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Noncytolytic Clearance of Sindbis Virus Infection from Neurons by Gamma Interferon Is Dependent on Jak/Stat Signaling[▽]

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The alphavirus Sindbis virus (SINV) causes encephalomyelitis in mice by infecting neurons of the brain and spinal cord. The outcome is age dependent. Young animals develop fatal disease, while older animals recover from infection. Recovery requires noncytolytic clearance of SINV from neurons, and gamma interferon (IFN-γ) is an important contributor to clearance in vivo. IFN-γ-dependent clearance has been studied using immortalized CSM14.1 rat neuronal cells that can be differentiated in vitro. Previous studies have shown that differentiated, but not undifferentiated, cells develop prolonged SINV replication and respond to IFN-γ treatment with noncytolytic clearance of virus preceded by suppression of genomic viral RNA synthesis and reactivation of cellular protein synthesis. To determine the signaling mechanisms responsible for clearance, the responses of SINV-infected differentiated neurons to IFN-γ were examined. IFN-γ treatment of SINV-infected differentiated CSM14.1 cells, AP-7 olfactory neuronal cells, and primary dorsal root ganglia neurons triggered prolonged Stat-1 Tyr_{701} phosphorylation, Stat-1 Ser_{727} phosphorylation, and transient Stat-5 phosphorylation. Inhibition of Jak kinase activity with Jak inhibitor I completely reversed the neuroprotective and antiviral activities of IFN-γ in differentiated cells. We conclude that activation of the Jak/Stat pathway is the primary mechanism for IFN-γ-mediated clearance of SINV infection from mature neurons.

Alphaviruses in the family *Togaviridae* are enveloped, plus-strand, mosquito-borne RNA viruses that can cause encephalomyelitis. Sindbis virus (SINV), the prototype alphavirus, causes arthritis and rash in humans (39, 48) and encephalomyelitis in mice, a small-animal model for study of the pathogenesis of acute encephalitis (32). Age is an important determinant of outcome, and neonatal mice die within the first few days after infection, while older mice clear SINV from the central nervous system (CNS) within 6 to 8 days without signs of paralysis or neurological damage (33, 40). Maturity of the infected neuron determines the level of virus replication and the susceptibility to SINV-induced cell death independent of the immune response (28, 43, 46, 56). Immature neurons replicate SINV to higher titers and are susceptible to virus-induced apoptosis, while mature neurons are intrinsically more resistant to SINV replication and survive virus infection (3, 4, 43). Recovery from infection requires immune-mediated clearance of virus from these surviving infected neurons.

Because mature neurons are terminally differentiated cells with limited capacity for regeneration, recovery that does not result in neuronal damage requires noncytolytic, rather than the more traditional cytolytic, immune mechanisms for virus clearance. Antibody is produced, T cells begin to infiltrate the CNS 3 to 4 days after infection, and virus clearance begins shortly thereafter (19, 50, 52). Type I interferon (IFN) is essential for initial control of virus replication (5, 6, 17, 69), and both humoral (6, 43, 77) and cellular (3, 37) arms of the adaptive immune response play important roles in clearance. Mice deficient in all components of adaptive immunity (SCID or $Rag^{-/-}$) develop persistent nonfatal infection, and passive transfer of SINV antibody results in clearance of infectious virus from the CNS and decreased viral RNA without neurologic damage, indicating an important role for antibody in noncytolytic clearance (34). However, mice deficient in antibody (μMT) are able to reduce levels of SINV in the cortex and hippocampus of the brain compared to SCID mice and to clear infectious virus from the brain stem and spinal cord through local production of IFN-γ, indicating a role for T cells in clearance from some, but not all, types of neurons (3). Studies with mice deficient in production of both IFN-γ and antibody indicate a synergistic role for these mediators in clearing SINV from the CNS, but the mechanisms are not known (5).

To identify mechanisms of immune-mediated clearance, various in vitro systems have been developed. CSM14.1 neuronal cells that have been differentiated in vitro become persistently infected with SINV, and treatment with IFN-γ results in virus clearance and improved cell survival (4, 79). Characterization of the response of infected differentiated neurons to treatment with IFN-γ has shown a transient increase in synthesis of viral RNA and protein 6 h after treatment followed by cessation of viral protein and RNA synthesis and restoration of host cell protein synthesis (4). However, the signaling pathways leading to these IFN-γ-mediated changes are unknown.

