Virus Research 180 (2014) 49–58

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Study of interferon-β antiviral activity against Herpes simplex virus type 1 in neuron-enriched trigeminal ganglia cultures

Ana Maria Low-Calle[a], Jeanette Prada-Arismendy[a], Jaime E. Castellanos[a,b,*]

[a] Grupo de Virologia, Universidad El Bosque, Carrera 9 No. 131 A-02, Edificio de Biblioteca, Laboratorio 205, Bogotá D.C., Colombia
[b] Grupo de Patogénesis Infecciosa, Facultad de Odontología, Universidad Nacional de Colombia, Carrera 30 No. 45-03, Edificio 210 – Oficina 301, Bogotá D.C., Colombia

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 9 December 2013
Accepted 9 December 2013
Available online 24 December 2013

Keywords:
HSV-1
Interferon-β
Trigeminal ganglion
Socs3
Jak-Stat

ABSTRACT

Herpes simplex virus type 1 (HSV-1) causes a lytic infection in epithelial cells before being captured and moved *via* retrograde axonal transport to the nuclei of the sensory neurons of the trigeminal ganglion or dorsal root, where it establishes a latent infection. HSV-1 infection induces an antiviral response through the production of Beta Interferon (IFN-β) in infected trigeminal ganglia. The aim of this work was to characterize the response induced by IFN-β in neuron-enriched trigeminal ganglia primary cultures infected with HSV-1. An antiviral effect of IFN-β in these cultures was observed, including reduced viral production and increased cell survival. In contrast, viral infection significantly decreased both *double stranded RNA dependent protein kinase* (*Pkr*) transcription and Jak-1 and Stat-1 phosphorylation, suggesting a possible HSV-1 immune evasion mechanism in trigeminal cells. Additionally, HSV-1 infection upregulated *Suppressor of Cytokine Signaling-3* (*Socs3*) mRNA; upregulation of *socs3* was inhibited in IFN-β treated cultures. HSV-1 infection increased the number of Socs3 positive cells and modified the intracellular distribution of Socs3 protein, in infected cells. This neuron-enriched trigeminal ganglia culture model could be used to elucidate the HSV-1 viral cycle in sensory neurons and to study cellular antiviral responses and possible viral evasion mechanisms that underlie the choice between viral replication and latency.

1. Introduction

Human Herpes virus 1 (HHV-1) also known as Herpes simplex virus type 1 (HSV-1) is a human pathogen that infects and replicates in epithelial cells from the orofacial region, causing vesicles and ulcers on the skin and mouth. This lytic cycle of viral replication leads to epithelial cell death (Roizman et al., 2007; Steiner et al., 2007). During the lytic phase, HSV-1 expresses nearly 80 gene products that are transcribed in a tightly regulated cascade of three gene types: immediate early (IE, α genes), early (E, β genes) and late genes (L, γ genes). IE genes, such as *Infected Cell Protein 0*, are transcribed from the moment the viral DNA enters the nucleus until 2–4 hours post infection (h.p.i.). E genes are involved in viral DNA replication and are transcribed between 4 and 8 h.p.i.; the E genes include *thymidine kinase* and polymerase. Finally, L genes are divided in two groups: Leaky late or γ1, which are expressed early during infection, independently of the viral replication, in this group are included glycoprotein B, ICP34.5, glycoprotein D and ICP5 (Weir, 2001; Roizman et al., 2007). The second genes group, the true late o γ2 genes are expressed later during viral infections, and depend on previous viral DNA replication for their expression between 8 and 12 h.p.i. include other viral glycoproteins and structural proteins that assemble new infectious virions (Honess and Roizman, 1974; Roizman et al., 2007).

After viral replication in oral epithelial cells, HSV-1 travels by retrograde axonal transport and enters the nucleus of sensory neurons in the trigeminal ganglion (TG). There, HSV-1 establishes acute infections by undergoing viral replication followed by the establishment of latency. Latency is a process in which viral assembly in the neurons is inhibited, viral gene expression shuts down and only partial transcription of a few viral genes remains active. In this latent stage, viral DNA remains dormant in the patient, with occasional reactivations that promote expression of the viral genes needed to produce new virions. These virions travel back down through the anterograde neuronal transport pathway and can cause

Abbreviations: HSV-1, Herpes simplex virus type 1; TG, trigeminal ganglion; IFN, interferon; gB, HSV-1 glycoprotein B; Oas, 2′–5′ oligoadenylate synthetase; RNase-L, latent ribonuclease or 2′,5′-oligoisoadenylate synthetase-dependent RNAse; Pkr, double stranded RNA-dependent protein kinase; Jak, Janus kinase; p-Jak, Jak phosphorylated form; Stat, Signal Transduction and Activator of Transcription; p-Stat, Stat phosphorylated form; Socs, suppressor of cytokine signaling; CM, complete medium.

* Corresponding author at: Grupo de Patogénesis Infecciosa, Facultad de Odontología, Universidad Nacional de Colombia, Carrera 30 No. 45-03, Building 210 – Office 301, Bogotá D.C., Colombia. Tel.: +57 1 3165555; fax: +57 1 6489066.
E-mail addresses: anamalowc@gmail.com (A.M. Low-Calle), jpradaarismendy@yahoo.es (J. Prada-Arismendy), jecastellanosp@unal.edu.co, castellanosjaime@unbosque.edu.co (J.E. Castellanos).

0168-1702/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2013.12.022

recurrent infections in the epithelia (Kramer et al., 1998; Efstathiou and Preston, 2005; Perng and Jones, 2010).

It has been reported that an immune response in the TG participates in HSV-1 infection control, and that Interferon (IFN) type I activity has a central role as an antiviral molecule. Examples of this role have previously been described. For example, IFN-α and -β are highly expressed in the TG of HSV-1 infected mice (Sokawa et al., 1980; Peng et al., 2005). In addition, *in vivo* HSV-1 infections in the presence of IFN blocking antibodies or in mice lacking the IFN receptor have been related to higher susceptibility to HSV infections or death (Halford et al., 1997; Leib et al., 1999). Likewise, the protective effect of IFN treatment, which resulted in an increase in survival percentages of mice during HSV-1 acute *in vivo* infections, has also been demonstrated (Cui and Carr, 2000; Al-Khatib et al., 2004, 2005; Austin et al., 2005).

In vitro cell models have shown that HSV-1-infected and IFN-β-treated TG cultures express several IFN stimulated genes (ISGs) that have antiviral activity, such as 2′–5′ oligoadenylate synthetase (*Oas*), latent ribonuclease (*Rnasel*, 2′,5′-oligoisoadenylate synthetase-dependent RNAse) and double stranded RNA-dependent protein kinase (*Pkr*) (Sokawa et al., 1980; Al-Khatib et al., 2003, 2004; Carr et al., 2003; Austin et al., 2005). Activation of these IFN-β antiviral genes begins with the binding of IFN-β to its receptor, which recruits and phosphorylates Janus kinases (Jak-1 and Tyk-2). These Janus kinases then phosphorylate the Signal Transduction and Activator of Transcription proteins (Stat-1 and Stat-2). This phosphorylation causes translocation of Stat-1 and Stat-2 to nucleus, triggering transcriptional activation of antiviral genes (Platanias, 2005; Bonjardim et al., 2009). This antiviral response is finally turned off through the activity of the suppressor of cytokine signaling (Socs) protein family. One of the proteins in this family, Socs3, associates with the IFN receptor, inhibiting Jak enzymatic activity and inducing Jak proteasomal degradation, which eventually shuts down antiviral signaling (Song and Shuai, 1998; Yoshimura et al., 2007; Croker et al., 2008).

Because IFN signaling and activity promote virus elimination, HSV-1 has evolved ways to inhibit this signaling pathway so the virus is more productive during epithelial infections; and this leads to the establishment of lifelong latent infections in sensory neurons (Johnson et al., 2008). It has been shown that HSV-1 infection can induce IFN-I receptor degradation, decrease Jak phosphorylation and block Stat-1 nuclear localization (Chee and Roizman, 2004; Johnson and Knipe, 2010). Additionally, HSV-1 infection in epithelial cells has also been found to evade the IFN antiviral response *via* early expression of Socs3. This impairment leads to more efficient viral replication (Yokota et al., 2001, 2004, 2005). These viral evasion mechanisms have previously been described in epithelial cells but not in TG cells.

