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Suppressor of Cytokine Signaling (SOCS) 1 & 3 Expression in HSV-1- Infected and Interferon-γ-treated Neuro-2A Cells

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SUPPRESSOR OF CYTOKINE SIGNALING (SOCS) 1 & 3 EXPRESSION IN HSV-1-INFECTED AND INTERFERON-γ-TREATED NEURO-2A CELLS

A thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Science.

By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Melinda Jones ENTITLED Suppressor of Cytokine Signaling (SOCS) 1 & 3 Expression in HSV-1-infected and Interferon-γ-treated Neuro-2A Cells BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Jones, Melinda. M.S. Microbiology and Immunology M.S. Program, Wright State University, 2012. Suppressor of Cytokine Signaling (SOCS) 1 & 3 Expression in HSV-1-Infected and Interferon-γ-treated Neuro-2A Cells.

This study examined the effects of HSV-1 infection and IFN-γ treatment on Neuro-2A cells. HSV-1 induces expression of SOCS1 and SOCS3 in infected cells, inhibiting the ability of these cells to produce the pro inflammatory, antiviral cytokine IFN-γ (Nowoslawski and Benveniste, 2011). SOCS1 and SOCS3 levels were determined in IFN-γ-treated cells, virus-infected cells, and cells that were both IFN-γ-treated and virus-infected. Results were compared with untreated, uninfected control cells. Flow cytometry data analysis showed a slight decrease in SOCS1 and SOCS3 protein levels in cells treated with IFN-γ for 6 hours compared to control cells. A significant decrease in SOCS1 and SOCS 3 levels was found in cells treated with IFN-γ for 18 hours. Up regulation of SOCS1 and SOCS3 expression was established in virus-infected Neuro-2A cells but this increase was not statistically significant when compared to control cells. However, culturing cells with IFN-γ for 6 hours prior to virus infection led to a significant (50%) decrease in SOCS1 and SOCS3expression compared to cells treated with IFN-γ alone. This showed that HSV-1 was not able to overcome the antiviral effects of IFN-γ to up regulate SOCS1 and SOCS3 expression. Cytopathic effects assays were performed to determine cell viability among cells treated with IFN-γ for 18 hours, cells infected with virus for 48 hours, and cells treated with IFN-γ for 18 hours followed by 48

hour HSV-1 infection. Pre-treating cells with IFN-γ for 18 hours prior to infection with HSV-1 (0.1 MOI) yielded a cell viability level similar to that of control cells (untreated/uninfected). This indicates that IFN-γ is providing antiviral protection to most, if not all of the cells. Applying these studies in a human cell line and eventually in an animal model would be necessary to find the efficacy of IFN-γ as a HSV-1 therapeutic.

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List of Abbreviations

BSA = Bovine serum albumin

DMEM = Dulbecco's Modified Eagle's Medium

FCS = Fetal calf serum

HSV-1 = Herpes simplex virus type 1

IFN-γ = Interferon-gamma

IFNGR1/2 = Interferon gamma receptor 1/2

JAK = Janus kinase

KIR = Kinase inhibitory region

MOI = Multiplicity of infection

NLS = Nuclear localization signal

PAMPs = Pathogen-associated molecular patterns

PBS = Phosphate buffered saline

PCR = Polymerase chain reaction

PKR = Protein Kinase R

SH2 = Src-homology domain

SOCS1 = Suppressor of cytokine signaling-1

SOCS3 = Suppressor of cytokine signaling-3

STAT = Signal transducer and activator of transcription

Acknowledgement

I would like to thank Dr. Nancy Bigley for the guidance and support she has provided me during my time at Wright State University. Her knowledge and insight has been vital to the completion of this project. I would also like to thank Dr. Barbara Hull and Dr. Cheryl Conley for their suggestions and contributions toward my thesis. Lastly, I want to thank my fellow lab members who have made this an enjoyable journey.

Dedication

I would like to dedicate this thesis project to my friends and family for their unwavering support and to everyone who has come into my life and taught me something, no matter how small.

Introduction

Herpes simplex virus type-1 (HSV-1) is a dsDNA virus that affects approximately 70-90% of the population (Cunningham et al., 2006). Although the primary infection is efficiently controlled by the immune system in healthy individuals, the virus will persist in a latent state for the life of the host. During latency, in which the virus resides in the trigeminal ganglia, viral gene expression is minimal (Rautemaa et al., 2002). Reactivation of the virus from neuronal latency is a common and potentially devastating occurrence, responsible for a variety of pathological conditions including cold sores, herpes simplex encephalitis (HSE), and herpes keratitis (Knickelbein et al., 2008; Sheridan et al. 2007)

The human body has intricate mechanisms for controlling viral invasions. One such mechanism is the use of interferons. Interferon gamma (IFN-γ) is a type II interferon that is rapidly produced after the recognition of pathogen-associated molecular patterns (PAMPs) by an infected host cell (Bonjardim et al. 2009). It is produced mainly by helper T cells and natural killer cells. The release of this cytokine activates cells of the innate immune system that attempt to control the infection by killing infected cells and inhibiting viral replication (Cunningham et al., 2006). This finely-tuned antiviral reaction also signals a cessation response which is vital in preventing over-inflammation. This is accomplished through the induction of suppressors of cytokine signaling (SOCS). SOCS proteins negatively regulate inflammatory signaling pathways by facilitating the ubiquination and proteosomal degradation of proteins associated with these pathways

(Nowoslawski and Benveniste 2011). There are currently eight known members of the SOCS family of proteins (SOCS1-7, CIS). All members contain a central SH2 domain and a C-terminal SOCS box (Figure 1). SOCS1 and SOCS3 also contain a kinase inhibitory region (KIR) which has been shown to interact directly with the JAK autophosphorylation loop and inhibit IFN-γ signaling (Croker et al., 2008).

This is important to the study because it is known that herpes simplex virus type 1 is one of a number of viruses that is able to exploit host SOCS proteins for their benefit (Nowoslawski and Benveniste 2011). Several cell lines have demonstrated an increase in SOCS1 and SOCS3 levels upon infection with HSV-1 (Yokota et al., 2005; Frey et al., 2009; Mahller et al., 2008). Increased SOCS1 and SOCS3 levels leads to reduced IFN-γ signaling via inhibition of the JAK/STAT pathway (Figure 2). The process by which HSV-1 increases SOCS expression has not been elucidated.

The interplay between these two proteins is important in balancing the beneficial antiviral and harmful pro-inflammatory effects of IFN-γ signaling in the host cell. SOCS1 –deficient cells are resistant to viral infections (Fenner et al., 2006). However, SOCS1 and SOCS3-deficient mice are vulnerable to inflammatory diseases including autoimmune disorders (Fenner et al., 2006; Qin et al., 2012).

As expected from this negative feedback mechanism, experiments with several cell lines have shown down regulation in SOCS1 and SOCS3 expression when treated with IFN-γ. These lines include keratinocytes, fibroblasts, and astrocytes (Frey et al., 2009; Stark et al., 2004). However, cell lines in which SOCS genes are constitutively

expressed often exhibit IFN-γ resistance (Fojtova et al., 2007; Vlotides et al., 2004). Neurons are a cell type that constitutively expresses both SOCS1 and SOCS3. For this reason, SOCS expression by neuronal cells treated with IFN-γ may differ from that of other cell types.