The IFN-γ receptor is expressed on many cells, including neurons, and has two subunits, the ligand-binding IFN-γR1

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chain and the signaling IFN-γR2 chain (74). The cytoplasmic domains of both chains are necessary for signal transduction and are constitutively associated with Janus tyrosine kinase 1 (Jak1) and Jak2, which phosphorylate signal transducers and activators of transcription (Stats) (74). Stat-1 phosphorylated by Jak1 at Tyr_{701} dissociates from the receptor complex and forms homodimers that are translocated to the nucleus, where they bind gamma-activated site elements to initiate gene expression. Transcription is dependent on the presence of a C-terminal transcriptional activation domain in Stat-1α and is regulated in a cell-type-dependent way by Ser_{727} phosphorylation in the nucleus and recruitment of CREB-binding protein/p300, N-myc-interacting protein Nmi-1, and other coregulators to this region (30, 54, 57, 64, 83, 89). Gamma-activated site regulatory elements have been identified in over 200 genes, suggesting the broad array of responses that can be initiated by IFN-γ (16, 74).

However, the Jak1 and -2/Stat-1 pathway is not the only mechanism by which IFN-γ initiates intracellular responses. Additional Stats can be activated in a cell-type-dependent manner (7, 51, 90), and Stat-independent signaling is important for the effects of IFN-γ in some cells (63). In the current studies we have examined the pathways involved in the IFN-γ-mediated initiation of virus clearance from persistently infected differentiated neurons and demonstrate the activation of Stat-1 and Stat-5. Inhibition of Jak tyrosine phosphorylation blocks the effect of IFN-γ on SINV replication in differentiated neurons, indicating an essential role of Jak/Stat signal transduction in the noncytolytic control of SINV replication in neurons.

MATERIALS AND METHODS

Neuronal cell cultures. The rat CSM14.1 nigral neuronal cell line, immortalized with a temperature-sensitive simian virus 40 T antigen, was a gift from Dale E. Bredesen (Buck Institute for Age Research, Novato, CA) (14, 88). CSM14.1 cells were grown at the permissive temperature of 31°C in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U penicillin/ml, 100 μg streptomycin/ml, and 2 mM glutamine. At 95% confluence cells were differentiated for 3 weeks by shifting to 39°C and DMEM–1% FBS. Under these conditions, CSM14.1 cells stop dividing and gradually differentiate to develop a mature neuronal phenotype (4, 79).

The rat AP-7 odora olfactory neuronal cell line, immortalized with a temperature-sensitive simian virus 40 T antigen, was a gift from Dale Hunter (Tufts University, Boston, MA) (55). AP-7 cells were grown at 33°C in 7% CO_2 in DMEM–10% FBS. At about 25% confluence, cells were differentiated for 7 days by shifting to 39°C and 5% CO_2 in DMEM–10% FBS supplemented with 1 μg/ml insulin, 20 μM dopamine, 100 μM ascorbic acid (Sigma, St Louis, MO), 100 U penicillin/ml, 100 μg streptomycin/ml, and 2 mM glutamine.

Rat dorsal root ganglia (DRG) neurons were obtained from E15 Sprague-Dawley rat embryos and were cultured as previously described (43, 44, 78). In brief, embryos were removed and placed in L15 medium and spinal columns were separated and trimmed to expose the spinal cords. The DRG bundles were plucked, trypsinized, and differentiated for 4 weeks in minimal essential medium–10% FBS supplemented with 20% glucose, 2 mM glutamine, 100 U penicillin/ml, 100 μg streptomycin/ml, 5 μg nerve growth factor/ml (Invitrogen), and 5 μl 2-deoxy-5-fluorouridine/ml (Sigma).

Virus infection and IFN-γ treatment. SINV strain 633 (76) expressing enhanced green fluorescent protein from a second subgenomic promoter was used (4, 23). Viral RNA was transcribed and transfected into BHK cells to produce stock virus that was assayed by measuring plaque formation on BHK cells. Cells were infected at a multiplicity of infection of 1 (determined in BHK cells) with virus diluted in DMEM–1% FBS. For treatment with IFN-γ, medium was supplemented with 100 or 500 U/ml recombinant rat IFN-γ (PBL Biomedical Laboratories, New Brunswick, NJ).