By contrast, in previous reports, TG cultures used for the study the HSV-1 infection had small numbers of sensory neurons (*i.e.* 1.5%) (Carr et al., 2003); these cells are where latency is established, and they are the most susceptible cells to herpes infection. For these reasons, here we studied the role of IFN-β during HSV-1 infections in mouse TG neuron-enriched primary cultures. Additionally, to better understand the putative mechanisms used by HSV-1 to evade the antiviral response elicited by TG cells, we also assessed IFN-β signaling pathway activation, ISGs and *Socs3* gene expression.

2. Materials and methods

2.1. Mouse trigeminal ganglia cultures, IFN-β treatment, HSV-1 infection and plaque assay

According to a previously published protocol for dorsal root ganglia cultures (Castellanos and Hurtado, 1999), TG were dissected from adult female ICR mice and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with fetal bovine serum 10%, v/v, (Gibco), penicillin 100 IU/ml and streptomycin 100 μg/ml (complete medium, CM). Ganglia were dissociated for 1 h at 37 °C in a solution containing collagenase 2 mg/ml (Gibco) and dispase 5 mg/ml (Sigma) that was prepared in CM. Tissue suspensions were mechanically disrupted using a flame-narrowed Pasteur pipette to dissociate cells. After centrifugation, the cell pellet was suspended in CM and seeded in 12- or 24-well dishes that had been treated with poly-L-lysine 10 μg/ml (Sigma). Cultures were maintained for 96 h with Cytosine β-D-arabinofuranoside (AraC) 10 μM (Sigma). The CM was replaced every day. Twenty-four hours before infection, the cell culture medium was replaced with AraC-free medium. The cultures were then treated with IFN-β (PBL Interferon Source) at different concentrations (1, 100 and 1000 U/ml) and time periods (15 min post-infection for Western blot experiments only and 6 h before infection for all other experiments).

HSV-1 was isolated from a patient with herpetic labial vesicles and was inoculated and titrated in Vero cells (ATCC). The titer obtained was 1×10^7 PFU/ml, and the virus was inoculated at a MOI of 1 in TG cultures for 1 h at 37 °C (taking into account 11,000 cells per each TG in culture). The virus was then discarded, and fresh medium was added for the defined time period. Vero cell lysate was used as a mock inoculation control. Viral titers were obtained in Vero cells; 100,000 cells were seeded per well in 24-well dishes using FBS 1% (v/v) medium. The cells were then infected in triplicate with serial dilutions of supernatant for 4 h at 37 °C. After infection, the inoculum was washed and replaced with carboxymethylcellulose 3% (w/v) in DMEM and incubated for 5 additional days. Staining was performed with 0.1% (w/v) crystal violet solution to count the viral plaques.

2.2. Cell viability and IFN-β treatment

Approximately one dissociated TG per well was seeded and treated with IFN-β 1, 100 or 1000 U/ml or medium, for 6 h and was then washed and infected with HSV-1 (MOI of 1) and incubated for 28 h. The supernatants were collected and titrated by plaque assay on Vero cells. The monolayers were incubated for 2 h with MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide) 0.5 mg/ml (Sigma) and were then washed. Formazan crystals were solubilized with dimetyl sulfoxide and $A_{570\,nm}$ was read in 96-well dishes with spectrophotometer (Stat Fax 2100, Awareness Technology).

2.3. Murine fibroblasts cell cultures, IFN-treatment and HSV-1 infection

L929 murine fibroblast cell line (ATCC) was cultured in DMEM (Sigma) supplemented with fetal bovine serum (FBS) 10%, v/v, (Gibco), penicillin 100 IU/ml and streptomycin 100 μg/ml. 200,000 cells were seeded in 12 well culture plates for 24 h in CM supplemented with 10% FBS. L929 cells were treated with IFN-β 1000 U/ml in CM or CM alone for 6 h. IFN-β was then washed and murine fibroblasts were inoculated with mock or HSV-1 (MOI of 1) in CM supplemented with 5% FBS for 1 h. Then inoculum was replaced by CM supplemented with 10% FBS, and cells were incubated for 12 h. RNA extraction was performed followed by DNaseI treatment and RT-qPCR.

2.4. Reverse transcription and real-time PCR

Total RNA was isolated from the TG or Murine Fibroblasts cultures using Trizol Reagent (Invitrogen) following the manufacturer's instructions. RNA was solubilized in diethyl pyrocarbonate 0.1% (v/v) treated water. Isolated RNA was quantified by $A_{260\,nm}$

(Nanodrop ND1000, ThermoScientific) and then treated with DNAse I (Sigma), and cDNA was synthesized from 500 ng of RNA per sample using random primers and 250 U of MMLV (Promega). Relative expression was then quantified using the DyNAmo HS SYBR Green qPCR kit (Finnzymes) in a real-time thermocycler (CFX96 Biorad). The PCR program was as follows: 10 min initial denaturation at 95 °C followed by 40 cycles of 95 °C for 30 s and 60 °C for 1 min. Amplicon specificity was verified with a dissociation curve created by reading the fluorescence between 100 and 60 °C. The following primers designed by Marin et al. (2007) were used: Oas-F:ATG TTA ATA CTT CCA GCA AGC, Oas-R:GCA AAG ACA GTG AGC AAC TCT; *Pkr*-F: GGT TAG GGT GAC CCA CAG ACA, Pkr-R:TGT GAC TTC CCA CAC AGG AG; *Rnase*-F:GAG TCG GGA AGG CAT AAA CA, *Rnase*-R: CCT CCT GGC TAT CCA AAC AA; Socs3-F: ACC TTT GAC AAG CGG ACT CT, *Socs3*-R:GGA GCC TGG CAC CAG TAT AG, Actin-F:ATC CTC TTC CTC CCT GGA GA, Actin-R: TGC CTG GGT ACA TGG TGG TA; *ICP0*-F: GAC CCT CCA GCC GCA TAC GA, *ICP0*-R:TTC GGT CTC CGC CTC AGA GT (Lynas et al., 1989); *TK*-F:AAA CCA CCA CCA CGC AAC, *TK-R:* ACA CCC GCC AGT AAG TCA TC (Al-Khatib et al., 2002); *gB*-F: CGT TTC GCA GGT GTG GTT C *gB-R:*ATG TCG GTC TCG TGG TCG (Al-Khatib et al., 2002). β-Actin mRNA was used as a housekeeping normalizer gene. In murine fibroblasts, were evaluated only Socs3 and β-Actin as a control of non-neuronal cell culture. Relative gene expression was evaluated by comparing gene expression levels from virus-infected cultures against mock-infected cultures. Viral genes were quantified using the formula reported by Pfaffl (2001). Relative expression of cellular genes was calculated using the mathematical method described by Schefe et al. (2006).

2.5. Western blot

Two dissociated TGs were seeded per well in 12-well dishes. Cultures were inoculated with HSV-1 or mock-infected for 12 and 18 h and were then treated for 15 min with IFN-β 1000 U/ml. Monolayers were scraped with Laemmli buffer supplemented with phosphatase inhibitors (sodium orthovanadate 1 mM and sodium fluoride 2 mM) and boiled for 5 min. Protein extracts were separated by SDS-PAGE and transferred to a PVDF membrane (16 h, 4 °C). The membrane was then soaked for 1 h in blocking solution at RT (Bovine Serum Albumin 1% (w/v), non-fat milk 1% (w/v), Tween-20 0.1% (v/v), pH 8.0). Primary antibodies were then prepared in blocking solution and were added to the blot. The following primary antibodies were used: anti-Stat-1 (Millipore 07-307, dilution 1:100), phospho-Stat-1 (Millipore 07-307, dilution 1:600), Jak-1 (R&D Systems MAB42601, dilution 1:500), phospho-Jak-1 (Millipore AB3850, dilution 1:100) and mouse anti β-Actin (Sigma A-5316, dilution 1:2500). The membranes were incubated with their respective secondary biotinylated antibodies (anti-mouse Vector BA-9200 or anti-rabbit Vector BA-1000) at a 1:200 dilution. Finally, peroxidase-coupled streptavidin (1 μg/ml) (Vector SA-5004) was added for 30 min. The proteins were visualized with Super signal West Pico chemiluminescence reagent (Thermo Scientific) on film (CL-XPosure Thermo Scientific), and densitometry analysis was performed using Image J V1.44 software. The relative intensity percentage was calculated as follows: the intensity absolute value of phosphorylated bands at every time post infection was divided by its corresponding β-actin band intensity.