The hypothesis of this study was that treatment of Neuro-2A cells with IFN-γ prior to infection with HSV-1 would improve cell viability compared to cells infected with virus alone. IFN-γ would cause down regulation of SOCS1 and SOCS3 expression and provide protection from the virus. Basal SOCS1 and SOCS3 levels would be established followed by levels determined after IFN-γ treatment, HSV-1 infection, and after IFN-γ-treatment/HSV-1-infection. Cells would be treated with IFN-γ for 6 and 18 hours to determine the most effective length of treatment. The Neuro-2A cell line was selected for this study due to the neuron being the site of viral latency and reactivation. Figure 3 shows an immunofluorescent image of Neuro-2A cells stained with Phalloidin which binds actin filaments and is commonly used in cellular imaging.

Literature Review

HSV-1

Herpes simplex virus type 1 is a dsDNA virus that undergoes cycles of latent and lytic infection. It is estimated that HSV-1 affects approximately 70%-90% of adults worldwide (Cunningham et al., 2006). Primary infection is usually controlled by the

host's immune system. Reactivation of the virus is common and exhibits as cutaneous or mucocutaneous disease (Arduino and Porter, 2006). These occurrences are often mild and are referred to as "cold sores." Severe but less frequent manifestations include herpes simplex encephalitis (HSE) and herpes stromal keratitis (HSK), with the latter being the most common cause of corneal blindness (Sheridan et al., 2007).

HSV-1 usually infects the oral mucosa and replicates in stratified squamous epithelium (Cunningham et al., 2006). Virus attached to host cell surface by way of envelope glycoproteins gB, gC, gD, gH, and gL. Additional glycoproteins gE and gI facilitate cell-cell spread (Rajcani and Vojvodova, 1998). After entering the stratified squamous epithelium, virus moves via retrograde microtubule-associated transport to the cell body of a neuron in the trigeminal ganglion where a life-long latent infection is established. Sporadic reactivation of HSV-1 results in anterograde transport to the original site of infection where viral replication and shedding occurs (Cunningham et al., 2006; Diefenbach et al., 2008).

The in vivo environment during an HSV-1 infection involves a complex immune response associated with many different cell types. Upon viral infection, activated NK cells and T cells secrete large amounts of IFN-γ. This prevents neighboring cells from becoming infected by inducing in them an antiviral state (Rottenberg and Kristensson, 2002). This is extremely important in the brain because neuronal cells lack the ability to express MHC class I molecules for viral antigen presentation. Interferon is sufficient for viral clearance in some diseases (Rodriguez et al., 2003). However, HSV-1 has evolved a

mechanism to ward off the antiviral effects of IFN-γ by up regulating the levels of SOCS1 and SOCS3. With high levels of these proteins, IFN-γ signaling is decreased, diminishing its antiviral capacity.

SOCS1 and SOCS3

Suppressors of cytokine signaling (SOCS) proteins are inhibitors of cytokine signaling pathways. The expression of SOCS proteins can be induced by cytokine stimulation in a classical "negative feedback" loop (Croker et al., 2008). Eight members comprise this family of proteins (CIS and SOCS1-SOCS7), each of which has a central SH2 domain, an amino-terminal domain, and a carboxy-terminal domain known as the SOCS box (Figure 1). SOCS1 and SOCS3 have an additional kinase inhibitory region (KIR) that functions as a pseudosubstrate to inhibit kinase activity (Nowoslawski and Benveniste, 2011). These two SOCS proteins are involved in IFN-γ regulation.

Interferon gamma

Interferon-γ is a powerful cytokine that influences the intracellular environment by recognizing a distinct cell surface heterodimer receptor identified as interferon gamma receptor 1/2 (IFNGR1/2) (Bonjardim et al., 2008). Once IFN-γ binds to its receptor, Janus kinases (JAKs) are activated to initiate intracellular signaling (Remy et al., 1999). JAK proteins phosphorylate tyrosine residues on the receptors, creating docking sites for a pair of signal transducer and activator of transcription (STATs). These dimerize and

translocate into the nucleus and bind to specific nucleotide sequences on the promoters of target genes (O'Sullivan et al., 2006). This allows for transcription of antiviral genes such as the enzyme protein kinase R (PKR) which is a serine-threonine kinase that binds to and deactivates dsRNA structures (Rottenberg and Kristensson, 2002). IFN-γ also stimulates bactericidal activity in phagocytes, stimulates antigen presentation through class I and II major histocompatibility complex (MHC) molecules, induces chemokines, orchestrates leukocyte-endothelium interactions, and affects cell proliferation and apoptosis (Rodriguez et al., 2003). Cellular division is often inhibited in virus-infected cells and in some situations, apoptosis is induced.

Materials and Methods

Cell Line

The CCL-131 murine Neuro-2A cell line (ATCC) is a neuroblastoma cell line derived from the brain of a strain A albino mouse. Neuro-2A cells were cultured in 25 cm^2 vented cap cell culture flasks and incubated at 37°C in 5% CO_2. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% heat inactivated fetal bovine calf serum (FCS) and 1% penicillin/streptomycin. Cells were passaged twice a week at a subcultivation ratio of 1:6. Culture flasks, DMEM, FCS, and Pen/Strep solution were purchased from Fisher Scientific (Pittsburg, Pennsylvania).

Cell Viability

Neuro-2A cells were subcultured at a ratio of 1:6 and incubated for 48 hours. At this time, the flasks were approximately 40% confluent. They were then treated with IFN-γ (100U/ml), infected with HSV-1 (0.1 MOI), or both. After an allotted time, cells were removed from the cell culture flask using a dissociation reagent (Cell Stripper). Cells were diluted with Trypan Blue and loaded onto a hemocytometer to obtain cell viability counts. Cell Stripper and Trypan Blue were purchased from Fisher Scientific.

Immunofluorescent Staining

Neuro-2A cells were grown in slide chambers (Fisher Scientific) to approximately 40% confluency. Cells were then treated with IFN-γ for 6 or 18 hours or with virus for 24 hours. Following treatment, medium was aspirated and cells were rinsed with 1% bovine serum albumin (BSA) suspended in phosphate buffered saline (PBS). Cells were then fixed using 4% Paraformaldehyde for 15 minutes and permeabilized using 0.1% Triton-X/PBS for 10 minutes, both at room temperature. Blocking buffer, comprised of 5% Goat Serum diluted in 1% BSA, was added for 1 hour at room temperature. After blocking, SOCS1 and SOCS3 primary antibodies were added at 10μg/million cells and the slide chambers were incubated overnight at 4˚C. Following incubation, cells were rinsed with 1% BSA. Secondary antibody (FITC) and Phalloidin were added at a 1:100 dilution for 1 hour. After a final rinse with 1% BSA, VectaShield mounting medium was applied and slides were covered with a coverslip. Slides were then analyzed with fluorescence

microscopy using the Spot Scope fluorescent microscope. SOCS1 and SOCS3 antibodies were generously donated by Dr. Howard Johnson from the University of Florida (Gainesville, Florida); FITC conjugated secondary antibody and Texas Red-Phalloidin X was purchased from Life Technologies (Gaithersburg, Maryland); VectaShield mounting medium supplied by Fisher Scientific.

Flow Cytometry

Cells were grown to approximately 40% confluency, at which time IFN-γ was added with or without virus. After treatment, cells were removed using Cell Stripper and centrifuged to obtain a cell pellet. The supernatant was removed and the cell pellet was resuspended in 1 mL medium. A hemocytometer was used to obtain a cell count. Dilutions were made to achieve the required one million cells for each flow sample.