Analysis of protein expression and phosphorylation. Infected and IFN-γ-treated CSM14.1 cells were washed with ice-cold phosphate-buffered saline, lysed, and scraped in radioimmunoprecipitation assay buffer (1% NP-40, 0.1% sodium dodecyl sulfate [SDS], 0.1% Na deoxycholate, 10 mM Tris-Cl [pH 7.0], 150 mM NaCl, 1 mM EDTA) with 25 μg aprotinin/ml (Sigma) at various times after infection and IFN-γ treatment. All samples were stored at −80°C, and 30 μg of protein from each sample was denatured by boiling for 5 min in 6× SDS loading buffer (0.5 M Tris [pH 6.8], 30% glycerol, 10% SDS, 0.12% bromophenol blue, 6% β-mercaptoethanol), followed by 10% SDS-polyacrylamide gel electrophoresis. For immunoblotting, proteins were transferred to polyvinylidene difluoride membranes blocked with 5% milk in Tris-buffered saline containing 0.1% Tween 20. Membranes were incubated with rabbit polyclonal or mouse monoclonal antibodies, diluted 1:1,000 in primary dilution buffer (Tris-buffered saline–5% bovine serum albumin), overnight with gentle rocking at 4°C. Rabbit polyclonal antibodies against the following antigens were used: Stat-1, Stat-1 phosphorylated at Y701 [phospho-(Y_{701}) Stat-1], phospho-(Ser_{727}) Stat-1, Stat-3, phospho-Stat-3, and phospho-Stat-5 α/β (Cell Signaling Technology, Beverly, MA). β-Actin was detected with a mouse monoclonal antibody (Chemicon, Temecula, CA). Secondary horseradish peroxidase-conjugated antibodies (Amersham Biosciences) were diluted 1:1,000 to 1:2,000.

Inhibitors. Jak inhibitor I (Calbiochem, La Jolla, CA) was diluted in dimethyl sulfoxide to produce a 10 mM stock, then diluted in cell culture medium to produce working solutions of 0.1 μM to 10 μM. Medium containing inhibitor was added 6 h after infection and removed when IFN-γ was added at 24 h after infection.

RESULTS

Stat-1 phosphorylation after infection and after IFN-γ treatment. Peak SINV replication in differentiated CSM cells at 48 h is followed by gradual spontaneous control of virus replication (4). To determine whether SINV infection activates Jak/Stat-1 signaling in mature neurons, differentiated CSM14.1 cells were infected with SINV and analyzed at different times after infection for total and phosphorylated (Tyr_{701}) Stat-1 (Fig. 1A). Stat-1 protein levels declined 4 to 6 h after infection and then increased steadily from 9 to 72 h. Low levels of phosphorylated Stat-1 could be detected 24 to 48 h after infection and remained through 72 h.

When SINV-infected, differentiated CSM cells are treated with IFN-γ 24 h after infection, virus replication decreases rapidly and cellular protein synthesis is restored (4). To determine whether treatment with IFN-γ can activate Stat-1 in infected cells, Stat-1 phosphorylation was assessed before and after treatment (Fig. 1B). Robust Stat-1 phosphorylation was induced within 6 h after IFN-γ treatment and was sustained for at least 48 h. To compare the responses to IFN-γ of infected cells to those of uninfected cells, levels of Stat-1 and phosphorylated Stat-1 in differentiated CSM14.1 cells that were and were not previously infected with SINV were assessed (Fig. 1C). Stat-1 phosphorylation in both SINV-infected and uninfected differentiated CSM14.1 cells was substantial within 2 h after treatment, indicating the intrinsic ability of these cells to respond to IFN-γ with activation of the Jak/Stat-1 pathway. Consistent with the fact that Stat-1 activation increases expression of Stat-1 mRNA (12, 45), Stat-1 protein levels were increased in response to IFN-γ.

Because Ser_{727} phosphorylation is necessary for transcriptional activation by Stat-1, differentiated CSM cells were also assessed for this modification after IFN-γ treatment (Fig. 2). Sustained Ser_{727} phosphorylation was observed within 2 h after IFN-γ treatment in infected cells and was sustained for at least 24 h.