2.6. Socs3 immunofluorescence

A cell suspension of eight TG was seeded in 24-well dishes with coverslips and infected with mock or HSV-1 (MOI of 1). 24 h later, cultures were fixed with precooled methanol for 10 minutes, then air dried for 2 min, and kept under −20 °C until further use. Coverslips were blocked, then probed with mouse monoclonal anti-glycoprotein B HSV-1 (1:100) (Abcam ab6506) for 16 h at 4 °C or with mouse monoclonal anti-Neurofilament (1:1000) (Sigma A-5316) in FBS 5% and PBS, for 1 h at room temperature. Then coverslips were incubated with rabbit polyclonal anti-Socs3 (1:100) (Abcam ab16030) in FBS 5% and PBS, for 16 h at 4 °C. Subsequently secondary antibodies were incubated anti-rabbit Alexa Fluor 594 conjugated (1:400) (Molecular Probes A11012) and biotinylated anti-mouse secondary antibody (1:200) (Vector BA-9200) for 30 min at room temperature, followed by incubation with Fluorescein-Streptavidin (Vector SA-5001) 2 μg/ml in PBS. Finally, monolayers were incubated with Hoescht (1:2000) (Sigma 861405) for 2 min. Coverslips were mounted in Vectashield (Vector H-1000) and kept at 4 °C in darkness. First, total cells and Socs3 positive cells were counted in HSV-1 and Mock infected conditions. In a different experiment, total amount of neurofilament positive and negative cells, and Socs3 positive cells were counted. Two independent experiments were performed by triplicate.

2.7. Statistical analysis

Data that were normally distributed were analyzed using ANOVA and Dunnet *post hoc* tests. Data that were non-normally distributed were analyzed using Mann–Whitney tests with SPSS V14.0 software. *p*-Values below 0.05 were considered significant.

2.8. Ethics

This research project was approved by the Universidad El Bosque Ethics' Committee. This research was performed under ethical regulations according to Colombian Health Ministry's Resolution No. 08430 of 1993. In addition, the research was performed taking into account the Universal declaration of animal rights from the International League of Animal Rights (Law 84 of 1989, Geneva, Switzerland) and according to ethical animal research principles from the International Council for Laboratory Animals.

3. Results

The TG cultures used in this paper were previously characterized by assessment of neurofilament positive and total amount of cells present per well (1 TG culture per well), after 96 h of incubation with AraC and 24 h later with CM only. It was established that were 11,000 cells per TG culture. In culture, TG neurons represented $14.7 \pm 2.8\%$ of the total cells, and non-neuronal cells constituted $85.3 \pm 2.7\%$ of the total cells. Thus, our culture system was neuron enriched (Fig. 1) and perhaps represents an improved culture model with which to study HSV-1 infection.

First, we assessed IFN-β activity against HSV-1. Viral infection for 28 h diminished cell viability to 45.4% (Fig. 2a), and the cytopathic effect was visible under phase contrast microscopy (data not shown). However, treatment with IFN-β 1000 U/ml completely rescued these cells, 107% survival, even in the presence of viral infection (Fig. 2a). HSV-1 replication in the TG cultures at 28 h.p.i. produced viral titer of 6.9×10^4 PFU/ml. Viral titers decreased in a dose-dependent manner in response to IFN-β treatment, the viral titers were 9.1×10^3 PFU/ml and 6.2×10^3 PFU/ml when the cultures were treated with IFN-β 100 and 1000 U/ml, respectively (Fig. 2b).

3.1. HSV-1 infection altered the expression of IFN-stimulated genes

Treatment with IFN-β 1000 U/ml up-regulated cellular *Pkr* expression by 4.3 folds in HSV-1-infected TG cultures. However, in untreated HSV-1-infected cultures, *Pkr* expression declined significantly, by 0.18-fold which is even lower than *Pkr* expression in untreated mock-infected cultures (Mann–Whitney $p < 0.05$)

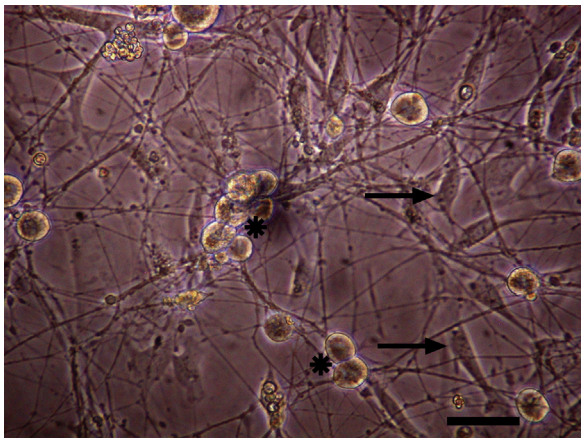

Fig. 1. Phase contrast micrographs of TG cultures after 96 h culture with AraC and 24 h culture without AraC. Note the round and refractive morphology of neurons (*) and the morphology of fibroblasts and Schwann cells (arrows). Scale bar represents 40 μM.

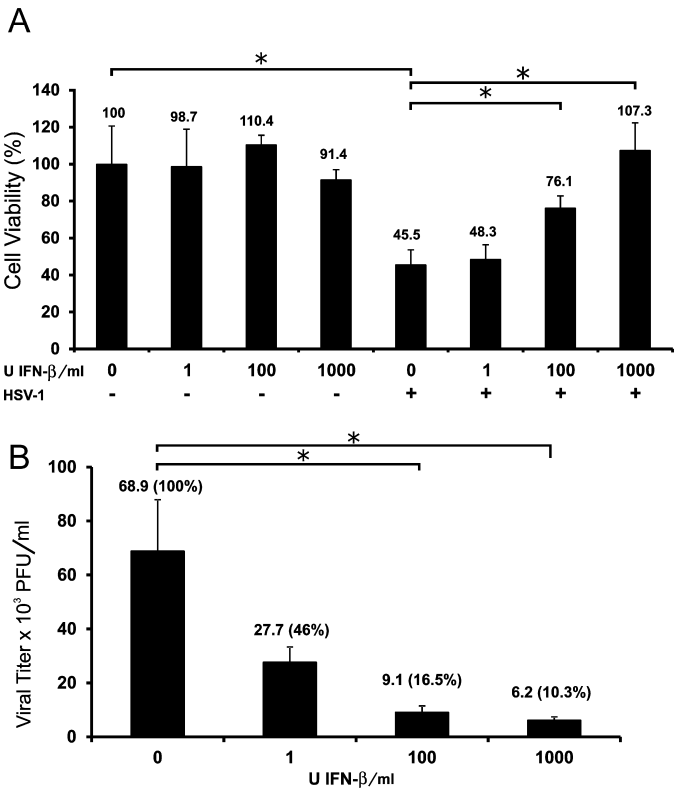

Fig. 2. IFN-β increased cell viability and decreased virus production after HSV-1 infection in TG cultures. (A) Cell viability percentage of mock or HSV-1 infected TG cultures for 28 h; after treatment with IFN-β 0, 1, 100 or 1000 U/ml. 100% cell viability was established according to $A_{570\,nm}$ of mock untreated. Bars represent mean ± standard error of the mean of 3 independent experiments performed my duplicate. (*) Represents $p < 0.05$ of Mann–Whitney test. (B) Viral titers obtained from supernatants at 28 h.p.i. in 6 h IFN-β treated and HSV-1 infected TG cultures. Bars represent mean ± standard error of the mean of 3 independent experiments. Numbers above bars represent viral titer PFU/ml multiplied by 10^3. Viral production percentage in parenthesis was calculated with the viral titers obtained from infected untreated cultures supernatants, which corresponds to 100% of viral production. (*) Represents $p < 0.05$ of ANOVA Dunnet *Post Hoc* test with titers from untreated supernatants as control condition.

(Fig. 3a). By contrast, infection of untreated cultures induced an 8.2-fold up-regulation of *Rnasel* mRNA, but this increase was counteracted during IFN-β treatment (Fig. 3b). Finally, in untreated cultures, HSV-1 infection up-regulated *Oas1a* expression 2.8-fold; and in IFN-β treated cultures, infection up-regulated *Oas1a* expression 10-fold (Fig. 3c).

3.2. HSV-1 infection induced Socs-3 mRNA and protein cell pattern change

The relative expression of *Socs3* in IFN-β-treated and HSV-1-infected cells was quantified. At 12 h.p.i. infected cultures exhibited 4-fold higher *Socs3* mRNA expression, whereas treatment of infected cultures with IFN-β decreased *Socs3* transcription to basal levels (Fig. 3d). Since, non-neuronal cells account for the majority of the cells in the culture, we assessed if this behavior was caused by these cells. In that way, we performed a parallel experiment with murine fibroblasts pre-treated or not with IFN for 6 h and then infected with HSV-1 for 12 h. In murine fibroblast cultures after HSV-1 infection Socs3 mRNA expression was lightly up-regulated (2.4-fold), while IFN decreased this expression by 1.1-fold (Fig. 3e).

