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Induction of Suppressor of Cytokine Signaling-3 by Herpes Simplex Virus Type 1 Contributes to Inhibition of the Interferon Signaling Pathway

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We showed previously that herpes simplex virus type 1 (HSV-1) suppresses the interferon (IFN) signaling pathway during the early infection stage in the human amnion cell line FL. HSV-1 inhibits the IFN-induced phosphorylation of Janus kinases (JAK) in infected FL cells. In the present study, we showed that the suppressor of cytokine signaling-3 (SOCS3), a host negative regulator of the JAK/STAT pathway, is rapidly induced in FL cells after HSV-1 infection. Maximal levels of SOCS3 protein were detected at around 1 to 2 h after infection. This is consistent with the occurrence of HSV-1-mediated inhibition of IFN-induced JAK phosphorylation. The HSV-1 wild-type strain VR3 induced SOCS3 more efficiently than did mutants that are defective in UL41 or UL13 and that are hyperresponsive to IFN. Induction of the IRF-7 protein and transcriptional activation of IFN-α4, which occur in a JAK/STAT pathway-dependent manner, were poorly induced by VR3 but efficiently induced by the mutant viruses. In contrast, phosphorylation of IRF-3 and transcriptional activation of IFN-β, which are JAK/STAT pathway-independent process, were equally well induced by the wild-type strain and the mutants. In conclusion, the SOCS3 protein appears to be mainly responsible for the suppression of IFN signaling and IFN production that occurs during HSV-1 infection.

Cells have various defense mechanisms that protect them from viral infection. In turn, viruses suppress or escape host responses by a variety of strategies. Interferon (IFN) is induced by viral infection and plays an important role in the defense of 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, and the MxA protein (10, 30, 35). The signal transduction pathway of IFN consists of Janus kinases (JAK), tyrosine protein kinases that interact with the intracellular domains of the receptors, and the STAT family proteins, transcription factors that are activated by their phosphorylation by JAK. This pathway, which is designated 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) (1, 9, 17, 25). Each cytokine employs a particular combination of the JAK and STAT proteins, which 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 the receptor, and they then phosphorylate STAT1 and STAT2. The transcription factor ISGF3, which consists of phosphorylated STAT1, phosphorylated STAT2, and IRF-9/p48/ISGF3γ, forms and then translocates into the nucleus and binds to IFN-stimulated response elements in the promoters of IFN-inducible genes (9, 12).

DNA and RNA viruses use various strategies to counteract the IFN-induced antiviral response (2, 11–13, 22). Blocking the JAK/STAT pathway, which is an entrance of IFN action, is a more efficient way to counteract the host defense reaction than inhibiting each of the IFN-induced effector molecules individually. It has been reported by Miller et al. (20, 21) that of the *Herpesviridae*, human cytomegalovirus downregulates the expression of Jak1 and IRF-9. Recently, we demonstrated that herpes simplex virus type 1 (HSV-1) suppresses IFN-induced JAK phosphorylation during the early infection stage in the human amnion cell line FL but not in the human monocytic cell line U937 (44). In the present study, we showed that HSV-1 induces a host inhibitor of the JAK/STAT pathway, specifically the suppressor of cytokine signaling-3 (SOCS3) protein. The SOCS family proteins are STAT-induced STAT inhibitors that constitute a negative feedback system of the JAK/STAT pathway. These proteins commonly share an N-terminal region of variable length, a central src homology 2 domain, and a C-terminal SOCS box. SOCS proteins are generally expressed at low levels in cells, and transcription of their genes is induced by various cytokines that activate the JAK/STAT pathway (3, 7, 14, 41). To date, eight SOCS family proteins (SOCS1 to 7 and CIS) have been identified. CIS, SOCS1, SOCS2, and SOCS3 have been reported to inhibit the signal transduction of various types of cytokines. Of these, SOCS1 and SOCS3 have been reported to inhibit the signal transduction of IFN (4, 36, 38).

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MATERIALS AND METHODS

Cells and viruses. The human amnion cell line FL was routinely cultured in RPMI-1640 containing 10% fetal bovine serum. The HSV-1 strain VR3 was obtained from the American Type Culture Collection (Manassas, Va.). UL41-defective (d41) and UL13-defective (d13) mutants derived from VR3 were prepared as described previously (37, 39). Unless otherwise mentioned, virus infection was performed at a multiplicity of infection of 5 (MOI 5). Virus titers in the culture supernatant were determined by a plaque-forming assay using Vero cells as an indicator. Virus inactivation by UV irradiation was performed according to a previous report (44).