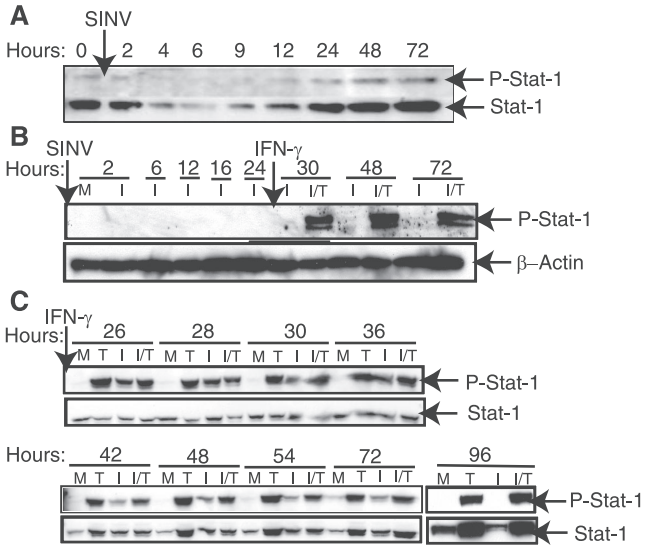

FIG. 1. Effect of SINV infection and IFN-γ treatment on Stat-1 Tyr_{701} phosphorylation in CSM14.1 cells. (A) Differentiated CSM14.1 cells were infected with SINV, and levels of Stat-1 and phosphorylated (Tyr_{701}) Stat-1 were assessed by immunoblotting in cell lysates collected before (0 h) and 2 to 72 h after infection. (B) Infected cells were treated or not treated with 100 U IFN-γ 24 h after infection. (C) Uninfected and infected cells were treated or not treated with IFN-γ 24 h after infection and analyzed 2 to 72 h after treatment. M, mock infected; T, uninfected, IFN-γ treated; I, SINV infected, untreated; I/T, SINV infected, IFN-γ treated.

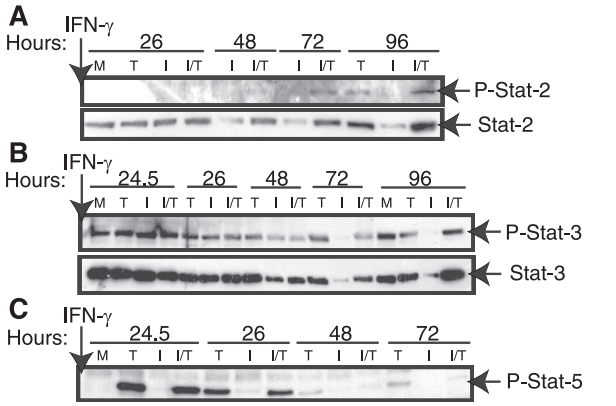

FIG. 3. Effect of SINV infection and IFN-γ treatment on Stat-2, Stat-3, and Stat-5 phosphorylation in CSM14.1 cells. Differentiated CSM14.1 cells were infected with SINV and treated with 100 U IFN-γ/ml 24 h after infection. Cell lysates were analyzed for levels of Stat-2 and phosphorylated Stat-2 (A), Stat-3 and phosphorylated Stat-3 (B), and phosphorylated Stat-5 (C) by immunoblotting. M, mock infected; T, uninfected, IFN-γ treated; I, SINV infected, untreated; I/T, SINV infected, IFN-γ treated.

Stat-2, Stat-3, and Stat-5 phosphorylation. Signaling through the IFN-α/β receptor results in the phosphorylation and heterodimerization of Stat-1 and Stat-2 through Jak phosphorylation cascades similar to IFN-γ-mediated pathways. Activation of other Stats may occur through secondary pathways or as part of the response to IFN-γ in some types of cells (51, 71, 85). To determine whether this occurred in CSM cells, the phosphorylation state of other Stats in infected and uninfected cells with and without treatment with IFN-γ was examined (Fig. 3). In infected cells, Stat-2 protein levels were reduced by 48 h and Stat-2 phosphorylation was not induced. In infected cells treated with IFN-γ, Stat-2 was phosphorylated 24 to 48 h after treatment and Stat-2 protein levels were maintained (Fig. 3A). Stat-3 was constitutively phosphorylated in differentiated CSM14.1 cells. Stat-3 protein levels in SINV-infected cells declined but were maintained in infected cells treated with IFN-γ (Fig. 3B). Stat-5 was not phosphorylated in infected cells but was rapidly phosphorylated in both infected and uninfected cells in response to IFN-γ treatment (Fig. 3C). However, unlike Stat-1 phosphorylation, Stat-5 activation was transient. By 24 h after IFN-γ treatment, phosphorylated Stat-5 was barely detectable in uninfected cells and was not detectable in infected cells.