Since Socs3 mRNA was increased after infection in TG cultures, to assess if Socs3 protein was also increased; immunofluorescence for Socs3 and viral antigen was performed 24 h.p.i. in mock and HSV-1 infected cultures, and Socs3 positive cells were counted (Fig. 4, panels A–J). The Socs3 cytoplasmic pattern (Fig. 4, solid arrow) was a homogeneous dotted protein pattern present continuously in the cytoplasm of mock (Fig. 4C, inset I) and some HSV infected cells (Fig. 4D, inset J); but no statistically significant difference was found in this pattern between virus (80.3%) and mock infected cultures (79.7%) (data not shown). The cluster pattern is a more intense cytoplasmic distribution observed in many cases with an intense spot or accumulated expression, in one side of the cytoplasm close to the nucleus (asterisk Fig. 4D, inset J).This cluster pattern was more frequently observed in infected cultures (12.7%) compared to mock infected (1.3%) (Mann–Whitney $p < 0.05$), mainly present in viral antigen positive cells (yellow in merged pictures Fig. 4B).

Further, we assessed if the increased Socs3 expression observed in infected cultures, was due to the presence of neuronal or non-neuronal cells. In consequence, Socs3 and Neurofilament immunofluorescence and cell counting were performed in virus and mock infected cultures (Fig. 4, panels L–U).

In virus and mock infected cultures a significant higher percentage of Socs3 positive neurons was found (Fig. 4, panel V). In addition, a significant higher percentage of neurons with Socs3 cluster pattern, compared to non-neuronal cells, was found in infected cultures (Fig. 4V). In mock infected cultures this cluster pattern disappeared in both neuron and non-neuronal cells (present only in 3.3% of neurons and non-neuronal cells), which means that this cluster mainly exists in infected cultures and neurons.

These results suggest for the first time, that HSV-1 infection of TG cultures increased the percentage of Socs3 positive neuronal and non-neuronal cells, being sensory neurons the most frequent cell type in which this expression occurred. In addition, virus infection also changed the Socs3 protein distribution pattern, mainly in neurons (Fig. 4V).

3.3. Viral gene transcription is impaired by IFN-β treatment

In this *in vitro* model, all three types of viral lytic genes were expressed at 6 and 12 h.p.i., but IFN-β treatment induced transcriptional inhibition of the immediate early (*Icp0*), early (*Tk*) and late

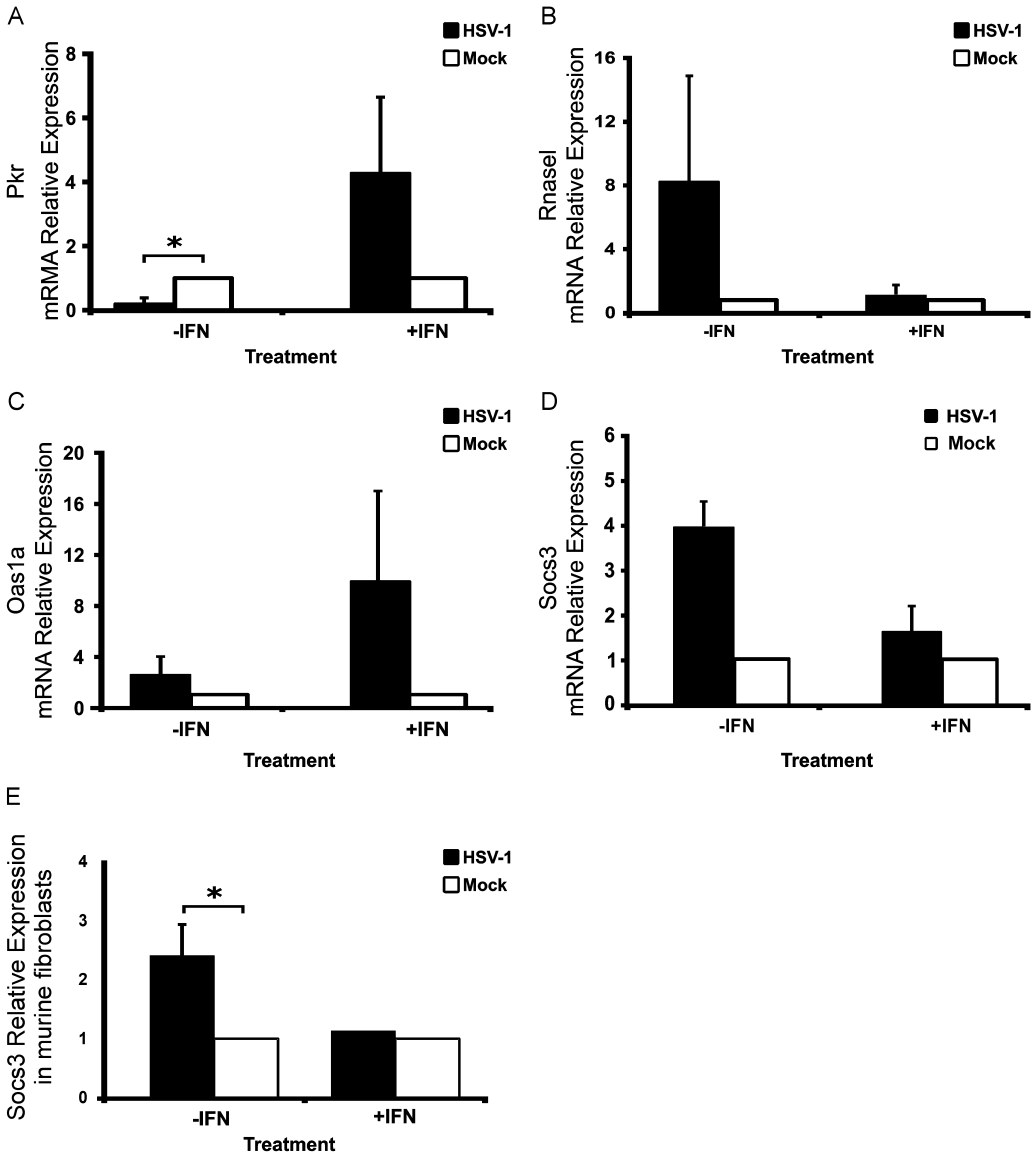

Fig. 3. HSV-1 infection induced *Rnasel* and o*as1a* expression and decreased *Pkr* constitutive expression. In addition, HSV-1 induced *socs3* expression and IFN-β treatment decreased that expression. (A) *Pkr* relative expression. (B) *Rnasel* relative expression. (C) *Oas1a* relative expression. (D) *Socs3* relative expression. In TG cultures treated for 6 h with IFN-β 1000 U/ml and infected for 12 h with HSV-1. (E) *Socs3* relative expression. In murine fibroblasts cultures treated for 6 h with IFN-β 1000 U/ml and infected for 12 h with HSV-1. Relative expression was calculated comparing virus infected against mock infected cultures which have a relative expression of 1. Relative expression was normalized against β-actin expression. Bars represent the mean ± standard error of the mean for 2 independent experiments by duplicate. (*) Represents $p < 0.05$ of Mann–Whitney test.

(*gB*) viral genes (Fig. 5).These data demonstrate an efficient antiviral response to IFN-β in these cultures.

3.4. Viral replication of HSV-1 partially inhibits the IFN-β Jak-Stat signaling pathway

IFN-β treatment of mock-infected TG cultures induced Jak-1 and Stat-1 phosphorylation. It was evidenced that in HSV-1-infected and IFN-β-treated cultures there was a reduction in phosphorylation signal of Jak-1 and Stat-1 compared to the mock-infected treated cultures (Fig. 6). This finding suggests a putative Herpes virus evasion mechanism against the IFN-β antiviral activity.