Plasmids and transient transfection. Human SOCS3 cDNA was prepared by reverse transcription-PCR (RT-PCR) using cellular RNA derived from FL cells infected with HSV-1 for 10 min as a template. The primer set for preparing full-length SOCS3 cDNA was as follows: sense, 5′-ATGGTCACCCACAGCA AGTT-3′; antisense, 5′-CTTAAAGCGGGGCATCGTACTG-3′. The resulting PCR product was ligated into a mammal expression vector, pTargeT vector (Promega, Madison, Wis.) and cloned.

The plasmid was transfected into cells by using SuperFect reagent (Qiagen, Hilden, Germany) according to the manufacturer's instruction manual. After 24 h of transfection, the cells were treated with human IFN-α (Serotec, Oxford, United Kingdom) at a final concentration of 1,000 IU/ml.

Western blotting. Preparation of total cell lysates, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and Western blotting were carried out as described previously (43, 44). Rabbit anti-SOCS3 antibody was purchased from IBL (Gunma, Japan). Rabbit anti-STAT1, anti-IRF-3, and anti-IRF-7 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif.). Rabbit anti-phospho-STAT1 (Tyr701) antibody was from Cell Signaling (Beverly, Mass.). Alkaline phosphatase-conjugated anti-rabbit or mouse immunoglobulin antibodies (BioSource, Camarillo, Calif.) and bromochloroindolylphosphate-nitroblue tetrazolium were used as secondary antibodies and the enzyme substrate for Western blotting, respectively. The resulting protein bands were scanned on a flatbed scanner and quantified by using the NIH Image program (National Institutes of Health, Bethesda, Md.).

RT-PCR. Total cellular RNA was prepared by using the RNeasy Mini kit (Qiagen). RT-PCR was performed with the OneStep RT-PCR kit (Qiagen). The quantitative nature of the PCR was validated by the linearity of the determination curve at various concentrations of RNA. The primer sets used to detect SOCS3, SOCS1, and CIS mRNA have been described elsewhere (29, 33). The primer sets used for IFN-β and IFN-α4 have been described previously (28). The following primer sets were used for 2-5AS: sense, 5′-CCAGGAAATTAGGAG ACAGC-3′; antisense, 5′-TGGCAGGGAGGAAGCAGGAG-3′. The primers for IRF-3 were the following: sense, 5′-GACCCTCACGACCCACATAA-3′; antisense, 5′-ACCCCACCAGCCGCAGGCCC-3′. The primers for IRF-7 were as follows: sense, 5′-GAGCCCTTACCTCCCCTGTTAT-3′; antisense, 5′-CCA CTGCAGCCCCTCATAG-3′. The primer set for actin was purchased from Clontech, and that for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been described previously (42).

RESULTS

Suppression of the JAK/STAT pathway by HSV-1. In an earlier study, our group showed that HSV-1 suppresses the IFN-induced phosphorylation of JAK, STAT1, and STAT2 during the early infection stage in FL cells (44). In the present study, we further examined the effects on the JAK/STAT pathway of IFN-sensitive HSV-1 mutants that are defective in tegument proteins UL41 and UL13 (designated d41 and d13, respectively). These mutants display higher sensitivity to IFN compared to the wild-type strain, VR3 (37, 39). Figure 1 shows the effects of virus infection on IFN-α-induced STAT1 phosphorylation in FL cells. Infection (after 3 h) with the wild-type strain, VR3, almost completely suppressed IFN-α-induced STAT1 phosphorylation (Fig. 1A) and 2-5AS mRNA expression (Fig. 1B). This inhibition was observed after 2 h of infection and continued until cytopathic effects were observed (data not shown) (44). In contrast, infection with d41 and d13 only partially inhibited IFN-α-induced STAT1 phosphorylation and 2-5AS mRNA expression. Compared to the uninfected control, both responses were still nevertheless weaker and delayed by d41 or d13. d41 inhibited the IFN-α-induced responses more weakly than d13. These results indicate that the suppression of the JAK/STAT pathway by HSV-1 largely contributes to viral resistance to IFN and that the viral tegument proteins UL41 and UL13 take some part in this inhibition.