Cells were centrifuged and rinsed twice with 1% BSA/PBS. Cells were then fixed with 4% Paraformaldehyde for 15 minutes at 4°C. After fixing, cells were rinsed three times. Next, cells were permeabilized using 0.1% Triton-X/PBS for 15 at 4°C. Cells were then rinsed three times. Blocking buffer (5% goat serum/3% BSA/PBS) was added to cells for 1 hour at room temperature. Following blocking, SOCS1 and SOCS3 primary antibodies were added (10 μg) for 45 minutes at 4˚C. Cells were then rinsed three times. FITC-conjugated secondary antibody, diluted in blocking buffer at a 1:100 dilution, was added to cells for 45 minutes at 4˚C. Cells were then rinsed three times and suspended in ice

cold PBS with 10% Fetal Calf Serum and 1% sodium azide. Samples were analyzed on the Accuri C6 flow cytometer.

Interferon-γ Treatment

Cells were grown to approximately 40% confluence in cell culture flasks. IFN-γ was added to flasks at a concentration of 100units/ml for 6 or 18 hours. IFN-γ was purchased from Fisher Scientific.

Virus Infection

Cells were grown to approximately 40% confluence in culture flasks. Cells were then removed using Cell Stripper and pelleted out. After centrifugation, the supernatant was aspirated and the cell pellet was resuspended in 1 mL of medium. A hemocytometer was used to obtain cell counts so multiplicity of infection (MOI) could be accurately calculated. Cells were added to sterile media and cell culture flasks. HSV-1 was administered to flasks at 0.1 MOI.

Results

Basal Levels of SOCS1 and SOCS3 are Similar in Neuro-2a Cells

Immunofluorescent staining and flow cytometry was performed on Neuro-2A cells to determine basal SOCS 1(Figure 4) and SOCS 3 (Figure 5) expression compared to a Negative Isotype control. Images show comparable fluorescence (Figure 6) which is supported by the flow data (Figure 7). Compared to the negative isotype control, SOCS1 expression levels ranged between 63.89% and 67.68%. SOCS3 levels ranged between 64.13% and 65.37%. The distribution of the proteins differed between the two types. SOCS1 was found mostly in the nuclear region while SOCS3 appeared to be strictly cytoplasmic.

Six Hour Interferon-γ Treatment Had No Significant Impact on SOCS Levels or Cell Viability

Neuro-2A cells were treated with IFN-γ (100U/ml) for 6 hours to determine the effect on SOCS expression. Immunofluorescent staining was performed and fluorescence was not as intense as untreated cells (Figure 8). Flow cytometry showed a decrease in expression with SOCS1 ranging between 55.42% and 64.19% and SOCS3 ranging between 53.57% and 62.41% (Figure 9). This was a 9% decrease for both SOCS1 and SOCS3. ANOVA analysis (Sigma Plot) showed this decrease to be of no statistical significance compared to basal levels.

Eighteen Hour Interferon-γ Treatment Had a Significant Impact on SOCS Levels but No Significant Impact on Cell Viability.

Neuro-2A cells were treated with IFN-γ for 18 hours to determine the effect on SOCS protein levels. Immunofluorescent staining was performed and fluorescent levels were so low, the microscope could not record an image. Flow cytometry showed a decrease in levels with SOCS1 ranging between 25.61% and 33.89% while SOCS3 ranged between 26.27% and 35.59% (Figure 10). This was a greater than 50% decrease from basal levels for both SOCS1 and SOCS3. ANOVA analysis (Sigma Plot) showed this to be a significant decrease. Cell viability experiments showed no loss of viability with treatment (93% viable compared to 94% for control cells).

SOCS1 and SOCS3 Levels are Increased with HSV-1 Infection

Neuro-2A cells were infected with 0.1 MOI of virus. After 24 hours, SOCS1 and SOCS 3 expression was assessed using immunofluorescent staining and flow cytometry. Fluorescence was more intense in virus infected cells compared to control (Figures 11, 12). Flow analysis revealed SOCS1 expression levels ranging between 59.8% and 78.58% and SOCS3 levels ranging between 63.40% and 86.44% (Figure 13). While it was shown that HSV-1 does increase SOCS1 and SOCS3 levels, ANOVA analysis (Sigma Plot) found this not to be statistically significant from basal levels.

IFN-γ Treatment for 6 Hours Followed by 24 Hour Virus Infection Led to Further Reduction in SOCS1 and SOCS3 Protein Levels

Cells were treated with IFN-γ for 6 hours followed with virus infection at 0.1 MOI for 24 hours. Flow cytometry revealed a dramatic decrease in both SOCS1 and SOCS3 expression. SOCS1 levels ranged between 15.51% and 20.84%. SOCS3 ranged between 20.30% and 25.29% (Figure 14). This is a 73% decrease for SOCS1 and a 64% decrease for SOCS3 from basal levels. Expression was lower than that of 18-hour IFN-γ-treated cells by 17% and 13% for SOCS1 and SOCS3 respectively. Pre-treating cells with IFN-γ for 6 hours prior to HSV-1 infection led to a decrease in SOCS1 and SOCS expression by 79% and 84% respectively compared to HSV-1 infection alone.

IFN-γ for 18 Hours Prior to HSV-1 Infection Gives Protection From Virus

Cell viability experiments were conducted for Neuro-2A cells. Cells were treated with IFN-γ for 18 hours, infected with HSV-1 for 48 hours, or treated with IFN-γ for 18 hours then infected with HSV-1 for 48 hours. Results were compared to control of untreated/uninfected cells. Experiments were performed in quadruplicate. Control cells exhibited a cell viability of 94%. Cells treated with IFN-γ for 18 hours showed viability of 93%. Virus infected cell viability was 74%. Cells treated with IFN-γ for 18 hours prior to virus infection had a cell viability of 91%. These data illustrate the ability of IFN-γ to protect Neuro-2A cells from the cytotoxic effects of HSV-1 when added prior to infection.

Discussion

In this study it was shown through that Neuro-2A cells had similar basal levels of SOCS1 and SOCS3. However, immunofluorescent images showed that the distribution of the proteins differed. Antibodies to SOCS1 fluoresced more in the nucleus while antibodies to SOCS3 seemed to fluoresce only in the cytoplasm. The nuclear localization signal (NLS) located between the src-homology 2 domain and the SOCS box of SOCS1 but not other SOCS family members explain these results (Baetz et al, 2008).

Treating Neuro-2A cells with IFN-γ at a concentration of 100U/ml caused SOCS1 and SOCS3 protein levels to decrease. The 18 hour treatment showed a significant decrease (p-value<0.05). There was expected to be an initial increase in SOCS protein levels because it is a negative regulator of IFN-γ signaling however, this was not the case. The concentration of IFN-γ used might have been too high or the time point tested may have been too late. Another possibility is IFN-γ resistance. Neuronal and malignant cells constitutively express SOCS genes and cells with constitutive SOCS expression may be less sensitive to IFN-γ signaling (Fojtova et al., 2007).

Up regulation of SOCS1 and SOCS3 protein levels was achieved with HSV-1 infection, although ANOVA analysis (Sigma Plot) found the data to not be of statistical significance. Due to the short half-life of SOCS proteins (1-2 hours) and the disputed length of the HSV-1replication cycle, time lapse experiments would need to be

performed to ascertain the time point following infection that SOCS levels were the highest (Vuong et al., 2004).

Because IFN-γ treatment caused a decrease in SOCS expression, experiments were performed to determine the effects on cells treated with IFN-γ for 6 hours prior to HSV-1 infection. These cells had SOCS1 and SOCS3 protein levels that were decreased by approximately 54% from cells that were IFN-γ-treated only. These data indicated that the virus was not able to up regulate the SOCS levels enough to overcome the antiviral effects of the interferon. A cytopathic effects assay was performed to establish if pre-treatment with IFN-γ could indeed offer protection to virus-infected cells.