Stat-1 and Stat-5 phosphorylation in differentiated AP-7 and DRG neurons. Cell types differ in their responses to IFN-γ (62), and in vivo studies have shown that types of neurons differ in their abilities to clear SINV infection in response to IFN-γ (3, 5). To determine whether the responses of infected differentiated CSM14.1 cells to IFN-γ were unique, AP-7 rat olfactory neuronal cells (55) and primary rat DRG neurons were studied. Differentiated AP-7 cells were infected with SINV, and virus production (Fig. 4A) and viability (Fig. 4B) in untreated cells and in cells treated with IFN-γ 24 h before or after infection were analyzed. Pretreatment of AP-7 cells with IFN-γ prevented SINV replication and cell death ($P = 0.035$). Treatment with IFN-γ 24 h after infection reduced SINV replication (day 3, $P = 0.00047$; day 7, $P = 0.0045$) and cell death ($P = 0.02$). These responses were similar to those observed with differentiated CSM14.1 cells (4).

Differentiated primary rat DRG neurons infected with SINV become persistently infected and respond to antiviral antibody with decreased virus replication (43, 78). Treatment of infected differentiated DRG neurons with IFN-γ reduced virus production almost 10-fold ($P = 0.031$) (Fig. 4C), thereby

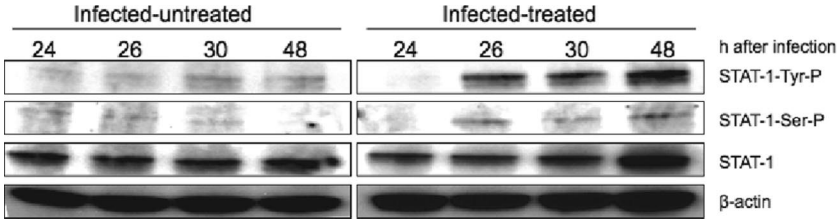

FIG. 2. Effect of SINV infection and IFN-γ treatment on Stat-1 Tyr_{701} and Ser_{727} phosphorylation. Differentiated CSM14.1 cells were infected with SINV, treated or not treated with 100 U IFN-γ/ml 24 h after infection, and analyzed for Tyr_{701} and Ser_{727} phosphorylation of Stat-1.

