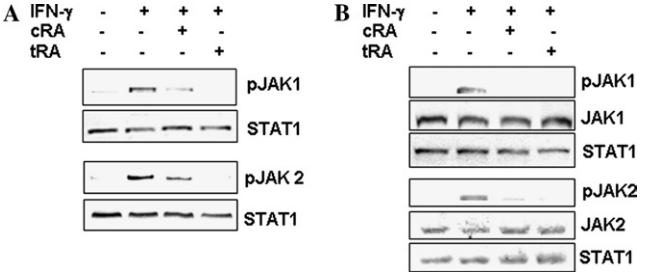


Fig. 3. Retinoic acid reduces IFN-γ-induced activation of JAK1 and JAK2. Primary cultured astrocytes (A) and C6 cells (B) were treated with cRA (20 μM) or tRA (20 μM) for 4 h prior to IFN-γ (10 U/ml). Thirty minutes later, activation of JAK1 and JAK2 was measured by Western blot, using antibodies specific for phospho-Tyr-JAK1 (pJAK1) and phospho-Tyr-JAK2 (pJAK2), respectively. Membranes were stripped and analyzed sequentially with STAT1 or JAK1/2. Data are representative of three independent experiments.

de novo protein synthesis since CHX (100 ng/ml) had no effect on RA-induced SOCS3 expression (Fig. 5C). SOCS3 protein expression was also detected in primary cultured astrocytes within 3 h after 20 μM tRA treatment, and the expression was sustained for up to 4 h (Fig. 5D). These results collectively suggest that SOCS3 transcription is at least one component that mediates the anti-inflammatory roles of RA.

## Discussion

A number of anti-inflammatory roles of RA have been reported so far. RA compounds have been clinically employed to treat arthritis and skin inflammation [18,19], although the mechanism of anti-inflammation remains to be determined. The findings in this study indicate that RA significantly suppresses IFN-γ-activated JAK/STAT pathways, prominent pro-inflammatory signaling pathways, resulting in suppression of the expression of inflammation-associated molecules, including interferon regulatory factor-1 (IRF-1).

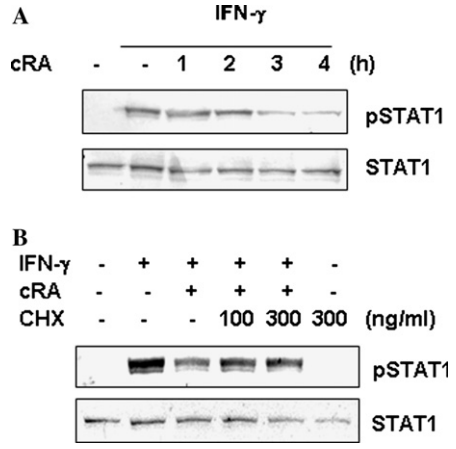


Fig. 4. Pre-treated retinoic acid reduces STAT activation through de novo protein synthesis. (A) C6 cells were pre-treated with 20 μM cRA for the indicated time-periods, followed by 10 U/ml IFN-γ for 30 min. pSTAT1 and STAT1 were analyzed by Western blot. (B) C6 cells were treated with the indicated doses of cycloheximide (CHX) for 30 min and 20 μM cRA for 4 h. Next, 10 U/ml IFN-γ was added to cells for 30 min. pSTAT1 and STAT1 were analyzed by Western blot. Data are representative of three independent experiments.

The inhibitory activity of pre-treated RA may require de novo protein synthesis, since an effect was evident only when cRA was added more than for 3 h prior to IFN-γ, and was reversed in the presence of cycloheximide (Fig. 4). SOCS may be candidates for mediators of the anti-inflammatory function of RA. RA-induced expression of SOCS3 was detected in RT-PCR and Western blot (Fig. 5). SOCS3 is proposed as negative regulators of IFN-γ signaling [12–14]. SOCS3 suppresses the tyrosine phosphorylation of JAK1 and 2 [25]. It is in agreement with the experimental results that show RA compounds reduced IFN-γ signaling at JAK phosphorylation. However, SOCS1 expression was not detected in RT-PCR (Fig. 5). Thus, the contribution of SOCS3 to RA-mediated suppression of IFN-γ signaling could be greater than that of SOCS1.