Treating Neuro-2A cells with IFN-γ for 18 hours prior to HSV-1 infection led to a cell viability count similar to that of untreated/ uninfected control cells. This cytokine prevents cells from becoming infected by inducing an antiviral state. This implies a wide spectrum of changes that aim to inhibit the uptake, transcription, translation, and release of infectious particles (Rottenberg and Kristensson, 2002). Future experiments need to be performed on the IFN-γ-treated/virus-infected cells to find what percentage is actually infected with the virus and if eventual clearance can be established. The biggest problem with treating viral infections in neuronal cells is that neurons are post mitotic. Viral clearance has to be accomplished in a way that does not destroy the cell. IFN-γ has the ability to clear certain viral infections without killing the host cell.

Determining if latency is occurring in these cells by performing polymerase chain reaction (PCR) would be important as well. It is not known at this point if IFN-γ is

keeping the Neuro-2A cells from becoming infected by HSV-1 or if it is causing the virus to immediately enter into a non-lytic, latent state.

It would also be important to better understand the localization patterns displayed by SOCS1 and SOCS3 since only SOCS1 has a nuclear localization signal. It stands to reason that herpes simplex virus exploits these two proteins somewhat differently. It is quite possible that one plays a more important role in infection and latency than the other.

These in vitro studies would further need to be applied in a murine model. The in vivo environment is vastly differently from an in vitro one with many other immune factors at play. Establishing the efficacy or any toxicity of IFN-γ using animal models would be necessary to proceed in using it as a possible prevention of HSV-1 pathogenesis in humans.

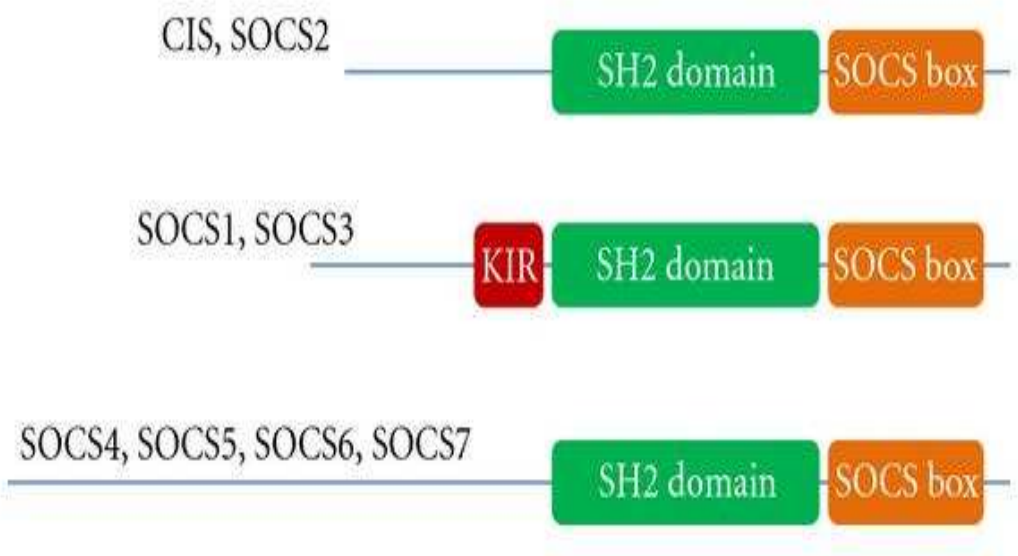

Figure 1: Schematic structure of suppressor of cytokine signaling (SOCS) proteins. SOCS proteins are characterized by a central SH2 domain, a docking motif to tyrosine-phosphorylated proteins, and a c-terminal SOCS box that recruits Elongin B/C complex. SOCS1 and SOCS3 also possess a KIR domain, which plays an important role in inhibition of JAK kinase activity. (Adapted from Fujimoto and Naka, 2010).

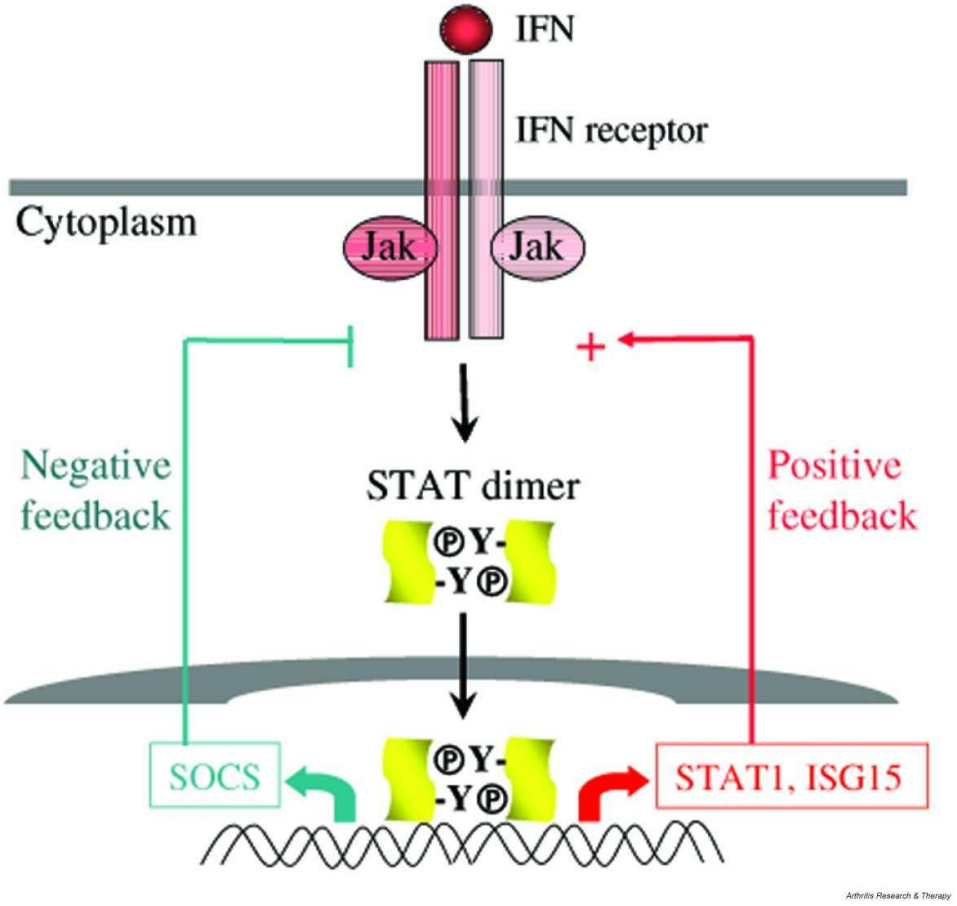

Figure 2: Activation of negative feedback loop by interferons via the JAK/STAT pathway. (Adapted from Ivashkiv and Hu, 2004).