4. Discussion

The culture model described in this work, has a higher percentage of sensory neurons than previously reported cultures (Carr et al., 2003). This could represent a better *in vitro* approximation of what may occur in TG cells during HSV-1 productive infection. However, is important to notice that in *in vitro* circumstances, the entire neuronal cell body and neurites are available for infection, which contrasts with the *in vivo* infection, where virions must enter the sensory nerve endings and travel along the axons to reach the cell body. In addition, the fact that fibroblasts and Schwann cells are the vast majority of cells in our model, means that they could be responsible for the most part of the observed response. However, since all types of cells get infected and neurons are the most infected type (72.9% of neurons and 48.3% of non-neuronal cells were positive for viral antigen) (Low-Calle et al., unpublished results), we can expect that most of the response is carried out by neuronal cells. It has been reported previously in immunocompetent mice models that a ganglia acute infection occurs after HSV-1 inoculation, expressing all classes of viral gene transcripts, viral antigen in both neuronal and non-neuronal cells and infectious virus production in

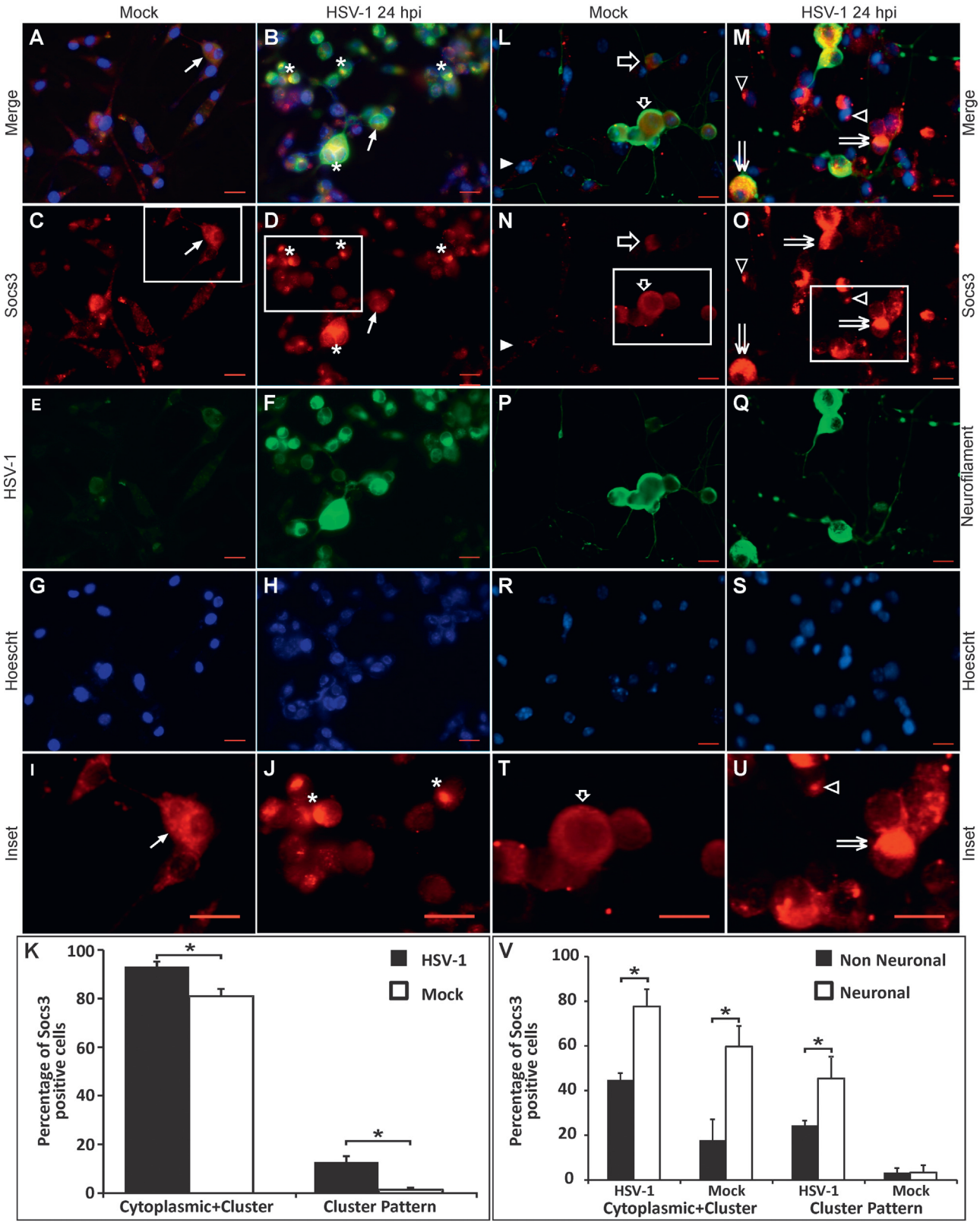

Fig. 4. Changes in Socs3 protein expression pattern after 24 h of HSV-1 infection in neuronal and non-neuronal cells. Panels A–J. HSV-1 induced Socs3 protein expression and changed expression pattern in some infected cells. Merge staining in (A) mock infected cells (B) HSV-1 infected cells. Socs3 protein expression pattern in (C) mock infected cells and (D) HSV-1 infected cells. (E) HSV gB negative cells (mock infected) and (F) HSV-1 gB positive cells. Nucleus Hoescht stained in (G) mock infected cells (H) HSV-1 infected cells. Inset magnification of Socs-3 positive cells in (I) mock infected cultures (J) HSV infected cultures. (Thin arrow) shows positive cells for Socs3 cytoplasmic pattern. (*) Shows Socs3 cluster pattern positive cells. (K) Plot of Socs3 positive cells percentage for cytoplasmic and cluster patterns and cluster pattern only. Bars represent the mean ± standard error of the mean for 2 independent experiments (*) Represents $p < 0.05$ of Mann–Whitney test. Scale Bar represents 20 μm. Panels L–U, HSV-1 induced Socs3 protein expression and changed the expression pattern, in neuronal cells and some non-neuronal cells. Merge staining in (L) mock and in (M) HSV-1 infected cells. Socs3 protein expression pattern in (N) mock and in (O) HSV-1 infected cells. (P) Neurofilament positive mock infected cells and (Q) neurofilament positive HSV-1 infected cells. Nucleus stained with Hoescht in (R) mock and (S) HSV-1 infected cells. Inset magnification of Socs-3 positive cells in (T) mock infected cultures and in (U) HSV infected

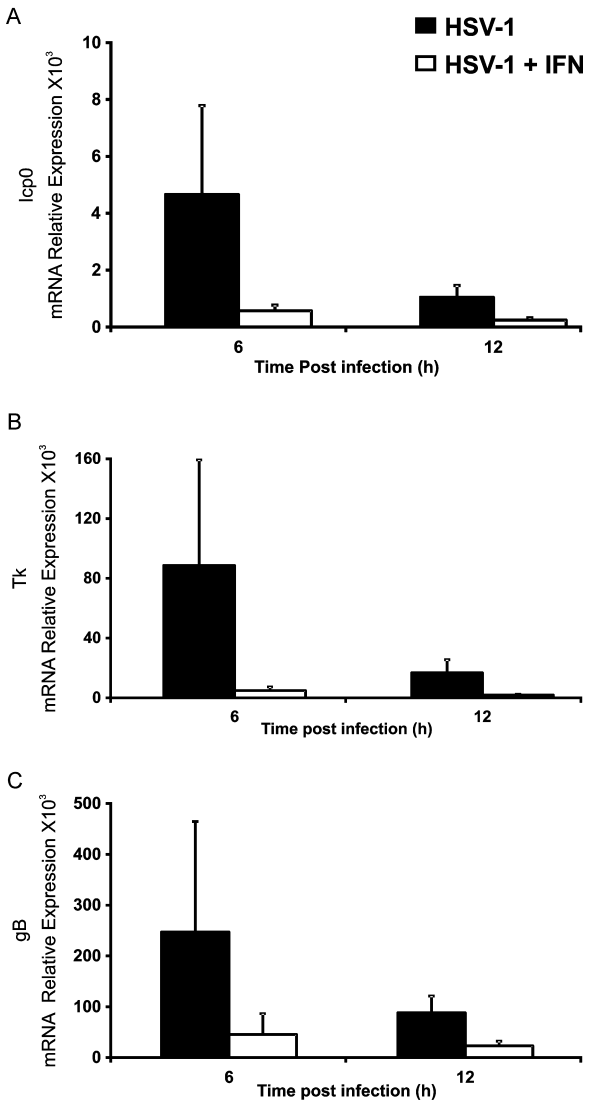

Fig. 5. IFN-β treatment decreased the expression of the 3 groups of viral genes IE, E and L. Icp0, Tk and gB. Viral genes relative expression was quantified in TG cultures treated for 6 h with IFN-β 1000 U/ml and then infected for 6 h and 12 h. Relative expression in virus infected cultures was obtained with β-actin as normalizer, and was compared to mock infected cultures which relative expression is 0 (cycle threshold = 40). (A) *Icp0* relative expression. (B) *Tk* relative expression. (C) *gB* relative expression. Bars represent the mean ± standard error of the mean for 2 independent experiments by duplicate.

the sensory ganglia, and this acute stage lasts only for 3–7 days (Liu et al., 1996; Kramer et al., 1998; Preston, 2000). On the other hand, latent infection of sensory neurons persists after virus clearance and it is established by 3 weeks post infection, in which infectious virus and viral antigen cannot be detected, and viral transcription is limited (Liu et al., 1996). In this way, our findings could be analogous to an acute infection stage, and the presence of viral antigens in non-neuronal cells could mean that the role of non-neuronal cells in trigeminal ganglia during HSV-1 acute infections might be important and needs further study.