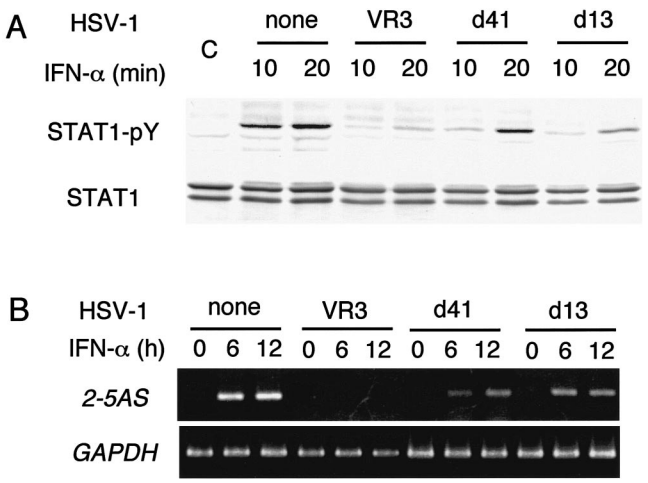

FIG. 1. Effects of infection with HSV-1 strain VR3, a UL41-deficient mutant (d41), or a UL13-deficient mutant (d13) on IFN-α-induced tyrosine phosphorylation of STAT1 (A) and induction of 2-5AS mRNA (B) in FL cells. FL cells were infected with HSV-1 VR3 or the d41 or d13 mutant at MOI 5 for 3 h. (A) The infected cells were treated with 1,000 IU of IFN-α/ml for 10 or 20 min. The cell lysates were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5% polyacrylamide slab gel and analyzed by Western blotting using anti-STAT1 and anti-tyrosine phosphorylated STAT1 (STAT1-pY) antibodies. C, uninfected control. (B) The infected cells were treated with 1,000 IU of IFN-α/ml for 6 or 12 h, after which total cellular RNA was extracted. 2-5AS mRNA levels were determined by RT-PCR. GAPDH mRNA levels were determined as a control.

SOCS3 is induced by HSV-1. To reveal the mechanism by which HSV-1 inhibits the JAK/STAT pathway, we examined the expression of the SOCS family proteins. Western blot analysis showed that SOCS3 protein levels were markedly upregulated by HSV-1 infection after 1 to 2 h of VR3 infection (Fig. 2A and B). This time course is in agreement with that for the observed inhibition of IFN signaling (44). With regard to SOCS3 mRNA levels, they increased between 10 and 20 min after infection, and maximal levels were observed at 30 and 60 min (Fig. 3A). The mRNA levels rapidly decreased after 2 h of infection. For VR3 and d41, SOCS3 mRNA levels were slightly higher at 4 h than at 2 h postinfection. The reason for this is unclear, but it could be a secondary response. On the other hand, SOCS3 protein levels were maintained at similar levels, even at around 12 h after infection (Fig. 2C). The induction of SOCS3 protein and mRNA is dependent on the virus MOI (Fig. 2D and 3B). The d41 and d13 mutant viruses only weakly upregulated the inhibitor, SOCS3, at the levels of mRNA (Fig. 3C) and protein (Fig. 2B). The lower induction of SOCS3 by the tegument-deficient mutants compared to the wild-type strain was consistent with the weaker inhibition of IFN-α-induced JAK/STAT signaling (Fig. 1). When the wild-type virus, VR3, was inactivated by UV irradiation, it did not induce SOCS3 protein (Fig. 2C). SOCS1 and CIS were little or not

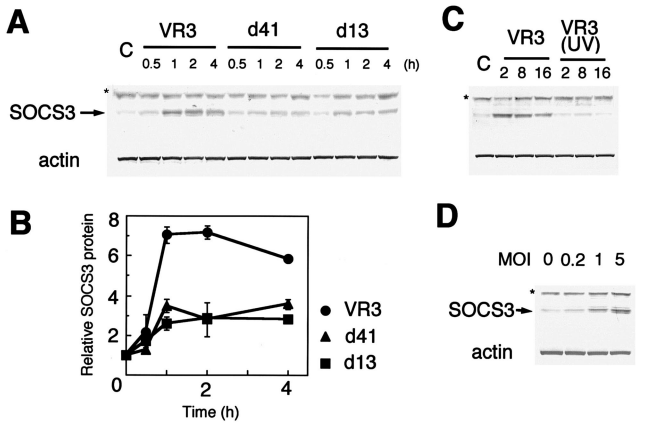

FIG. 2. Induction of SOCS3 protein by HSV-1 infection. (A and B) FL cells were infected with HSV-1 VR3 or the d41 or d13 mutant at MOI 5 for various periods. (A) The infected cell lysates were then analyzed by Western blotting using anti-SOCS3 antibody. Actin was employed as a control for protein loading. (B) Each band was quantified, and the results of the experiments performed in triplicate (means ± standard deviations) are shown. (C) FL cells were infected with HSV-1 VR3 or UV-inactivated virus for various periods. (D) FL cells were infected with VR3 at various MOIs for 2 h, and the levels of SOCS3 protein were then determined. The expression of SOCS3 protein was detected by Western blotting (arrow). *, nonspecific binding; C, uninfected control.

induced by HSV-1 and the mutants (Fig. 3C), which indicates that HSV-1 inhibits the JAK/STAT pathway by specifically inducing SOCS3.