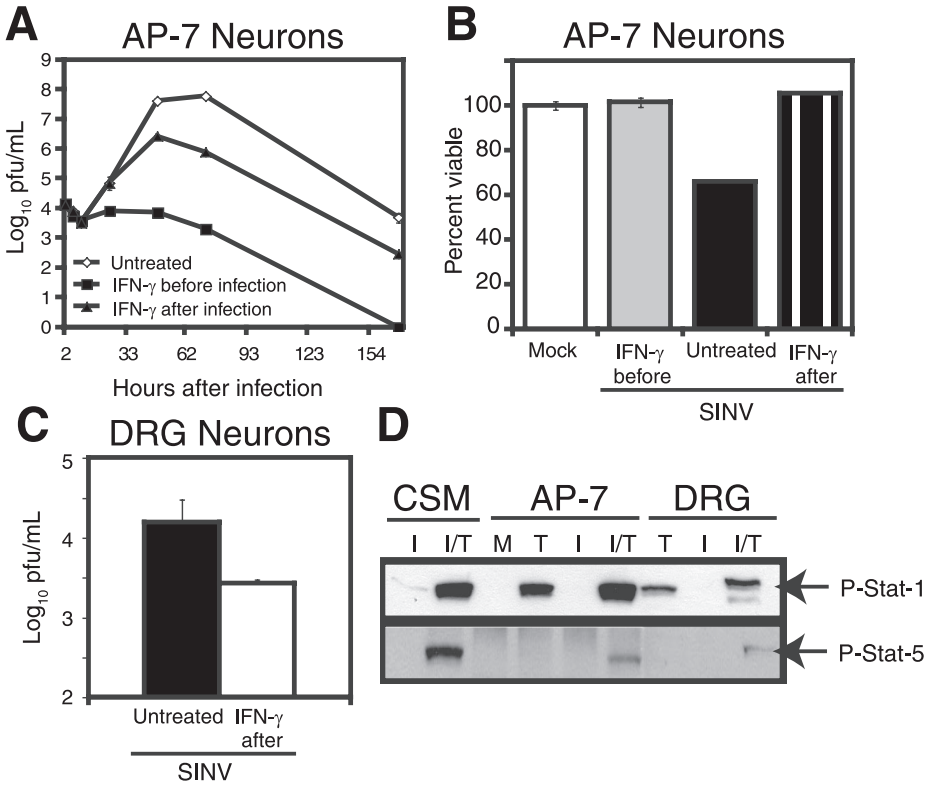

FIG. 4. SINV infection and IFN-γ treatment of differentiated AP-7 olfactory neuronal cells, DRG neurons, and CSM14.1 neuronal cells. (A) Differentiated AP-7 cells were infected with SINV and were or were not treated with 500 U/ml IFN-γ 2 h before or 24 h after infection. Supernatant fluids were assayed for virus production by plaque assay. (B) Cell viability was assessed by trypan blue exclusion 3 days after infection. (C) Differentiated DRG neurons were infected with SINV, and the effect of IFN-γ treatment (500 U) 24 h after infection on virus production was assessed at 7 days. Each point represents the average and standard error of the mean of three individual wells. (D) Differentiated CSM14.1, AP-7, and DRG cells were infected with SINV and treated with IFN-γ 24 h after infection. Lysates were collected 30 min after IFN-γ treatment (24.5 h after infection) and analyzed for Stat-1 and Stat-5 phosphorylation by immunoblotting. M, mock infected; T, uninfected, IFN-γ treated; I, SINV infected, untreated; I/T, SINV infected, IFN-γ treated.

demonstrating that primary neurons also respond to IFN-γ treatment after SINV infection has been established. Differentiated AP-7 and DRG cells, as well as CSM14.1 cells, responded to IFN-γ by activating Stat-1 and Stat-5 within 30 min after IFN-γ treatment (Fig. 4D).

Effects of inhibition of Jak/Stat signaling on virus replication and cell survival. To determine the role of activation of the Jak/Stat pathway in IFN-γ-mediated suppression of virus replication and prevention of cell death, differentiated SINV-infected CSM14.1 cells were treated with Jak inhibitor I for 18 h before IFN-γ treatment (Fig. 5). Jak inhibitor I inhibits activity of all Jak kinases (75), and treatment of uninfected cells with the highest dose (10 μM) did not significantly affect cell viability ($P = 0.31$) but blocked the ability of IFN-γ to improve survival of differentiated CSM cells after SINV infection in a dose-dependent fashion (10 μM, $P = 0.0072$; 1 μM, $P = 0.014$; 0.1 mM, $P = 0.07$) (Fig. 5A). The inhibitory effects of IFN-γ on virus replication were also eliminated by treatment (10 μM, $P = 0.0017$; 1 μM, $P = 0.013$; 0.1 μM, $P = 0.4$) (Fig. 5B), as well as Stat-1 and Stat-5 phosphorylation (Fig. 5C). Treatment of infected cells with Jak inhibitor I in the absence of IFN-γ did not significantly affect SINV replication (data not shown).

DISCUSSION

SINV-infected neurons can respond to IFN-γ by decreasing virus replication through modulation of both viral protein and RNA synthesis (4). The current studies have shown that IFN-γ treatment induced prolonged phosphorylation of Stat-1 and transient phosphorylation of Stat-5 in three different types of differentiated neuronal cells previously infected with SINV. Stat-1 was phosphorylated at both Tyr_{701} and Ser_{727} in response to IFN-γ treatment. Inhibition of the Jak/Stat pathway with Jak inhibitor 1 blocked the beneficial effects of IFN-γ treatment on cell viability and virus clearance in differentiated neurons. These studies show that the Jak/Stat signaling pathway induced by IFN-γ is able to induce expression of genes that control virus replication and improve viability in different types of mature neurons.