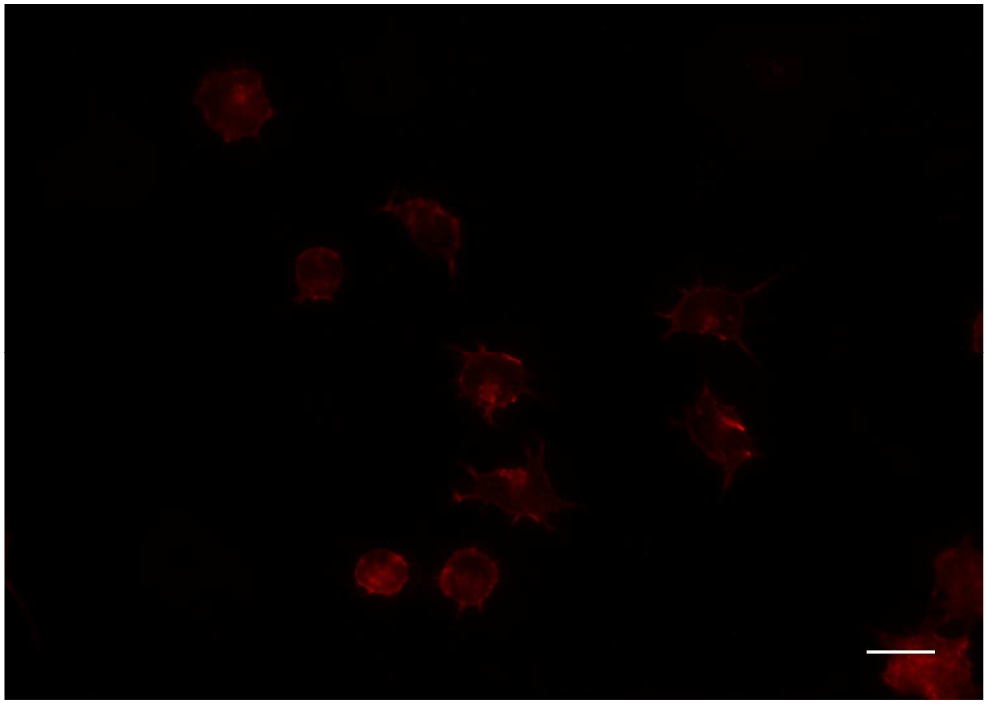

Figure 3: Immunofluorescent image of Neuro-2A cells stained with Phalloidin. (Scale bar = 50μm)

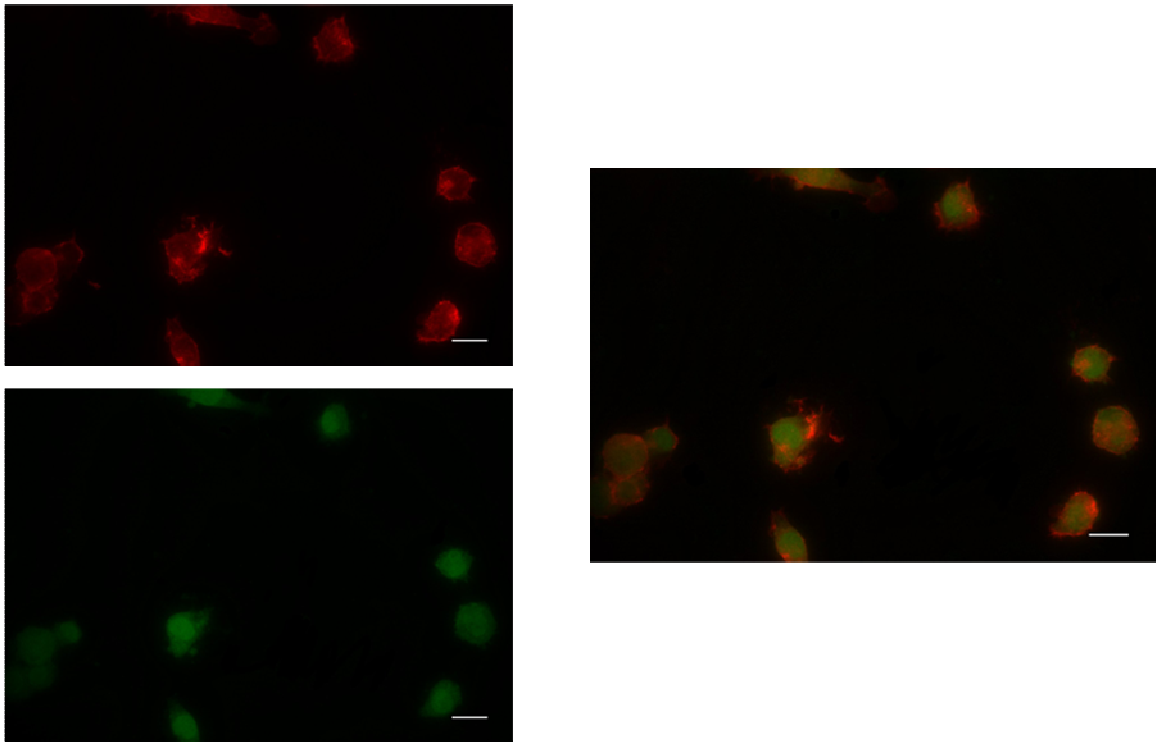

Figure 4: Immunofluorescent staining images of Phalloidin (top left), SOCS1 (bottom left), and Phalloidin/SOCS1 merged (right). (Scale bar = 50μm)

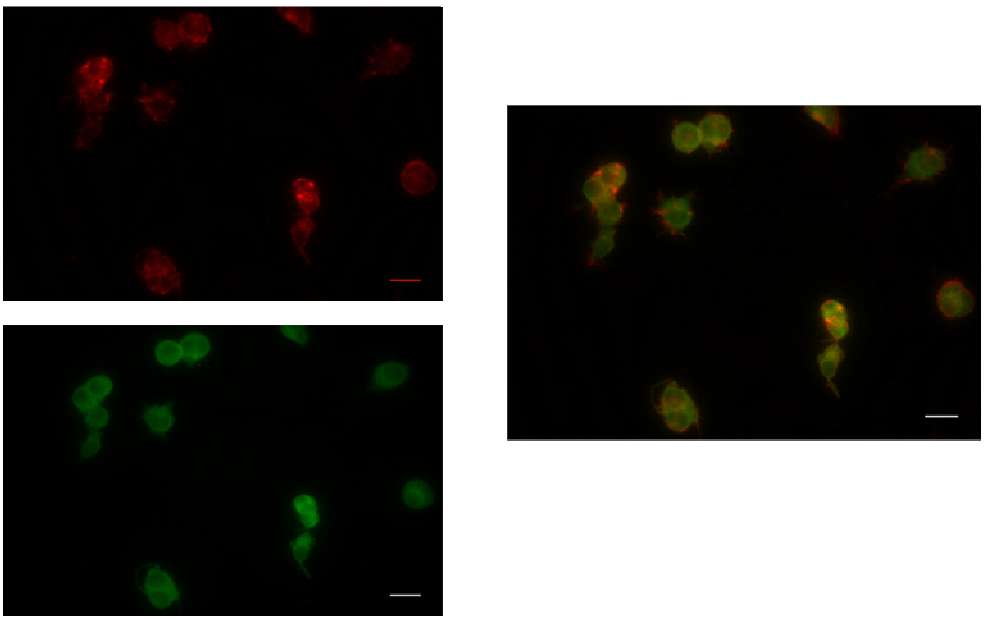

Figure 5: Immunofluorescent staining images of Phalloidin (top left), SOCS3 (bottom left), and Phalloidin/SOCS3 merged (right). (Scale bar = 50μm)