IFN signal transduction is inhibited by overexpression of SOCS3. To assess whether SOCS3 can repress IFN-induced JAK/STAT signaling, we transiently overexpressed SOCS3 in FL cells by transfection with the pSOCS3 expression plasmid and then treated them with IFN-α. IFN-α-induced STAT1 phosphorylation was clearly suppressed in cells transfected with pSOCS3 (Fig. 4). These results indicate that HSV-1-induced SOCS3 is sufficient for the suppression of IFN signaling.

Suppression of the JAK/STAT pathway leads to inhibition of IRF-7 induction. We also characterized the effect of the virus-induced SOCS3 protein on the system that produces IFN. IFN production via the JAK/STAT pathway has been well characterized (5, 18, 31). Initially, IFN-β is transcriptionally activated

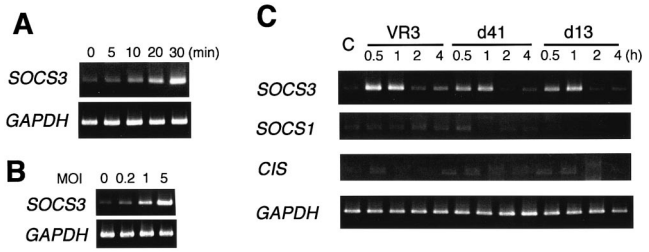

FIG. 3. Induction of SOCS3 mRNA by HSV-1 infection. (A) FL cells were infected with HSV-1 VR3 at MOI 5 for various periods. (B) FL cells were infected with VR3 at various MOIs for 30 min. The levels of SOCS3 mRNA were determined by semiquantitative RT-PCR. (C) FL cells were infected with HSV-1 VR3 or the d41 or d13 mutant at MOI 5 for various periods. The SOCS1, SOCS3, and CIS mRNA levels in the infected cells were analyzed by semiquantitative RT-PCR. GAPDH was determined as a control. C, uninfected control.

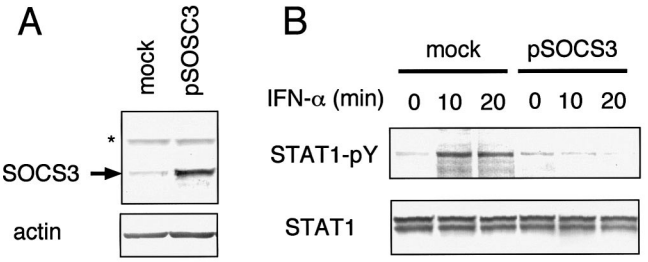

FIG. 4. Overexpression of SOCS3 suppresses IFN-α-induced Tyr-phosohorylation of STAT1. FL cells were transiently transfected with the SOCS3 expression plasmid (pSOCS3). (A) The expression of SOCS3 was determined by Western blotting. (B) After 24 h of transfection, the cells were treated with IFN-α (1,000 IU/ml) for 10 or 20 min. The cell lysates were then analyzed for the Tyr phosphorylation status of STAT1 by Western blotting. Actin and STAT1 were used as controls. *, nonspecific binding.

by phosphorylated IRF-3 and activated NF-κB. Both transcription factors are activated by various extracellular stimuli, including microorganisms and their components, in a JAK/STAT pathway-independent manner. We found that in FL cells challenged with VR3, d41, and d13, IRF-3 was equally well phosphorylated, as revealed by slower-migrating bands in Western blots compared to the unphosphorylated form (Fig. 5B). IFN-β mRNA induction was also upregulated to a similar extent by the three virus strains (Fig. 5A).