Consequently, we believe our findings are valuable, even though the vast majority of cells are non-neuronal type, because for the first time we propose the existence of putative viral evasion mechanisms in primary cells of TG origin in the context of a productive HSV-1 infection. There was no previous information regarding if these evasion mechanisms could appear in TG cells, since most HSV-1 and IFN proposed evasion mechanisms have been described in cell lines (Yokota et al., 2004, 2005; Johnson et al., 2008; Frey et al., 2009; Johnson and Knipe, 2010). Besides, in our model, a further description of the IFN-β induced response was made compared to previous studies.

4.1. IFN-β exerted an antiviral effect against HSV-1 in TG cultures

IFN-β can increase cell survival and impair viral production in neuron-enriched TG cultures. This antiviral role of IFN against HSV has been previously reported *in vitro* in TG cultures treated with IFN-β (Carr et al., 2003), in rat dorsal root ganglia cultures treated with IFN-α/β (Svennerholm et al., 1989) and after *in vivo* corneal transduction of IFN-expressing adenovirus (Cui and Carr, 2000; Al-Khatib et al., 2004, 2005; Austin et al., 2005). In addition, HSV-1 replication is more efficient in IFN-I receptor-deficient mice (Leib et al., 1999) and in anti-IFN-α/β antibody treated mice (Halford et al., 1997), indicating an IFN important role in controlling acute HSV-1 infections.

4.2. IFN-β treatment decreased HSV-1 viral gene expression

In our culture model, a lytic viral cycle occurs, with the transcription of IE, E and L genes, which is similar to previous findings by Bertke et al. (2011) where most neuronal cells populations in TG cultures, support productive HSV infections

On the other hand, Carr et al. (2003) previously showed that the transcription of these IE, E and L viral genes was decreased by IFN-β treatment, and IFN-β was the only type-I IFN capable of inhibiting every stage of viral gene expression in TG cultures. Moreover, L929 murine fibroblasts cells decreased viral products transcription and translation when they were transfected with IFN-β (Al-Khatib et al., 2002; Härle et al., 2002). IFN-α treated swine TG cultures showed a reduced number of infected cells (De Regge et al., 2010), which is similar to one of our previous findings (data not shown). The decreased viral gene transcription observed in our culture model led to lower viral protein expression (Low-Calle et al., unpublished results) and viral production, as well as to an improvement in TG cell survival.

4.3. ISGs expression in HSV-1 infected TG cultures

The results reported here suggest that the antiviral effect of IFN-β occurs *via* the up-regulation of ISGs such as *Pkr* and *Oas1a*. It has also been shown in both *in vivo* and *in vitro* models that IFN-β treatment increases *Oas* transcription (Al-Khatib et al., 2003, 2004; Carr et al., 2003). In contrast, the absence of *Oas* or *Pkr* abolishes the IFN-β antiviral effect, leading to increased susceptibility to HSV-1 infection in mice (Al-Khatib et al., 2004; Austin et al., 2005). Consequently, in TG cultures the IFN-β effect is mediated by Oas and its down-stream effector RNAse L.

RNase L is constitutively expressed in most mammalian tissues (Bisbal and Silverman, 2007), and it has been shown that *in vitro* IFN-β stimulation induces a slight up-regulation of *Rnasel* expression (Rusch et al., 2000). Despite the lack of *Rnasel* up-regulation in our IFN treated cultures; we found reduced levels of viral

cultures. Thick empty arrow, shows neuronal cells with cytoplasmic pattern, Double arrow shows neuronal cells with cluster pattern. Solid arrowhead, shows non-neuronal cells with cytoplasmic pattern and Empty arrowhead, shows non-neuronal cells with cluster pattern. (V) Plot of Socs3 positive cells per cell type. Shows the percentage of neuronal and non-neuronal cells with cytoplasmic and cluster patterns, and with cluster pattern only. Bars represent the mean ± standard error of the mean for 2 independent experiments (*) Represents $p < 0.05$ of Mann–Whitney test.

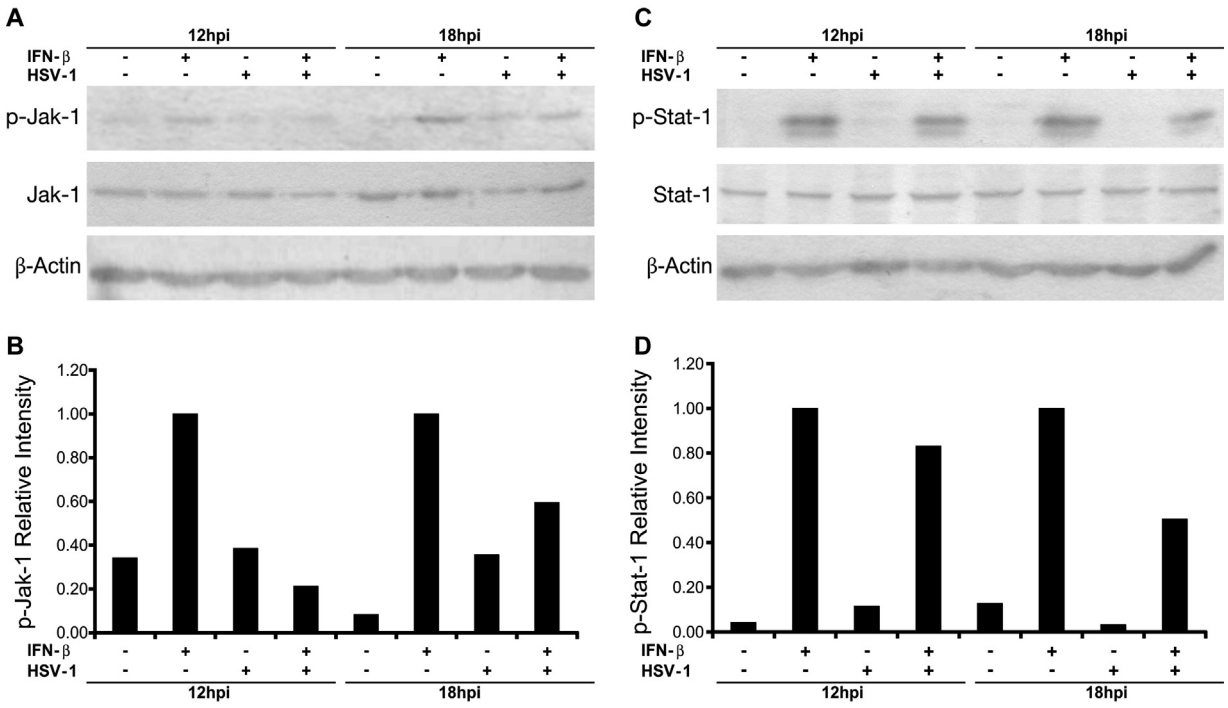

Fig. 6. HSV-1 infection partially impaired Jak-Stat signal transduction activation. TG primary cultures were infected for 12 h and 18 h and then IFN-β treated for 15 min. (A) Western blot for phospho-Jak-1 (p-Jak-1), Jak-1 and β-actin. (B) Densitometry analysis of p-Jak-1, bars represent p-Jak-1 relative intensity normalized against β-actin band intensity. (C) Western blot for p-Stat-1 (2 isoforms 91 and 84 kDa), Stat-1 and β-actin. (D) Densitometry analysis for p-Stat-1, bars represent p-Stat-1 relative intensity normalized against β-actin band intensity. Relative phosphorylation intensity was calculated comparing virus infected – IFN treated bands against mock infected – IFN treated phosphorylation bands. Mock infected – IFN treated represent a phosphorylation intensity of 1. Graphics show a representative experiment, but 2 independent experiments were performed with similar results.

transcripts. One explanation for this finding is that the constitutive levels of *Rnasel* are sufficient to set up the observed antiviral response. It has been shown that HSV-1 acute infection induces IFN-α and -β expression in the TG *in vivo* (Peng et al., 2005), then we can speculate that the increased transcription of *Oas* and *Rnasel* in untreated cells observed here, was due to IFN-I production triggered by HSV infection. An increased transcription of endogenous IFN-β was observed at 6 h.p.i. in infected TG cultures (data not shown).

