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Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 confers efficient viral replication

Shin-ichi Yokota[a], Noriko Yokosawa[a], Tamaki Okabayashi[a], Tatsuo Suzutani[b], Nobuhiro Fujii[a],*

[a]Department of Microbiology, Sapporo Medical University School of Medicine, South-1, West-17, Chuo-ku, Sapporo, 060-8556 Hokkaido, Japan
[b]Department of Microbiology, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan

Abstract

We showed previously that infection of herpes simplex virus type 1 (HSV-1) rapidly induced the suppressor of cytokine signaling-3 (SOCS3), a host negative regulator of the JAK/STAT pathway, in the amnion cell line FL. Thus, HSV-1 suppresses the interferon (IFN) signaling pathway at the step of IFN-induced phosphorylation of janus kinases during an early infection stage. In the present study, we examined SOCS3 induction by HSV-1 infection in several types of human cell lines. FL cells and the T-cell line CCRF-CEM strongly induced SOCS3 during HSV-1 infection. The virus rapidly propagated in both cell lines and produced a lytic infection. On the other hand, the monocytic cell lines U937 and THP-1, and the B-cell line AKATA showed neither SOCS3 induction nor suppression of IFN-induced STAT1 phosphorylation during HSV-1 infection. These cell lines resulted in a persistent or prolonged infection, which continuously produced a low titer of infectious virus. The induction of SOCS3 by HSV-1 should occur via STAT3 activation immediately after HSV-1 infection. SOCS3 induction was inhibited by the addition of a Jak3 inhibitor WHI-P131. Treatment with WHI-P131 or transfection of antisense oligonucleotides specific for SOCS3 dramatically suppressed replication of HSV-1 in FL cells. The suppression of viral replication by WHI-P131 was released in the presence of neutralizing anti-IFN-α and anti-IFN-β antibodies. In conclusion, suppression of IFN signaling by HSV-1-induced SOCS3 is required for efficient replication and lytic infection of HSV-1. The SOCS3 induction varied among cell lines, indicating that it is an important factor determining the cell type specificity of efficient HSV-1 replication.
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Introduction

Cells have various defense mechanisms that protect them from viral infection. Interferon (IFN) is induced by viral infection and plays an important role in defending the host cell from viral attack. When IFN binds to specific cell surface receptors on the host cells, it promotes the antiviral state through induction or activation of the 2′,5′-oligoadenylate synthetase (2-5AS)/RNase L system, the double-stranded RNA-activated protein kinase (PKR), and the MxA protein (Fujii, 1994; Samuel, 2001; Sen and Ransohoff, 1993). Signal transduction by IFN is mediated through the JAK/STAT pathway which consists of janus kinases (JAK),

protein tyrosine kinases that interact with the intracellular domains of various receptors, and the STAT family proteins, transcription factors that are activated through phosphorylation by JAK. The JAK/STAT pathway also transduces various cytokine signals. There are four JAK proteins (Jak1, Jak2, Jak3, and Tyk2) and seven STAT proteins (STAT1 to 4, STAT5a, STAT5b, and STAT6) (Aaronson and Horvath, 2002; Darnell et al., 1994; Leonard and O'Shea, 1998; O'Shea et al., 2002). Each cytokine employs a particular combination of the JAK and STAT proteins, which in turn determines the specificity of the cytokine responses. For instance, Jak1 and Tyk2 are associated with the IFN-α/β receptor complex. These JAK proteins are activated by phosphorylation after IFN-α/β binds to its receptor, and they phosphorylate STAT1 and STAT2. Phosphorylated STAT1 and phosphorylated STAT2 can bind to IRF-9/p48/ISGF3γ,

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* Corresponding author. Fax: +81 11 612 5861.
  E-mail address: fujii@sapmed.ac.jp (N. Fujii).

to form the transcription factor ISGF3, which then translocates into the nucleus and binds to IFN-stimulated response elements in the promoters of IFN-inducible genes (Darnell et al., 1994; Goodbourn et al., 2000; Samuel, 2001).