In general, virus-induced IFN-β stimulates the expression of

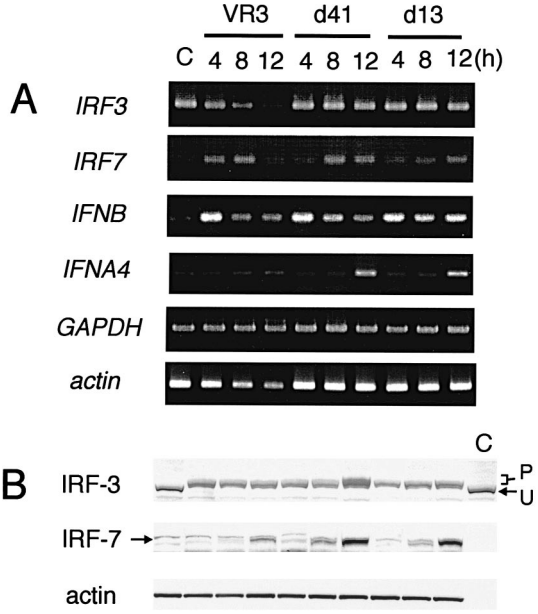

FIG. 5. Semiquantitative RT-PCR analysis of IRF-3, IRF-7, IFN-β, and IFN-α4 mRNA (A) and Western blotting analysis of IRF-3 and IRF-7 (B) in FL cells during infection with HSV-1 VR3 or the d41 or d13 tegument protein-deficient mutant. FL cells were infected with virus at MOI 5 for various periods. The phosphorylation status of IRF-3 was determined by Western blotting using an anti-IRF-3 antibody. Unphosphorylated and phosphorylated forms of IRF-3 are indicated by U and P, respectively. In contrast, total protein levels of IRF-7 are shown. C, uninfected control. Actin mRNA and protein (RT-PCR and Western blotting) and GAPDH mRNA (RT-PCR) were determined as controls.

IRF-7, a component of the transactivator of IFN-α genes, to produce large amounts of IFN, via the JAK/STAT pathway in an autocrine or a paracrine manner (5, 18, 31). In turn, IRF-7 mediates the induction of IFN-α and various IFN-inducible genes. In human cells, the expression of IFN-α4 is not activated but rather is inhibited by cooperative interactions between IRF-3 and NF-κB (32). Consequently, it appears that the induction of the IFN-α4 gene is IRF-7 dependent but not IRF-3 dependent. In other words, it is considered to be dependent upon the JAK/STAT pathway but not the IRF-3/NF-κB pathway. We found that infection of FL cells with the wild-type strain, VR3, poorly induced IRF-7 protein, even though IFN-β was induced (Fig. 5). However, the IRF-7 protein levels were increased by the two mutants defective in tegument proteins (Fig. 5B). These results indicate that the inhibition of the JAK/STAT pathway due to HSV-1 infection leads to significant blockage of JAK/STAT-dependent IFN production and to suppression of the antiviral effectors that are regulated by IFN. Consistent with this finding is that IFN-α4 mRNA levels were upregulated by the two mutants but not the wild-type strain.

The levels of IRF-3, IRF-7, and actin mRNAs decreased about 12 h after infection with the wild-type strain but not the mutant strains (Fig. 5A). This may have been due to the degradation of mRNA by the action of the viral host-shutoff (vhs) protein, which is encoded by the UL41 gene (15). The protein kinase that is encoded by the UL13 gene has also been reported to be involved in the regulation of vhs activity (26).

DISCUSSION

We previously reported that HSV-1 infection leads to inhibition of the IFN signal transduction pathway, as we observed marked suppression of IFN-induced phosphorylation of Jak1, Tyk2, Jak2, STAT1, and STAT2 (44). These results indicate that HSV-1 inhibits the JAK/STAT pathway at a point that precedes the JAK phosphorylation step. In the present study, we showed that SOCS3, a host JAK/STAT inhibitor, is transcriptionally induced by HSV-1 infection. SOCS3 inhibits JAK phosphorylation through binding to the cytokine receptors (3, 14). To our knowledge, only one report showing that virus induces SOCS3 has been published to date. Namely, Bode et al. (4) demonstrated that human hepatitis C virus core protein transcriptionally induces SOCS3, which suppresses the IFN-induced antiviral state. In the case of HSV-1 infection studied here, maximal protein levels of SOCS3 were detected a few hours after virus infection. This finding is consistent with the kinetics of the HSV-1-mediated IFN signal transduction suppression described previously (44). It has been shown that HSV-1 activates IFN-inducible genes under experimental conditions in which de novo cellular protein synthesis was inhibited (23, 24). These observations are consistent with our finding that SOCS3 is synthesized de novo following HSV-1 infection. In addition, UV-treated VR3 did not induce SOCS3, which correlates with its poor inhibition of the JAK/STAT pathway (44). In the previous report, we initially speculated that the virus-mediated inhibition of the JAK/STAT pathway requires viral protein synthesis. However, we found that SOCS3 induction occurs too rapidly (within 10 min) at the mRNA level for it to be driven by the de novo synthesis of viral proteins. Two mutant viruses that are defective in one of the tegument proteins UL41 and UL13, which are hypersensitive to IFN (37, 39), weakly induced SOCS3 compared to the parental wild-type strain. Accordingly, we now postulate that the induction of SOCS3 occurs after endocytosis or uncoating but not at the step of virus attachment to host cells. The UL41 gene product is an RNase, the vhs protein, which rapidly degrades host and virus mRNA and thereby causes protein synthesis shutoff (15). The UL13 gene encodes a protein kinase whose exact function is currently unknown. However, it is proposed that the protein kinase regulates vhs activity (26). The impairment of SOCS3 induction by these mutants should contribute to their hypersensitivity to IFN. However, we found that the mutant viruses are still able to weakly induce SOCS3. Consequently, we propose that the two tegument proteins do not contribute directly to SOCS3 induction. The poor induction of SOCS3 by these mutants may instead relate to the fact that they replicate with a lower efficacy (about 1 log unit less of virus titer) than the parental virus (data not shown).