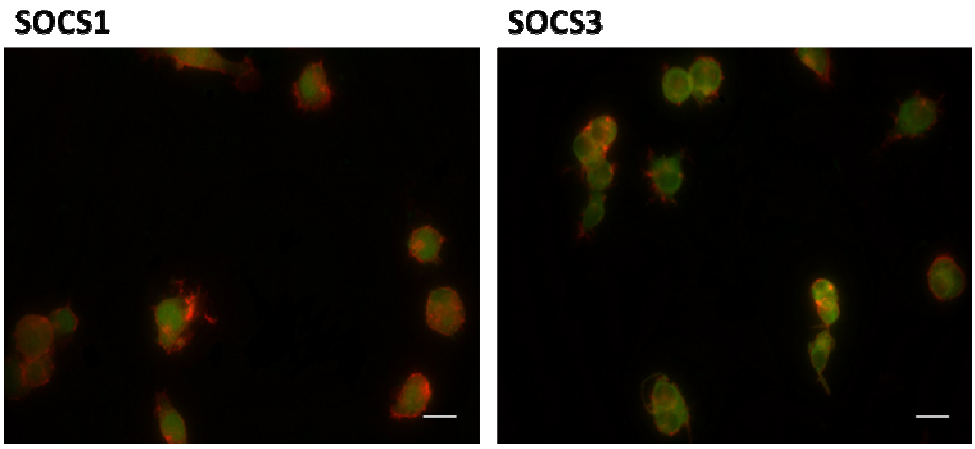

Figure 6: Immunofluorescent staining images comparing basal SOCS1 (left) and SOCS3 (right) protein levels. (Scale bar = 50μm)

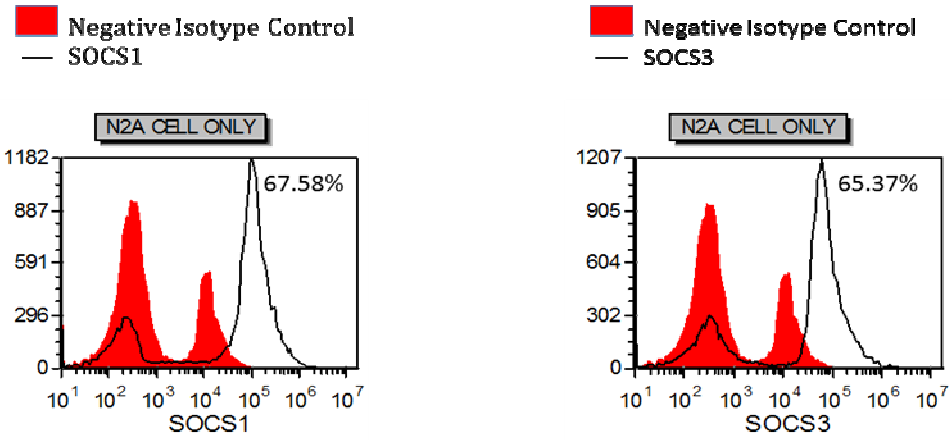

Figure 7: Flow cytometry histograms showing basal SOCS1 (left) and SOCS3 (right) expression compared to Negative Isotype control. The expression of both proteins was similar.

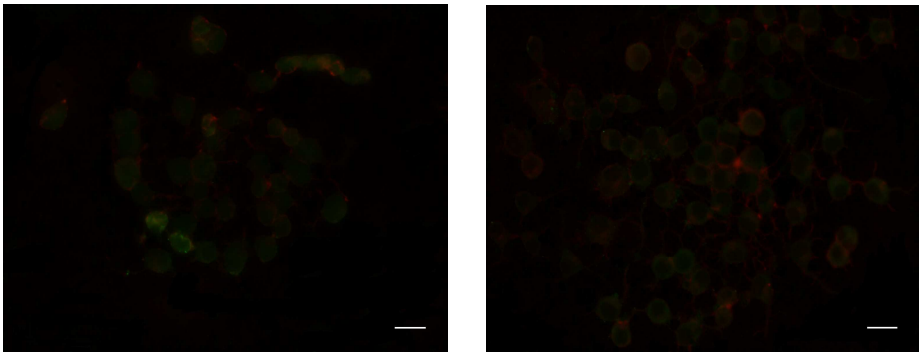

Figure 8: Immunofluorescent staining images showing SOCS1 (left) and SOCS3 (right) protein levels after 6-hour IFN-y treatment (100U/ml). (Scale bar = 50μm)

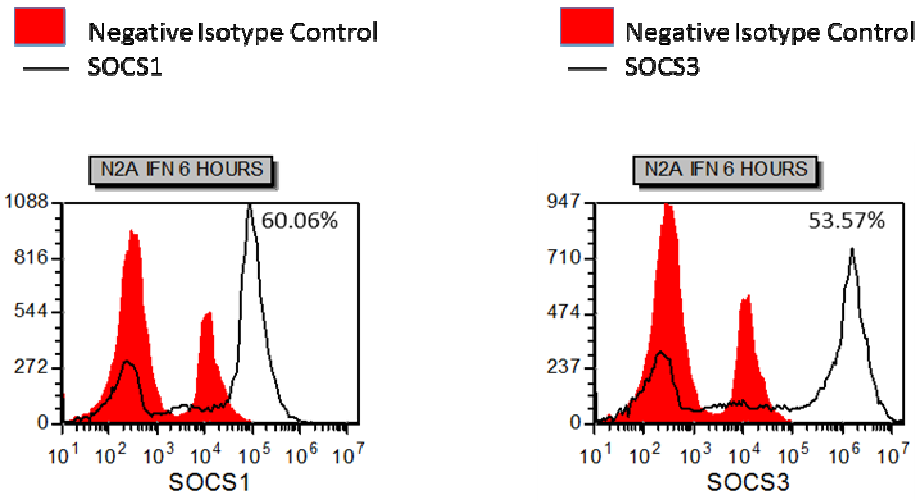

Figure 9: Flow cytometry histograms showing SOCS1 (left) and SOCS3 (right) expression after 6-hour IFN-y treatment (100U/ml). Expression was not significantly decreased from basal levels for either SOCS1 or SOCS3.

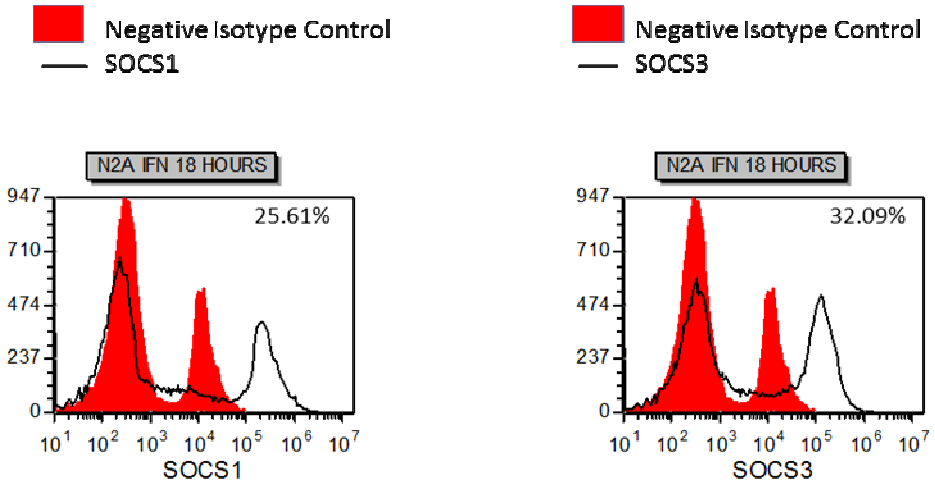

Figure 10: Flow cytometry histograms showing SOCS1 (left) and SOCS3 (right) expression after 18-hour IFN-y treatment (100U/ml). Expression was significantly decreased from basal levels for both SOCS1 and SOCS3.