The expression of SOCS3 is tightly regulated. The SOCS3 promoter contains STAT-binding sites [26]. Although JAK/STAT pathways are major pathways that induce SOCS expression [12–14], STAT-independent expression has additionally been reported. Interleukin-10 (IL-10) enhances SOCS3 mRNA expression in human neutrophils without activation of tyrosine and serine phosphorylation of STAT1 and STAT3 [27]. In macrophages and dendritic cells, bacterial DNA CpG-DNA induces SOCS3 expression via MAPK pathways [28]. RA does not induce tyrosine phosphorylation of either STAT1 or STAT3 in C6 cells (data not shown), and appears to trigger SOCS expression via a STAT-independent pathway. Since RA binds to transcription factors belonging to the nuclear receptor superfamily that regulates the transcription of retinoid-responsive genes, specifically, retinoic acid receptor (RAR) and retinoid X receptor (RXR) [29]. Activated RAR and RXR bind to the retinoic acid response element (RARE) located in the promoter region of retinoid response genes and trigger diverse cellular functions, including regulation of cell growth and differentiation [29,30]. However, the existence of a RARE or RXRE sequence in the promoter regions of SOCS3 has not been identified to date. Therefore, the underlying mechanisms that mediate SOCS3 expression in RA-treated cells remain to be elucidated in the future study.

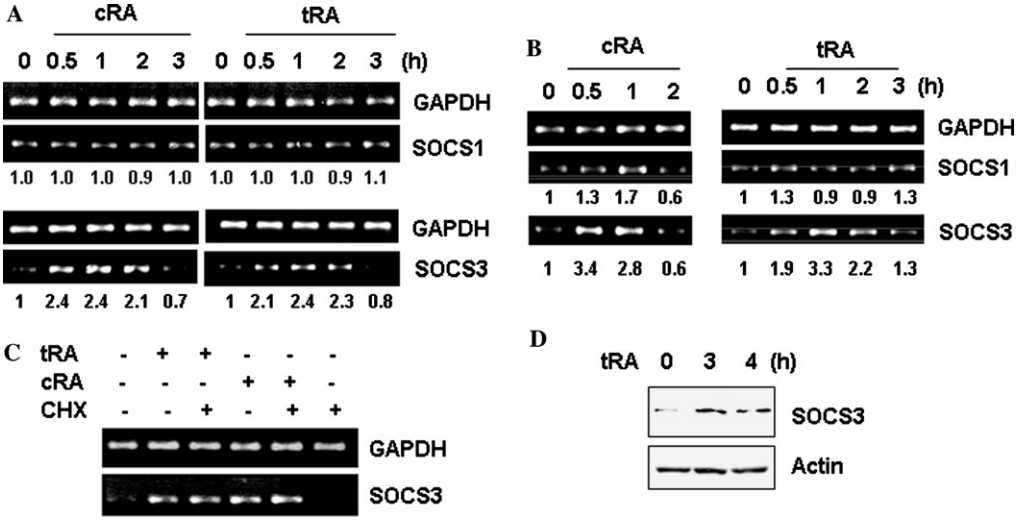


Fig. 5. Retinoic acid induces SOCS1 and SOCS3 mRNA expression. Primary cultured astrocytes (A) and C6 cells (B) were treated with cRA (20 μM) or tRA (20 μM) for the indicated time-periods. SOCS1 and SOCS3 transcription was determined by RT-PCR, as described in Materials and methods. The values represent normalized intensities of bands of SOCS1 or SOCS3 against those of GAPDH. (C) C6 cells were pre-treated with 100 ng/ml cycloheximide (CHX) for 30 min, followed by 20 μM tRA for 1 h. SOCS3 mRNA expression was measured by RT-PCR. (D) Primary cultured astrocytes were treated with tRA (20 μM) for 3 or 4 h. SOCS3 expression was detected in Western blot. Data are representative of more than three (A–C) or two (D) independent experiments.

In addition to SOCS, there may be other mechanisms that mediate the anti-inflammatory effect of RA. One of the anti-inflammatory mechanisms of RA may comprise activation and/or induction of phosphatases, such as SHP and MAP kinase phosphatase 1 (MKP-1). RA increases the phosphatase activity and expression of SHP-1 in human myeloid leukemia cells [31,32], and induces MKP-1 in bronchial epithelial cells and mesangial cells [33,34]. SHP-1 and SHP-2 associate with and regulate JAK family tyrosine kinases [35,36], and MKP-1 dephosphorylates STAT1 [37]. We also found that phosphatase activity increased in primary cultured astrocytes and microglia in response to tRA (data not shown), although we could not characterize the type of phosphatases at this point. Therefore, these phosphatases may mediate the anti-inflammatory effects of RA, particularly, the effect of co-treated RA in primary cultured astrocytes. In addition to JAK/STAT pathways, RA compounds block NF-κB-mediated transcription [38–40]. In mesangial cells, RA reduces NO production by suppressing nuclear levels of p50 and p65 subunits of NF-κB [21]. In dermal microvascular endothelial cells, neutrophils, and macrophages, RA inhibits the DNA-binding activity of NF-κB [38–40]. RXR may compete with NF-κB to recruit co-activators, such as CBP/p300 and SRC-1 [40]. The anti-oxidant effect of RA may participate in the anti-inflammatory mechanism in polymorphonuclear leukocytes, hippocampal neurons, and mesangial cells [17,41,42]. In hippocampal neurons, RA reduces neuronal ROS content by inducing the expression of SOD-1 and SOD-2 [41]. RA suppresses $H_2O_2$-triggered JNK activation and the activator protein 1 (AP-1) pathway in mesangial cells [42]. Therefore, the anti-inflammatory effect of RA may be achieved via diverse mechanisms, which require further investigation.