We also suggest that the induction of SOCS3 by HSV-1 blocks the IFN production system. The IFN production system has been well characterized (5, 18, 31). Initially, IFN-β is transcriptionally activated by phosphorylated IRF-3 and activated NF-κB in a JAK/STAT pathway-independent manner. Virus-induced IFN-β then stimulates the expression of IRF-7, a component of the transactivator of IFN-α genes, via the JAK/STAT pathway (5, 18, 31). IRF-7 subsequently mediates the induction of IFN-α, such as human IFN-α4, and various IFN-inducible genes. This cycle results in the production of large amounts of IFN-α and the establishment of a strong antiviral state. SOCS3, which is induced by HSV-1, may suppress the JAK/STAT-dependent production of large amounts of IFN-α. We found that after HSV-1 infection, IFN-β is upregulated but IRF-7 and IFN-α4 levels are poorly induced (Fig. 5). However, HSV-1 activates IFN- (namely JAK/STAT pathway-) independent signal transduction, including IRF-3 phosphorylation and upregulation of IFN-β (27; also this study). These events were equally well induced by the wild-type virus, VR3, and the mutant viruses d41 and d13. In contrast, the IFN-dependent signal transduction activated by IFN-β is markedly suppressed after infection with VR3 but only partially blocked by infection with the mutant virus particles.

SOCS3 is an important regulator of cytokine signaling. SOCS3 induction would influence not only the IFN system but would also have a dramatic impact on the immune system in a manner that would favor HSV-1 replication. For example, SOCS3 promotes Th2 development by inhibiting interleukin-12 (IL-12)-mediated STAT4 activation in T cells (34). It also negatively regulates IL-2 signaling (6) and IL-2 production via CD28 signaling (19). Furthermore, it inhibits IL-6 signaling in macrophages (8, 16, 40). These SOCS3-mediated events would suppress the ability of the host to clear the virus. We conclude that the induction of SOCS3 by the virus plays a key role in host-virus interactions, as it may directly promote an active and productive infection by the virus. We are currently elucidating the details of the molecular mechanism by which SOCS3 is induced and the effects that it has on both the virus and the host.