According to recent reports, herpes simplex virus type 1 (HSV-1) suppresses the IFN signaling pathway at multiple sites in order to evade host defense mechanisms. We reported that IFN-induced JAK phosphorylation was inhibited in FL cells during the early infection stages of HSV-1 (Yokota et al., 2001) and suppressor of cytokine signaling-3 (SOCS3), which is a host negative regulator of the JAK/STAT pathway, was rapidly induced under these conditions (Yokota et al., 2004b). The UL41 gene product (virion host shutoff protein) contributes to IFN resistance (Suzutani et al., 2000). Chee and Roizman (2004) reported that HSV-1-blocked-IFN signaling in part by reduced levels of Jak1 and STAT2 proteins through the action of the UL41 gene product. In addition, HSV-1 suppresses IFN production. Melroe et al. (2004) reported that HSV-1 inhibited the production of IFN-β, which is partly mediated by degradation of IFN regulatory factor 3 (IRF-3). The immediate early protein ICP0 contributes to this. Lin et al. (2004) reported that ICP0 inhibited IRF-3- and IRF-7-mediated gene activation, including IFN-β production, without degradation of proteins, such as IRF-3, CBP, and TBK-1. They also showed that hyperphosphorylation of IRF-3 did not occur during HSV-1 infection. In contrast, our previous report showed that phosphorylation of IRF-3 and induction of IFN-β mRNA occurred during HSV-1 infection (Yokota et al., 2004b). Mogensen et al. (2004) reported that production of proinflammatory cytokines, including IFN-α/β, was suppressed by HSV-1, and the suppression was caused by participation of the immediate early gene products, ICP4 and ICP27. Suppression of IFN-induced effectors by HSV-1 has been also reported. Phosphorylated eIF-2α, which shuts off protein synthesis, is dephosphorylated by cooperation of viral $\gamma_1 34.5$ protein and host protein phosphatase 1α (He et al., 1997). So, the IFN-induced protein synthesis shutoff is released by HSV-1. In addition to $\gamma_1 34.5$, the late gene Us11 also contributes to IFN resistance (Mulvey et al., 2004). Promyelocytic leukemia protein (PML), which forms nuclear body ND10 and contributes to anti-viral activity, is degraded by viral ICP0 (Chee et al., 2003; Everett et al., 1998).

Previously, we showed that SOCS3 was rapidly induced in FL cells by HSV-1 infection (Yokota et al., 2004b). The induced SOCS3 protein reaches maximal levels around 1 to 2 h post-infection and inhibits IFN-induced phosphorylation of JAK. We consider this event to be crucial in HSV-1 inhibition of IFN system because it occurs most rapidly after infection as compared to the strategies described above which should take at least several hours to occur. The SOCS family proteins are STAT-induced STAT inhibitors that provide negative feedback regulation of the JAK/STAT pathway. These proteins commonly share an N-terminal region of variable length, a central src homology 2 (SH2) domain, and a C-terminal SOCS box. SOCS proteins are generally expressed at low levels in cells and their gene transcription is induced by various cytokines that activate the JAK/STAT pathway (Alexander, 2002; Cooney, 2002; Krebs and Hilton, 2001; Yasukawa et al., 2000). To date, eight SOCS family proteins (SOCS1 to 7 and CIS) have been identified. Of these, SOCS1 and SOCS3 are reported to inhibit signal transduction by IFN (Bode et al., 2003; He et al., 2003; Sen and Ransohoff, 1993; Song et al., 1998; Yokota et al., 2004b). SOCS3 has been reported to inhibit various cytokines including IFNs, such as interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-6, growth hormone, prolactin, insulin, oncostatin M, leptin, erythropoietin, and ciliary neurotropic factor (Alexander, 2002; Cooney, 2002; Krebs and Hilton, 2001; Yasukawa et al., 2000). SOCS3 associates with cytokine receptors and inhibits activation, namely phosphorylation, of JAKs (Nicholson et al., 2000; Schmitz et al., 2000). SOCS1 directly interacts with JAK and inhibits their enzymatic activity (Yasukawa et al., 1999).

Our previous paper described induction of SOCS3 in FL cells (Yokota et al., 2004b). Currently, it is unclear whether the induction of SOCS3 by HSV-1 is general phenomenon in other cell types or not. In the present study, we examined SOCS3 induction in various cell lines and found that the inducibility of SOCS3 was correlated with rapid viral replication in the early stages of infection.

Results

Inducibility of SOCS-3 by HSV-1 varies among cell lines and correlates with viral replication

Previously, we described SOCS3 induction during HSV-1 infection in FL cells (Yokota et al., 2004b). In the present study, we examined the mRNA expression of JAK/STAT negative regulators in various types of cell lines, in the presence or absence of HSV-1 infection (Fig. 1A). FL and CCRF-CEM showed a dramatic increase in SOCS3 mRNA following virus infection (9.27-fold and 15.30-fold, respectively). TALL-1 showed a weak increase (4.70-fold) upon infection. U937, THP-1, and AKATA did not show any SOCS3 induction. Protein expression levels of SOCS3 were consistent with the results of semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) (Fig. 1B). Induction of SOCS3 protein was also observed in FL cells (4.46-fold compared to untreated control) and CCRF-CEM cells (5.33-fold). TALL-1 showed very weak increase (1.65-fold). However, the other cell lines express SOCS3 protein, under normal culture condition, they did not show significant changes in SOCS3 protein levels. The basal expression levels SOCS3 were 1.19 (TALL-1), 0.79 (CCRF-CEM), 1.10 (AKATA), 1.05 (THP-1), and 1.47 (U937), relative to level in FL cells, which was set as 1.00. The quantitative values were mean values deduced from three time experiments. CIS mRNA was downregulated in TALL-1 and CCRF-CEM and upregulated in THP-1 cells by HSV-1 infection. SOCS1 mRNA was observed in AKATA and U937 cells, and their expression levels did not change by