IFN-γ signaling leads to cell-type-specific responses that depend on the levels of the IFN-γ receptor chains, the signaling pathways activated in the target cell, and the availability of coregulatory factors (35, 54, 65). During SINV-induced encephalomyelitis, IFN-γ is produced as a part of the adaptive immune response, which begins 3 to 4 days after infection (84). Therefore, for clearance of SINV from the CNS, IFN-γ must

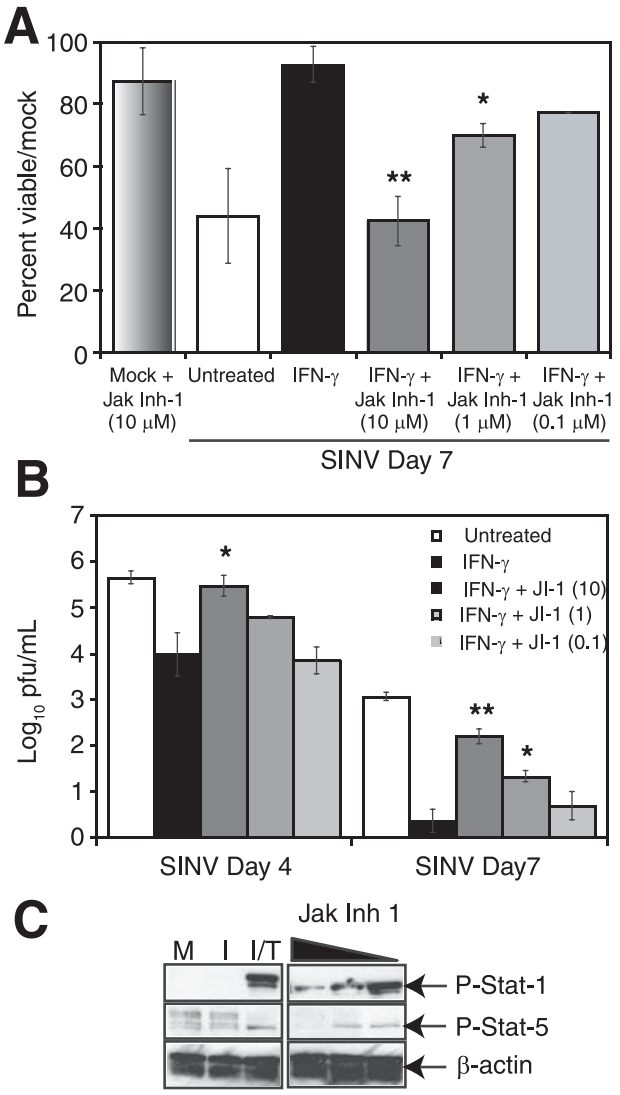

FIG. 5. Effect of Jak inhibitor I on virus replication and cell viability of SINV-infected, IFN-γ-treated, differentiated CSM14.1 cells. (A) Infected cells were treated with Jak inhibitor I (Inh-1) (10 μM, 1 μM, and 0.1 μM) for 16 h before IFN-γ treatment (500 U/ml) 24 h after infection. Cell viability was determined by trypan blue exclusion 7 days after infection. (B) Supernatant fluids collected 4 and 7 days after infection were analyzed for virus production by plaque assay. Each point represents the average and standard error of the mean of three individual wells. (C) Cell lysates were collected 2 h after IFN-γ treatment and analyzed for Stat-1 and Stat-5 phosphorylation by immunoblotting. M, mock infected; T, uninfected, IFN-γ treated; I, SINV infected, untreated; I/T, SINV infected, IFN-γ treated.

induce an antiviral response in neurons that are already infected, rather than protect neurons from becoming infected. The IFN-γ receptor is constitutively expressed in many neuronal populations, and IFN-γ can induce differentiation and improve survival, as well as induce an antiviral state (1, 4, 8, 24, 31, 58, 66, 67, 80). The importance of a direct role for IFN-γ in control of virus replication is increasingly recognized. Noncytolytic control of hepatitis B virus replication in hepatocytes is dependent primarily on production of IFN-γ (21, 49). Studies of mice deficient in production of IFN-γ or in expression of the IFN-γR have shown an important role for IFN-γ in protection from infection with a number of neurotropic viruses (27, 67, 73). Pretreatment of neuroblastoma cells and primary neonatal olfactory bulb neurons with IFN-γ induces phosphorylation of Stat-1 and an antiviral state that protects them from vesicular stomatitis virus infection (9).