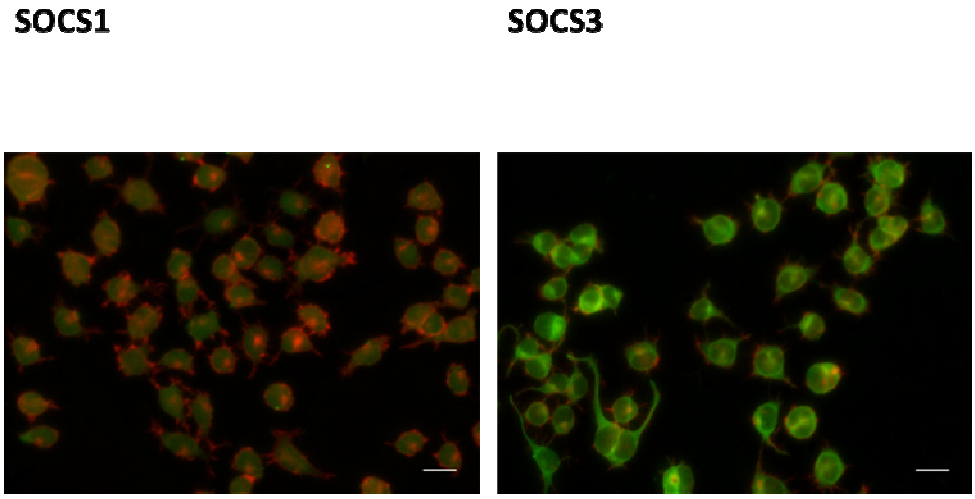

Figure 11: Immunofluorescent staining images of SOCS1 (left) and SOCS3 (right) 24 hours post HSV-1 infection. (Scale bar = 50μm)

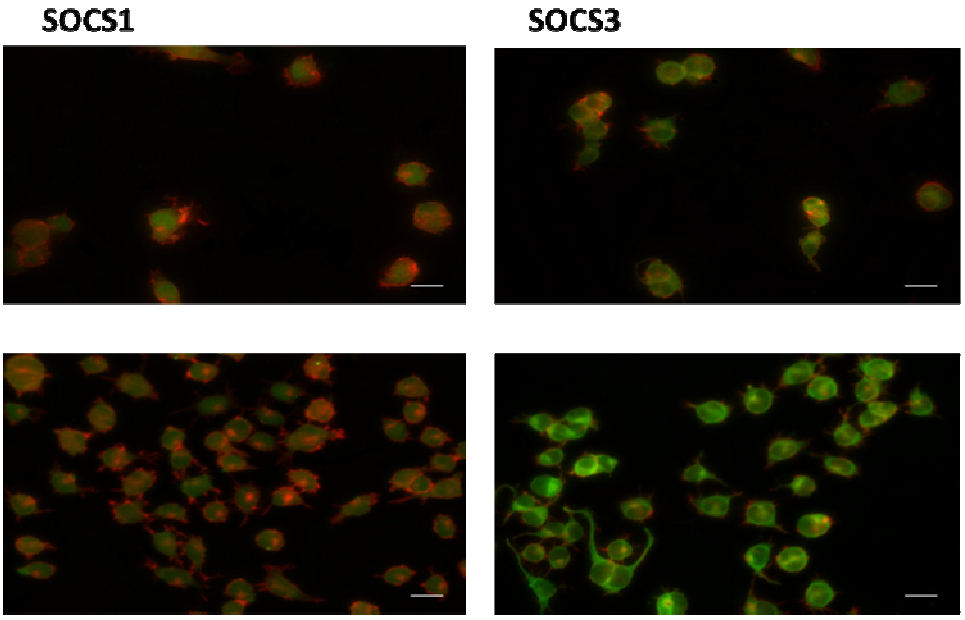

Figure 12: Immunofluorescent staining images of SOCS1 (left) and SOCS3 (right) before (top) and after (bottom) infection with HSV-1 (0.1 MOI). (Scale bar = 50μm)

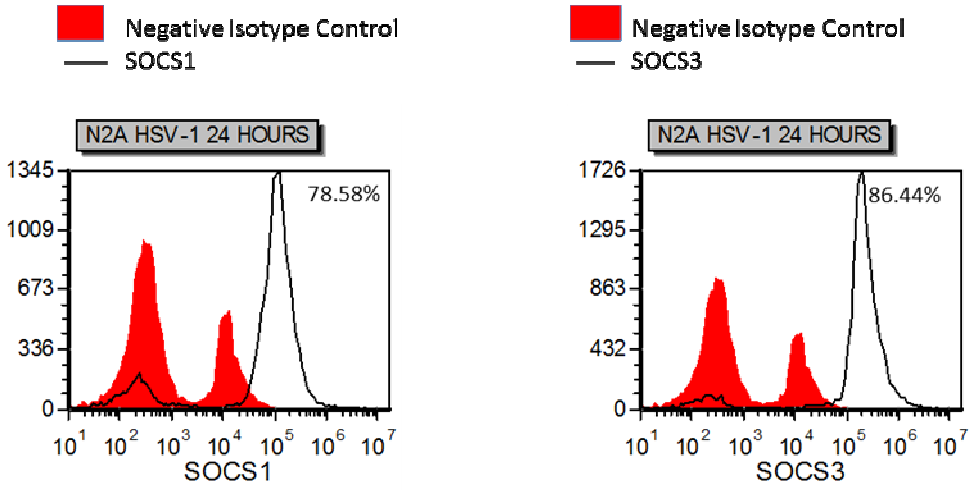

Figure 13: Flow cytometry histograms showing SOCS1 (left) and SOCS3 (right) expression 24 hours post HSV-1 infection (0.1 MOI). Expression was slightly increased from basal levels for both SOCS1 and SOCS3.

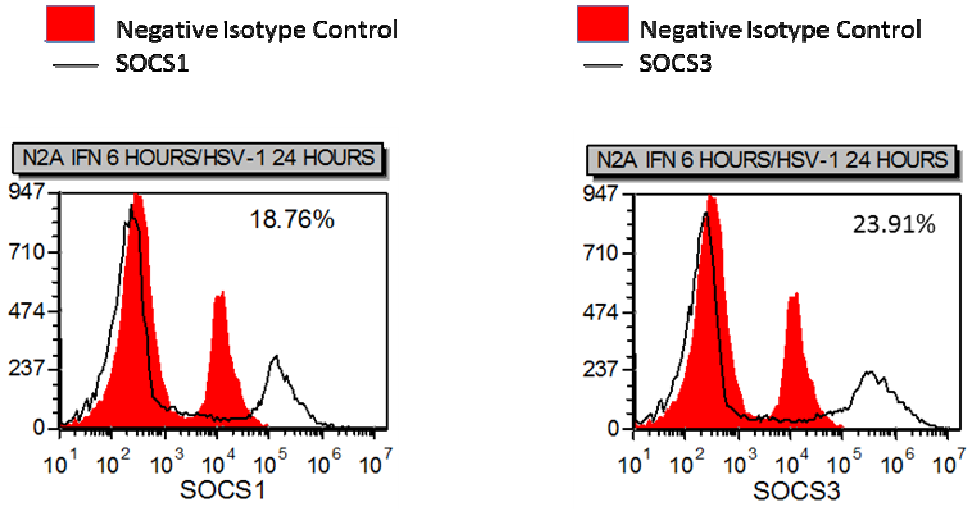

Figure 14: Flow cytometry histograms showing SOCS1 (left) and SOCS3 (right) expression after 6-hour IFN-y treatment (100U/ml) and 24-hour HSV-1 infection (0.1 MOI). Expression was significantly decreased from IFN-γ-treated only cells. (p-value<0.05)