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REFERENCES

1. **Aaronson, D. S., and C. M. Horvath.** 2002. A road map for those who don't know JAK-STAT. Science **296:**1653–1655.
2. **Alcami, A., and U. H. Koszinowski.** 2000. Viral mechanisms of immune evasion. Immunol. Today **21:**447–455.
3. **Alexander, W. S.** 2002. Suppressors of cytokine signalling (SOCS) in the immune system. Nat. Rev. Immunol. **2:**410–416.
4. **Bode, J. G., S. Ludwig, C. Ehrhardt, U. Albrecht, A. Erhardt, F. Schaper, P. C. Heinrich, and D. Haussinger.** 2003. IFN-α antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J. **17:**488–490.
5. **Braganca, J., and A. Civas.** 1998. Type I interferon gene expression: differential expression of IFN-A genes induced by viruses and double-stranded RNA. Biochimie **80:**673–687.
6. **Cohney, S. J., D. Sanden, N. A. Cacalano, A. Yoshimura, A. Mui, T. S. Migone, and J. A. Johnston.** 1999. SOCS-3 is tyrosine phosphorylated in response to interleukin-2 and suppresses STAT5 phosphorylation and lymphocyte proliferation. Mol. Cell. Biol. **19:**4980–4988.
7. **Cooney, R. N.** 2002. Suppressors of cytokine signaling (SOCS): inhibitors of the JAK/STAT pathway. Shock **17:**83–90.
8. **Croker, B. A., D. L. Krebs, J. G. Zhang, S. Wormald, T. A. Willson, E. G. Stanley, L. Robb, C. J. Greenhalgh, I. Forster, B. E. Clausen, N. A. Nicola, D. Metcalf, D. J. Hilton, A. W. Roberts, and W. S. Alexander.** 2003. SOCS3 negatively regulates IL-6 signaling *in vivo*. Nat. Immunol. **4:**540–545.
9. **Darnell, J. E., Jr., I. M. Kerr, and G. R. Stark.** 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science **264:**1415–1421.
10. **Fujii, N.** 1994. 2–5A and virus infection. Prog. Mol. Subcell. Biol. **14:**150–175.
11. **Garcia-Sastre, A.** 2001. Inhibition of interferon-mediated antiviral responses by influenza A viruses and other negative-strand RNA viruses. Virology **279:**375–384.
12. **Goodbourn, S., L. Didcock, and R. E. Randall.** 2000. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J. Gen. Virol. **81:**2341–2364.
13. **Gotoh, B., T. Komatsu, K. Takeuchi, and J. Yokoo.** 2002. Paramyxovirus strategies for evading the interferon response. Rev. Med. Virol. **12:**337–357.
14. **Krebs, D. L., and D. J. Hilton.** 2001. SOCS proteins: negative regulators of cytokine signaling. Stem Cells **19:**378–387.
15. **Kwong, A. D., and N. Frenkel.** 1989. The herpes simplex virus virion host shutoff function. J. Virol. **63:**4834–4839.
16. **Lang, R., A. L. Pauleau, E. Parganas, Y. Takahashi, J. Mages, J. N. Ihle, R. Rutschman, and P. J. Murray.** 2003. SOCS3 regulates the plasticity of gp130 signaling. Nat. Immunol. **4:**546–550.
17. **Leonard, W. J., and J. J. O'Shea.** 1998. Jaks and STATs: biological implications. Annu. Rev. Immunol. **16:**293–322.
18. **Mamane, Y., C. Heylbroeck, P. Genin, M. Algarte, M. J. Servant, C. LePage, C. DeLuca, H. Kwon, R. Lin, and J. Hiscott.** 1999. Interferon regulatory factors: the next generation. Gene **237:**1–14.
19. **Matsumoto, A., Y. Seki, R. Watanabe, K. Hayashi, J. A. Johnston, Y. Harada, R. Abe, A. Yoshimura, and M. Kubo.** 2003. A role of suppressor of cytokine signaling 3 (SOCS3/CIS3/SSI3) in CD28-mediated interleukin 2 production. J. Exp. Med. **197:**425–436.
20. **Miller, D. M., B. M. Rahill, J. M. Boss, M. D. Lairmore, J. E. Durbin, J. W. Waldman, and D. D. Sedmak.** 1998. Human cytomegalovirus inhibits major histocompatibility complex class II expression by disruption of the Jak/Stat pathway. J. Exp. Med. **187:**675–683.
21. **Miller, D. M., Y. Zhang, B. M. Rahill, W. J. Waldman, and D. D. Sedmak.** 1999. Human cytomegalovirus inhibits IFN-α-stimulated antiviral and immunoregulatory responses by blocking multiple levels of IFN-α signal transduction. J. Immunol. **162:**6107–6113.
22. **Mossman, K. L.** 2002. Activation and inhibition of virus and interferon: the herpesvirus story. Viral Immunol. **15:**3–15.
23. **Mossman, K. L., P. F. Macgregor, J. J. Rozmus, A. B. Goryachev, A. M. Edwards, and J. R. Smiley.** 2001. Herpes simplex virus triggers and then disarms a host antiviral response. J. Virol. **75:**750–758.
24. **Nicholl, M. J., L. H. Robinson, and C. M. Preston.** 2000. Activation of cellular interferon-responsive genes after infection of human cells with herpes simplex virus type 1. J. Gen. Virol. **81:**2215–2218.