Unexpectedly, HSV-1 infection caused a significant decrease in *Pkr* transcription (Fig. 3a). This observation suggests that the virus-induced impairment of the antiviral response in the TG cells is similar to what has been found in HSV-1 infected L929 fibroblasts (Al-Khatib et al., 2002). This possible evasion mechanism could contribute to improve viral replication not only by inhibiting the effect of PKR on cell protein synthesis but also by inhibiting PKR-mediated apoptosis, since this protein has been associated with apoptosis induction (Balachandran et al., 1998; Gil et al., 1999).

4.4. Viral Infection induced Socs3 expression

HSV-1-infected TG cultures up-regulated *Socs3* transcription, but IFN-β treatment inhibited this up-regulation. This finding suggests that in this culture, HSV-1 could establish an immune evasion mechanism exerted by late *Socs3* induction. This Socs-mediated mechanism of immune evasion has been reported previously during HSV-1 early infection in FL amniotic cell line, where the IFN-induced antiviral cell response was impaired (Yokota et al., 2004, 2005). In the same way, suppression of the IFN response by Socs1 up-regulation at later stages of HSV-1 infection has also been observed in a keratinocyte cell line (Frey et al., 2009).

In addition, we observed that HSV-1 infection increased Socs3 protein expressing cell percentage and changed its distribution pattern, mainly in virus infected cultures (cluster pattern, Fig. 4, panels D and J). We propose that this could be a novel viral evasion mechanism induced in TG cells. Since, non-neuronal cells account for the majority of the cells in the culture, we wanted to evaluate if Socs3 is up-regulated after viral infection in murine fibroblasts alone. Results in murine fibroblasts showed that HSV-1 infection also increased *Socs3* transcription, while IFN treatment decreased it, similar to what we found in TG cultures. However, Socs3 up-regulation in TG was almost twice higher (4-fold) compared to murine fibroblasts alone (2.4-fold). This difference could be due to the TG sensory neurons; because they were the cell type most frequently observed withSocs3 positive and with the "activated" cluster pattern (Fig. 4, panels L–V). In addition, they were the most frequent infected cell (72.9% of neurons were positive for viral antigen, data not shown).

The impaired Socs3 mRNA up-regulation in IFN-β treated viral infected TG cultures, might be a consequence of the reduced viral gene expression and replication observed after IFN treatment (Figs. 2b and 5), this same behavior was evidenced in murine fibroblast cultures after 6 h IFN pre-treatment and 12 h of viral infection (Fig. 3E).

This Socs3 increased expression, could represent a novel viral evasion mechanism where Socs3 protein expression is highly increased after viral infection at late times post infection (24 h) in TG cells. This means that the increased Socs3 expression (mRNA and protein) observed in infected cells negatively regulates the IFN-β Jak-Stat signal transduction, preventing its adequate phosphorylation (also observed in our cultures Fig. 5), and impairing the IFN-I induced innate response; which could help the establishment of more productive acute viral infections in TG cells. The Socs3 increased expression was consistent with the observation of a slight but important reduction in Jak and Stat phosphorylation in infected cultures; which we described here for the first time in HSV-1-infected TG cultures. This reduction could be a mechanism that the HSV-1 employs for immune evasion in the trigeminal ganglion.

This type of inhibition and evasion mechanism has been described previously in epithelial and FL infected cells using MOIs of 5 and 20 (Yokota et al., 2001, 2004; Johnson et al., 2008; Johnson and Knipe, 2010); our neuron enriched TG culture model used an MOI of 1. The Jak and Stat phosphorylation reduction that we observed coincided with the up-regulation of *Socs3*, and had a tendency to increase at later stages post-infection, which could be related to the intense Socs3 cluster pattern of expression observed at late 24 h.p.i.

The *Socs3* up-regulation causing an impaired Jak-Stat signaling pathway activation has been previously demonstrated in FL cell line (Yokota et al., 2001, 2004). Since theSocs3 protein is rapidly induced (between 1 and 2 h after IFN stimulation) (Zimmerer et al., 2007) and degraded after signaling (Croker et al., 2008), it is not likely that the Socs3 expression observed, could be the consequence of endogenous IFN-β expression observed only at 6 h.p.i. in infected TG cultures. Given that, it has been reported that HSV-1 infection or reactivation induced both IFN-α and IFN-β production in trigeminal ganglia (Tal-Singer et al., 1998; Peng et al., 2005), the Socs3 induction during HSV-1 productive infections in TG could improve viral replication and dissemination, facilitating both acute infections and recurrences.

5. Conclusions

From the present study, we conclude that HSV-1 replicates *via* a lytic cycle in this TG culture, producing virions, expressing lytic genes and decreasing cell survival. In addition, IFN-β treatment had an important anti-Herpes effect: it protected trigeminal neurons from viral induced death and reduced viral production. Additionally, we demonstrated that HSV-1 has two putative evasion mechanisms that have not been previously reported in TG cells: 1. a reduction in the constitutive expression of *Pkr*, and 2. the increased Suppressor of Cytokine Signaling-3 expression, mainly evidenced in trigeminal neurons, which causes a decreased Jak and Stat protein phosphorylation after IFN treatment. These two strategies could partially impair the antiviral activity of IFN-β produced in TG during acute or even recurrent infections, thus increasing the likelihood of viral infection, replication and transmission.

Acknowledgements

Ana Maria Low-Calle had a Colciencias Young Researcher Fellowship for two consecutive years. División de Investigaciones of Universidad El Bosque, and Facultad de Odontología, Universidad Nacional de Colombia funded this project.

References

Al-Khatib, K., Williams, B.R., Silverman, R.H., Halford, W.P., Carr, D.J., 2002. Absence of PKR attenuates the anti-HSV-1 activity of an adenoviral vector expressing murine IFN-beta. J. Interferon Cytokine Res. 22, 861–871.

Al-Khatib, K., Williams, B.R., Silverman, R.H., Halford, W., Carr, D.J., 2003. The murine double-stranded RNA-dependent protein kinase PKR and the murine 2′,5′-oligoadenylate synthetase-dependent RNase L are required for IFN-beta-mediated resistance against herpes simplex virus type 1 in primary trigeminal ganglion culture. Virology 313, 126–135.

Al-Khatib, K., Williams, B.R., Silverman, R.H., Halford, W., Carr, D.J., 2004. Distinctive roles for 2′,5′-oligoadenylate synthetases and double-stranded RNA-dependent protein kinase R in the in vivo antiviral effect of an adenoviral vector expressing murine IFN-beta. J. Immunol. 172, 5638–5647.

Al-Khatib, K., Williams, B.R., Silverman, R.H., Halford, W., Carr, D.J., 2005. Dichotomy between survival and lytic gene expression in RNase L- and PKR-deficient mice transduced with an adenoviral vector expressing murine IFN-beta following ocular HSV-1 infection. Exp. Eye Res. 80, 167–173.

Austin, B.A., James, C., Silverman, R.H., Carr, D.J., 2005. Critical role for the oligoadenylate synthetase/RNase L pathway in response to IFN-beta during acute ocular herpes simplex virus type 1 infection. J. Immunol. 175, 1100–1106.

Balachandran, S., Kim, C.N., Yeh, W.C., Mak, T.W., Bhalla, K., Barber, G.N., 1998. Activation of the dsRNA-dependent protein kinase, PKR, induces apoptosis through FADD-mediated death signaling. EMBO J. 17, 6888–6902.

Bertke, A.S., Swanson, S.M., Chen, J., Imai, Y., Kinchington, P.R., Margolis, T.P., 2011. A5-Positive primary sensory neurons are nonpermissive for productive infection with Herpes Simplex Virus 1 *in vitro*. J. Virol. 85, 6669–6677.

Bisbal, C., Silverman, R.H., 2007. Diverse functions of RNase L and implications in pathology. Biochimie 89, 789–798.

Bonjardim, C.A., Ferreira, P.C., Kroon, E.G., 2009. Interferons: signaling, antiviral and viral evasion. Immunol. Lett. 122, 1–11.