In summary, the results of this study provide initial evidence that RA compounds induce anti-inflammation by inhibiting activation of the JAK/STAT pathways. Furthermore, expression of SOCS3, negative feedback regulators of the JAK/STAT pathway, contributes to one of the anti-inflammatory mechanisms of RA. Since brain inflammation is a risk factor of neurodegenerative disease, the anti-inflammatory effect of RA may provide a novel therapeutic option for the treatment of brain injury and neurodegenerative disease.

## Acknowledgment

This work was supported by Korea Research Foundation Grant KRF-2003-015-E00171 to E. Joe.

## References

[1] P.J. Gebicke-Haerter, Microsc. Res. Tech. 54 (2001) 47–58.
[2] J.L. Ridet, S.K. Malhotra, A. Privat, F.H. Gage, Trends Neurosci. 20 (1997) 570–577.
[3] M.E. Hatten, R.K. Liem, M.L. Shelanski, C.A. Mason, Glia 4 (1991) 233–243.
[4] P. Vanguri, J. Neuroimmunol. 56 (1995) 35–43.
[5] E.M. Frohman, T.C. Frohman, M.L. Dustin, B. Vayuvegula, B. Choi, A. Gupta, N.S. van den, S. Gupta, J. Neuroimmunol. 23 (1989) 117–124.
[6] Z.H. Zhou, P. Chaturvedi, Y.L. Han, S. Aras, Y.S. Li, P.E. Kolattukudy, D. Ping, J.M. Boss, R.M. Ransohoff, J. Immunol. 160 (1998) 3908–3916.
[7] G.R. Stark, I.M. Kerr, B.R. Williams, R.H. Silverman, R.D. Schreiber, Annu. Rev. Biochem. 67 (1998) 227–264.
[8] J.E. Darnell Jr., I.M. Kerr, G.R. Stark, Science 264 (1994) 1415–1421.
[9] R. Starr, T.A. Willso, E.M. Viney, L.J. Murray, J.R. Rayner, B.J. Jenkins, T.J. Gonda, W.S. Alexander, D. Metcalf, N.A. Nicola, D.J. Hilton, Nature 387 (1997) 917–921.
[10] T.A. Endo, M. Masuhara, M. Yokouchi, R. Suzuki, H. Sakamoto, K. Mitsui, A. Matsumoto, S. Tanimura, M. Ohtsubo, H. Misawa, T. Miyazaki, N. Leonor, T. Taniguchi, T. Fujita, Y. Kanakura, S. Komiya, A. Yoshimura, Nature 387 (1997) 921–924.
[11] T. Naka, M. Narazaki, M. Hirata, T. Matsumoto, S. Minamoto, A. Aono, N. Nishimoto, T. Kajita, T. Taga, K. Yoshizaki, S. Akira, T. Kishimoto, Nature 387 (1997) 924–929.
[12] W.S. Alexander, Nat. Rev. Immunol. 2 (2002) 410–416.
[13] J.J. O'Shea, M. Gadina, R.D. Schreiber, Cell 109 (Suppl.) (2002) S121–S131.
[14] H. Yasukawa, A. Sasaki, A. Yoshimura, Annu. Rev. Immunol. 18 (2000) 143–164.
[15] M. Federici, M.L. Giustizieri, C. Scarponi, G. Girolomoni, C. Albanesi, J. Immunol. 169 (2002) 434–442.
[16] A. Suzuki, T. Hanada, K. Mitsuyama, T. Yoshida, S. Kamizono, T. Hoshino, M. Kubo, A. Yamashita, M. Okabe, K. Takeda, S. Akira, S. Matsumoto, A. Toyonaga, M. Sata, A. Yoshimura, J. Exp. Med. 193 (2001) 471–481.
[17] G. Witz, B.D. Goldstein, M. Amoruso, D.S. Stone, W. Troll, Biochem. Biophys. Res. Commun. 97 (1980) 883–888.
[18] C.E. Brinckerhoff, J.W. Coffey, A.C. Sullivan, Science 221 (1983) 756–758.
[19] G. Plewig, A. Wagner, Arch. Dermatol. Res. 270 (1981) 89–94.
[20] K. Mehta, T. McQueen, S. Tucker, R. Pandita, B.B. Aggarwal, J. Leukoc. Biol. 55 (1994) 336–342.
[21] P.K. Datta, E.A. Lianos, Kidney Int. 56 (1999) 486–493.
[22] H. Pyo, I. Jou, S. Jung, S. Hong, E.H. Jo, Neuroreport 9 (1998) 871–874.
[23] P.H. Driggers, D.L. Ennist, S.L. Gleason, W.H. Mak, M.S. Marks, B.Z. Levi, J.R. Flanagan, E. Appella, K. Ozato, Proc. Natl. Acad. Sci. USA 87 (1990) 3743–3747.
[24] A.M. Stevens, Y.F. Wang, K.A. Sieger, H.F. Lu, L.Y. Yu-Lee, Mol. Endocrinol. 9 (1995) 513–525.
[25] D. Stoiber, P. Kovarik, S. Cohney, J.A. Johnston, P. Steinlein, T. Decker, J. Immunol. 163 (1999) 2640–2647.
[26] C.J. Auernhammer, C. Bousquet, S. Melmed, Proc. Natl. Acad. Sci. USA 96 (1999) 6964–6969.
[27] M.A. Cassatella, S. Gasperini, C. Bovolenta, F. Calzetti, M. Vollebregt, P. Scapini, M. Marchi, R. Suzuki, A. Suzuki, A. Yoshimura, Blood 94 (1999) 2880–2889.
[28] A.H. Dalpke, S. Opper, S. Zimmermann, K. Heeg, J. Immunol. 166 (2001) 7082–7089.
[29] B. Blumberg, D.J. Mangelsdorf, J.A. Dyck, D.A. Bittner, R.M. Evans, E.M. De Robertis, Proc. Natl. Acad. Sci. USA 89 (1992) 2321–2325.
[30] L.J. Gudas, Cell Growth Differ. 3 (1992) 655–662.
[31] Y. Uesugi, I. Fuse, K. Toba, K. Kishi, S. Hashimoto, T. Furukawa, M. Narita, M. Takahashi, Y. Aizawa, J. Exp. Clin. Cancer Res. 19 (2000) 363–366.