25. **O'Shea, J. J., M. Gadina, and R. D. Schreiber.** 2002. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell **109**(Suppl.)**:**S121–S131.
26. **Overton, H., D. McMillan, L. Hope, and P. Wong-Kai-In.** 1994. Production of host shutoff-defective mutants of herpes simplex virus type 1 by inactivation of the UL13 gene. Virology **202:**97–106.
27. **Preston, C. M., A. N. Harman, and M. J. Nicholl.** 2001. Activation of interferon response factor-3 in human cells infected with herpes simplex virus type 1 or human cytomegalovirus. J. Virol. **75:**8909–8916.
28. **Remoli, M. E., E. Giacomini, G. Lutfalla, E. Dondi, G. Orefici, A. Battistini, G. Uze, S. Pellegrini, and E. M. Coccia.** 2002. Selective expression of type I IFN genes in human dendritic cells infected with *Mycobacterium tuberculosis*. J. Immunol. **169:**366–374.
29. **Sakai, I., K. Takeuchi, H. Yamauchi, H. Narumi, and S. Fujita.** 2002. Constitutive expression of SOCS3 confers resistance to IFN-α in chronic myelogenous leukemia cells. Blood **100:**2926–2931.
30. **Samuel, C. E.** 2001. Antiviral actions of interferons. Clin. Microbiol. Rev. **14:**778–809.
31. **Sato, M., H. Suemori, N. Hata, M. Asagiri, K. Ogasawara, K. Nakao, T. Nakaya, M. Katsuki, S. Noguchi, N. Tanaka, and T. Taniguchi.** 2000. Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-α/β gene induction. Immunity **13:**539–548.
32. **Schafer, S. L., R. Lin, P. A. Moore, J. Hiscott, and P. M. Pitha.** 1998. Regulation of type I interferon gene expression by interferon regulatory factor-3. J. Biol. Chem. **273:**2714–2720.
33. **Schuringa, J. J., A. T. Wierenga, W. Kruijer, and E. Vellenga.** 2000. Constitutive Stat3, Tyr705, and Ser727 phosphorylation in acute myeloid leukemia cells caused by the autocrine secretion of interleukin-6. Blood **95:**3765–3770.
34. **Seki, Y., H. Inoue, N. Nagata, K. Hayashi, S. Fukuyama, K. Matsumoto, O. Komine, S. Hamano, K. Himeno, K. Inagaki-Ohara, N. Cacalano, A. O'Garra, T. Oshida, H. Saito, J. A. Johnston, A. Yoshimura, and M. Kubo.** 2003. SOCS-3 regulates onset and maintenance of T_H2-mediated allergic responses. Nat. Med. **9:**1047–1054.
35. **Sen, G. C., and R. M. Ransohoff.** 1993. Interferon-induced antiviral actions and their regulation. Adv. Virus Res. **42:**57–102.
36. **Shen, X., F. Hong, V. A. Nguyen, and B. Gao.** 2000. IL-10 attenuates IFN-α-activated STAT1 in the liver: involvement of SOCS2 and SOCS3. FEBS Lett. **480:**132–136.
37. **Shibaki, T., T. Suzutani, I. Yoshida, M. Ogasawara, and M. Azuma.** 2001. Participation of type I interferon in the decreased virulence of the UL13 gene-deleted mutant of herpes simplex virus type 1. J. Interferon Cytokine Res. **21:**279–285.
38. **Song, M. M., K. Shuai, and J. Liao.** 1998. The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities enhancement of antiproliferative activity of gamma interferon by the specific inhibition of tyrosine dephosphorylation of Stat1. J. Biol. Chem. **273:**35056–35062.
39. **Suzutani, T., M. Nagamine, T. Shibaki, M. Ogasawara, I. Yoshida, T. Daikoku, Y. Nishiyama, and M. Azuma.** 2000. The role of the UL41 gene of herpes simplex virus type 1 in evasion of non-specific host defence mechanisms during primary infection. J. Gen. Virol. **81:**1763–1771.
40. **Yasukawa, H., M. Ohishi, H. Mori, M. Murakami, T. Chinen, D. Aki, T. Hanada, K. Takeda, S. Akira, M. Hoshijima, T. Hirano, K. R. Chien, and A. Yoshimura.** 2003. IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages. Nat. Immunol. **4:**551–556.
41. **Yasukawa, H., A. Sasaki, and A. Yoshimura.** 2000. Negative regulation of cytokine signaling pathways. Annu. Rev. Immunol. **18:**143–164.
42. **Yokota, S., H. Saito, T. Kubota, N. Yokosawa, K. Amano, and N. Fujii.** 2003. Measles virus suppresses interferon-α signaling pathway: suppression of Jak1 phosphorylation and association of viral accessory proteins, C and V, with interferon-α receptor complex. Virology **306:**135–146.
43. **Yokota, S., H. Yanagi, T. Yura, and H. Kubota.** 1999. Cytosolic chaperonin is up-regulated during cell growth. Preferential expression and binding to tubulin at G_1/S transition through early S phase. J. Biol. Chem. **274:**37070–37078.
44. **Yokota, S., N. Yokosawa, T. Kubota, T. Suzutani, I. Yoshida, S. Miura, K. Jimbow, and N. Fujii.** 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.