Jak1/Jak2 activation leading to Stat-1 Tyr_{701} phosphorylation and nuclear translocation followed by Ser_{727} phosphorylation is the pathway responsible for most IFN-γ-dependent gene expression (61, 62, 68, 74). Mice deficient in Stat-1 are more susceptible than wild-type mice to a variety of RNA virus infections but are often less susceptible than mice deficient in expression of both the IFN-α/β and IFN-γ receptors, indicating the presence of Stat-1-independent mechanisms of protection (6, 15, 18, 25, 29, 36, 53, 72). In the current studies, both Stat-1 and Stat-5 were activated in uninfected and infected neurons by treatment with IFN-γ. Although Stat inactivation usually occurs within a few hours after phosphorylation through the activity of nuclear and cytoplasmic tyrosine phosphatases and the suppression of continued Stat-1 phosphorylation by SOCS (26, 45, 86), Stat-1 phosphorylation in infected neurons was sustained for at least 72 h after IFN-γ treatment. Prolonged activation in response to IFN-α in cells sensitive to the antiproliferative effect of IFN has also been observed (20). It is possible that prolonged activation is important for the shutdown of virus replication that progresses over several days (4).

Stat-1-independent pathways can also be important in selected biological functions attributed to IFN-γ signaling (18, 61–63). We also observed IFN-γ-mediated activation of the Stat-5 pathway in SINV-infected differentiated neurons. Stat-5 was first recognized as a prolactin- and growth hormone-induced factor in mammary glands (11, 59) but is also commonly activated in immune cells through activation of Jak3, associated with the common γ chain of interleukin-2 family cytokines (47, 71). In neurons, Stat-5 can be activated by a variety of hormones and growth factors by Jak2-dependent and -independent mechanisms (38, 59, 87). IFN-γ has not previously been reported to activate Stat-5 in neurons although transgenic mice expressing IFN-γ in the CNS show activation of Stat-5 (82). The importance of Stat-5 activation, in addition to Stat-1 activation, is unclear. Stat-5 can induce expression of Bcl-xL and XIAP, important for neuronal survival, and may contribute to the neuroprotective properties of IFN-γ in CSM14.1 cells (11, 47, 59, 60, 87). The more rapid turnover of Stat-5 than of Stat-1 may be due to transcriptional activation domain-mediated proteasomal degradation or differential use of phosphatases or SOCS proteins available in neurons (30, 86).

Alphaviruses shut off synthesis of cellular proteins, and this can affect availability of cellular factors for response to stimuli such as IFN (13). Stat-1 protein levels were decreased after infection but returned to baseline, along with evidence of some Stat-1 activation, beginning 12 to 24 h after infection. This decrease may be a result of virus-induced degradation or decreased synthesis of Stat-1 protein. Because generalized virus-induced shutoff of host protein synthesis in differentiated CSM cells is not evident until 36 to 48 h after infection (4, 79), decreased synthesis of Stat-1 seems unlikely. However, the decreases in levels of Stat-2 and Stat-3 that were observed at later times are consistent with an effect of virus infection on

host protein synthesis. IFN-γ treatment of infected CSM cells has a marked effect on viral and cellular protein synthesis within a few hours after treatment (4).

The antiviral proteins responsible for IFN-γ-mediated control of SINV replication are poorly characterized. As in other virus infections of the CNS, a large number of IFN-stimulated genes are expressed in response to infection (40, 81). An increase in nitric oxide synthase is important for inhibition of vesicular stomatitis virus replication, but the pathway by which IFN-γ signaling leads to protection is not clear (10). There is no evidence that the well-characterized PKR, Mx, and RNase L pathways are essential for control of SINV replication (70). IFN-induced antiviral proteins that have been implicated include ISG-15 and zinc finger antiviral protein, but their importance in neurons and mechanisms of action are unclear (2, 22, 41, 42). Future studies will be required to determine the downstream effectors of Jak/Stat-induced suppression of virus replication in neurons.

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