[32] Y. Uesugi, I. Fuse, K. Toba, K. Kishi, T. Furukawa, T. Koike, Y. Aizawa, Eur. J. Haematol. 62 (1999) 239–245.
[33] H.Y. Lee, N. Sueoka, W.K. Hong, D.J. Mangelsdorf, F.X. Claret, J.M. Kurie, Mol. Cell. Biol. 19 (1999) 1973–1980.
[34] Q. Xu, T. Konta, A. Furusu, K. Nakayama, J. Lucio-Cazana, L.G. Fine, M. Kitamura, J. Biol. Chem. 277 (2002) 41693–41700.
[35] H. Jiao, K. Berrada, W. Yang, M. Tabrizi, L.C. Platanias, T. Yi, Mol. Cell. Biol. 16 (1996) 6985–6992.
[36] T. Yin, R. Shen, G.S. Feng, Y.C. Yang, J. Biol. Chem. 272 (1997) 1032–1037.
[37] R.C. Venema, V.J. Venema, D.C. Eaton, M.B. Marrero, J. Biol. Chem. 273 (1998) 30795–30800.
[38] J. Gille, L.L. Paxton, T.J. Lawley, S.W. Caughman, R.A. Swerlick, J. Clin. Invest. 99 (1997) 492–500.
[39] T. Hisada, I.M. Adcock, Y. Nasuhara, M. Salmon, T.J. Huang, P.J. Barnes, K.F. Chung, Eur. J. Pharmacol. 377 (1999) 63–68.
[40] S.Y. Na, B.Y. Kang, S.W. Chung, S.J. Han, X. Ma, G. Trinchieri, S.Y. Im, J.W. Lee, T.S. Kim, J. Biol. Chem. 274 (1999) 7674–7680.
[41] B. Ahlemeyer, E. Bauerbach, M. Plath, M. Steuber, C. Heers, F. Tegtmeier, J. Krieglstein, Free Radic. Biol. Med. 30 (2001) 1067–1077.
[42] T. Konta, Q. Xu, A. Furusu, K. Nakayama, M. Kitamura, J. Biol. Chem. 276 (2001) 12697–12701.