Carr, D.J., Al-khatib, K., James, C.M., Silverman, R., 2003. Interferon-beta suppresses herpes simplex virus type 1 replication in trigeminal ganglion cells through an RNase L-dependent pathway. J. Neuroimmunol. 141, 40–46.

Castellanos, J., Hurtado, H., 1999. Viral infection studied in adult sensory neurons. In: Haynes, L. (Ed.), The Neuron in Tissue Culture, International Brain Research Organization (IBRO) Handbook Series: Methods in Neuroscience, vol. 18. John Wiley & Sons, Chichester, UK, pp. 289–293.

Chee, A.V., Roizman, B., 2004. Herpes simplex virus 1 gene products occlude the interferon signaling pathway at multiple sites. J. Virol. 78, 4185–4196.

Croker, B.A., Kiu, H., Nicholson, S.E., 2008. Socs regulation of the JAK/STAT signalling pathway. Semin. Cell Dev. Biol. 19, 414–422.

Cui, B., Carr, D.J., 2000. A plasmid construct encoding murine interferon beta antagonizes the replication of herpes simplex virus type I in vitro and in vivo. J. Neuroimmunol. 108, 92–102.

De Regge, N., Van Opdenbosch, N., Nauwynck, H.J., Efstathiou, S., Favoreel, H.W., 2010. Interferon alpha induces establishment of alphaherpesvirus latency in sensory neurons in vitro. PLoS One 5, e13076.

Efstathiou, S., Preston, C.M., 2005. Towards an understanding of the molecular basis of herpes simplex virus latency. Virus Res. 111, 108–119.

Frey, K.G., Ahmed, C.M., Dabelic, R., Jager, L.D., Noon-Song, E.N., Haider, S.M., Johnson, H.M., Bigley, N.J., 2009. HSV-1-induced Socs-1 expression in keratinocytes: use of a Socs-1 antagonist to block a novel mechanism of viral immune evasion. J. Immunol. 183, 1253–1262.

Gil, J.J., Alcami, J., Esteban, M., 1999. Induction of apoptosis by double stranded-RNA-dependent protein kinase (PKR) involves the alpha subunit of eukaryotic translation initiation factor 2 and NF-κB. Mol. Cell. Biol. 19, 4653–4663.

Halford, W.P., Veress, L.A., Gebhardt, B.M., Carr, D.J.J., 1997. Innate and acquired immunity to herpes simplex virus type 1. Virology 236, 328–337.

Härle, P., Cull, V., Agbaga, M.P., Silverman, R., Williams, B.R., James, C., Carr, D.J., 2002. Differential effect of murine alpha/beta interferon transgenes on antagonization of herpes simplex virus type 1 replication. J. Virol. 76, 6558–6567.

Honess, R.W., Roizman, B., 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. 14, 8–19.

Johnson, K.E., Knipe, D.M., 2010. Herpes simplex virus-1 infection causes the secretion of a type I interferon-antagonizing protein and inhibits signaling at or before Jak-1 activation. Virology 396, 21–29.

Johnson, K., Song, B., Knipe, D.M., 2008. Role for herpes simplex virus 1 ICP27 in the inhibition of type I interferon signaling. Virology 374, 487–494.

Kramer, M.F., Chen, S.H., Knipe, D.M., Coen, D.M., 1998. Accumulation of viral transcripts and DNA during establishment of latency by herpes simplex virus. J. Virol. 72, 1177–1185.

Leib, D.A., Harrison, T.E., Laslo, K.M., Machalek, M.A., Moorman, N.J., Virgin, H.W., 1999. Interferons regulate the phenotype of wild-type and mutant herpes simplex viruses in vivo. J. Exp. Med. 189, 663–672.

Liu, T., Tang, Q., Hendricks, R.L., 1996. Inflammatory infiltration of the trigeminal ganglion after herpes simplex virus type 1 corneal infection. J. Virol. 70, 264–271.

Lynas, C., Laycock, K.A., Cook, S.D., Hill, T.J., Blyth, W.A., Maitland, N.J., 1989. Detection of herpes simplex virus type 1 gene expression in latently and productively infected mouse ganglia using the polymerase chain reaction. J. Gen. Virol. 70, 2345–2355.

Marin, L.M., Prada-Arismendy, J., Bohorquez, S.P., Castellanos, J.E., 2007. Changes in oligoadenylate synthetase, protein kinase R and RNaseL mRNA in trigeminal ganglia cultures infected by herpes simplex. Braz. Oral Res. 21 (Suppl. 1), 348.

Peng, W., Henderson, G., Inman, M., BenMohamed, L., Perng, G.C., Wechsler, S.L., Jones, C., 2005. The locus encompassing the latency-associated transcript of herpes simplex virus type 1 interferes with and delays interferon expression in productively infected neuroblastoma cells and trigeminal ganglia of acutely infected mice. J. Virol. 79, 6162–6171.

Perng, G.C., Jones, C., 2010. Towards an understanding of the herpes simplex virus type 1 latency-reactivation cycle. Interdiscip. Perspect. Infect. Dis. 2010, 262415.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.

Platanias, L.C., 2005. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat. Rev. Immunol. 5, 375–386.

Preston, C.M., 2000. Repression of viral transcription during herpes simplex virus latency. J. Gen. Virol. 81 (Pt 1), 1–19.

Roizman, B., Knipe, D.M., Whitley, R.J., 2007. Herpes simplex viruses. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology. , fifth ed. Lippincott, Williams, and Wilkins, Philadelphia, pp. 2501–2602.

Rusch, L., Zhou, A., Silverman, R.H., 2000. Caspase-dependent apoptosis by 2′,5′-oligoadenylate activation of RNase L is enhanced by IFN-beta. J. Interferon Cytokine Res. 20, 1091–1100.

Schefe, J.H., Lehmann, K.E., Buschmann, I.R., Unger, T., Funke-Kaiser, H., 2006. Quantitative real-time RT-PCR data analysis: current concepts and the novel gene expression's CT difference formula. J. Mol. Med. 84, 901–910.

Sokawa, Y., Ando, T., Ishihara, Y., 1980. Induction of 2′,5′-oligoadenylate synthetase and interferon in mouse trigeminal ganglia infected with herpes simplex virus. Infect. Immun. 28, 719–723.

Song, M.M., Shuai, K., 1998. The suppressor of cytokine signaling (Socs) 1 and Socs3 but not Socs2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. J. Biol. Chem. 273, 35056–35062.
Steiner, I., Kennedy, P., Pachner, A.R., 2007. The neurotropic herpes viruses: herpes simplex and varicella-zoster. Lancet Neurol. 6, 1015–1028.
Svennerholm, B., Ziegler, R., Lycke, E., 1989. Herpes simplex virus infection of the rat sensory neuron. Effects of interferon on cultured cells. Arch. Virol. 104, 153–156.
Tal-Singer, R., Podrzucki, W., Lasner, T.M., Skokotas, A., Leary, J.J., Fraser, N.W., Berger, S.L., 1998. Use of differential display reverse transcription-PCR to reveal cellular changes during stimuli that result in herpes simplex virus type 1 reactivation from latency: upregulation of immediate-early cellular response genes TIS7, interferon, and interferon regulatory factor-1. J. Virol. 72, 1252–12561.
Weir, J.P., 2001. Regulation of herpes simplex virus gene expression. Gene 271, 117–130.
Yokota, S., Yokosawa, N., Kubota, T., Suzutani, T., Yoshida, I., Miura, S., Jimbow, K., Fujii, N., 2001. Herpes simplex virus type 1 suppresses the interferon signaling pathway by inhibiting phosphorylation of STATs and janus kinases during an early infection stage. Virology 286, 119–124.
Yokota, S., Yokosawa, N., Okabayashi, T., Suzutani, T., Miura, S., Jimbow, K., Fujii, N., 2004. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 contributes to inhibition of the interferon signaling pathway. J. Virol. 78, 6282–6286.
Yokota, S., Yokosawa, N., Okabayashi, T., Suzutani, T., Fujii, N., 2005. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 confers efficient viral replication. Virology 338, 173–181.
Yoshimura, A., Naka, T., Kubo, M., 2007. Socs proteins, cytokine signalling and immune regulation. Nat. Rev. Immunol. 7, 454–465.
Zimmerer, J.M., Lesinski, G.B., Kondadasula, S.V., Karpa, V.I., Lehman, A., Raychaudhury, A., Becknell, B., Carson 3rd., W.E., 2007. IFN-alpha-induced signal transduction, gene expression, and antitumor activity of immune effector cells are negatively regulated by suppressor of cytokine signaling proteins. J. Immunol. 178, 4832–4845.