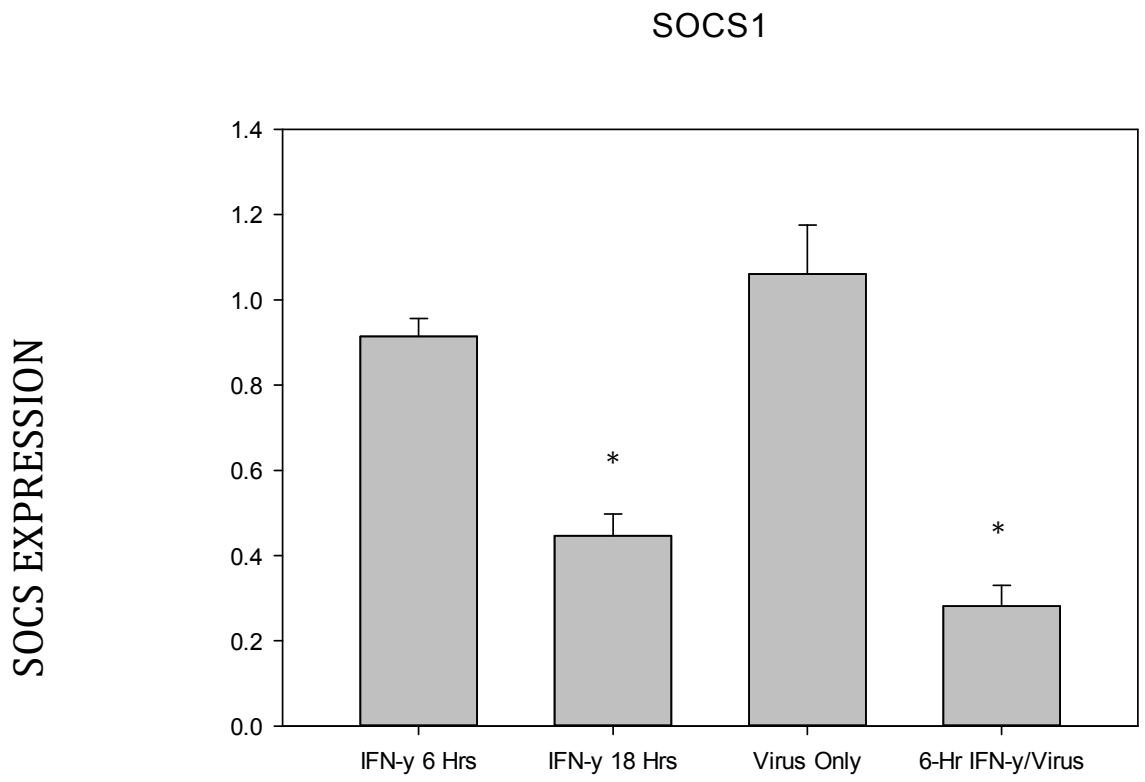

Figure 15: SOCS1 expression in Neuro-2A cells treated with IFN-y for 6 and 18 hours, infected with virus for 24 hours, and cells treated with IFN-y for 6 hours then virus infected for 24. Normalized to untreated/uninfected control cells. There was slightly more than a half fold decrease in SOCS1 expression with 18-hour IFN-γ treatment and an even greater decrease with IFN-γ treatment/virus infection. (*=p-value<0.05)

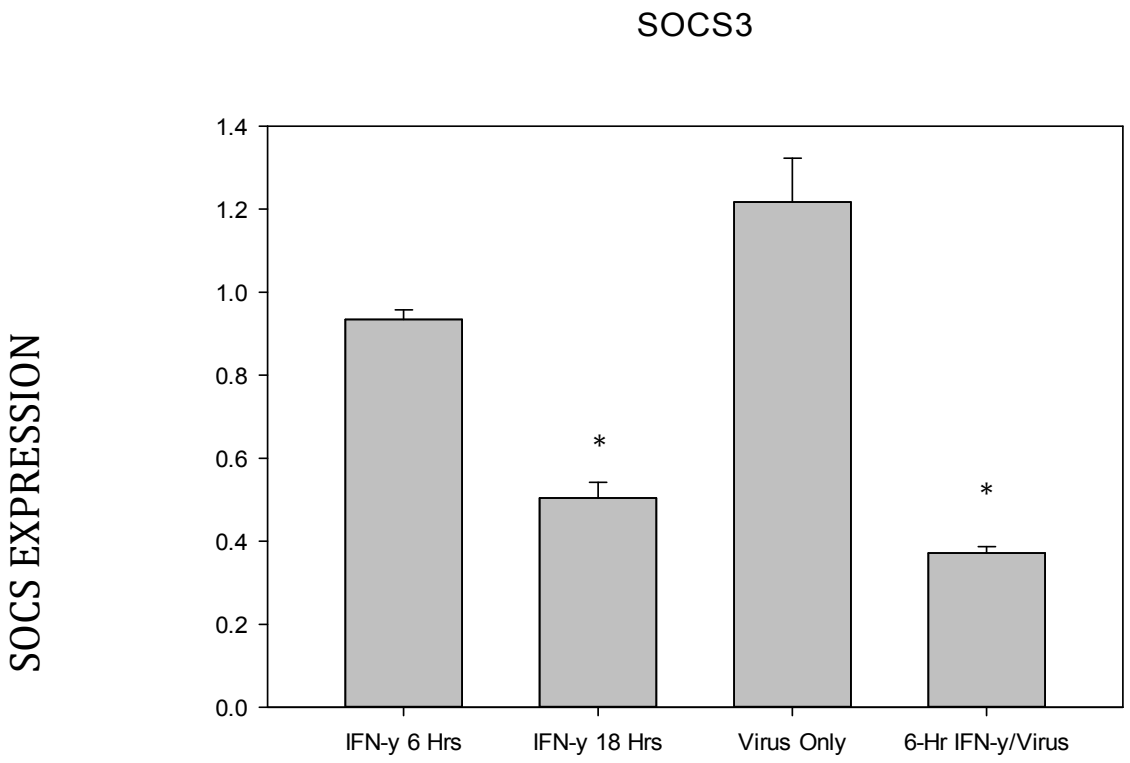

Figure 16: SOCS3 expression in Neuro-2A cells treated with IFN-y for 6 and 18 hours, infected with virus for 24 hours, and cells treated with IFN-y for 6 hours then virus infected for 24. Normalized to untreated/uninfected control cells. There was approximately a 50% decrease in SOCS3 expression with 18-hour IFN-γ treatment and an even greater decrease with IFN-γ treatment/virus infection. (*=p-value<0.05)

% VIABILITY	**CELL ONLY**	CELL W/IFN-γ (100U/ml) 18 HOURS	CELL W/VIRUS (0.1MOI) 48 HOURS	CELL W/IFNγ (100U/ml) 18 HOURS & VIRUS (0.1MOI) 48 HOURS
1	94	92	75	92
2	91	95	71	90
3	95	93	73	89
4	96	92	77	94
AVERAGE	94%(+/-2.16)	93%(+/-1.41)	74%(+/-2.58)	91%(+/-2.22)

Figure 17: Cell viability table showing four separate trials and the average cell viability count (last row) of those trials. IFN-γ treatment prior to infection yielded cell viability similar to that of untreated/uninfected control Neuro-2A cells.

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