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Short Communication

Interferon-γ–induced inhibition of neuronal vesicular stomatitis virus infection is STAT1 dependent

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In this report, the signaling pathways utilized by interferon (IFN)-γ in neurons and their respective roles in the inhibition of vesicular stomatitis virus (VSV) replication were studied. The authors have previously shown that IFN-γ treatment of NB41A3 neuroblastoma cells results in a 2-log inhibition of VSV production. This inhibition of VSV replication is dependent both *in vitro* and *in vivo* on nitric oxide (NO) production by NO synthase (NOS)-1. In NB41A3 neuroblastoma cells, IFN-γ was found to induce the signal transducer and activator of transcription (STAT) STAT1 phosphorylation, interferon regulatory factor (IRF)-1 expression, and p42/p44 mitogen-activated protein kinase (MAPK) phosphorylation; MAPK, however, was not required for inhibition of viral replication. Using olfactory bulb–enriched primary neuronal cultures, the inhibition of VSV replication was found to be STAT1 dependent, but did not require IRF-1. *Journal of NeuroVirology* (2004) **10,** 57–63.

Keywords: interferon-gamma; IRF-1; MAPK; neuroimmunology; STAT-1; VSV

Viral infections of terminally differentiated neurons within the central nervous system (CNS) provide a unique challenge for the host immune response. T cell–induced cytolytic responses to virally infected cells would be disadvantageous due to the potential for loss of motor function or cognition resulting from targeted elimination of neurons Therefore, the host is better served by innate immune responses, which are antigen nonspecific and rapidly induced by infections and cytokine action (Bi *et al*, 1995; Guidotti and Chisari, 2001). Work from our laboratory has demonstrated the requirement for rapid and early innate responses to control viral infections in the CNS (Plakhov *et al*, 1995; Christian *et al*, 1996; Reiss *et al*, 1996; Komatsu *et al*, 1996).

Vesicular stomatitis virus (VSV), a single-stranded negative-sense RNA virus, provides an excellent model for the study of the immune response to viral infections in neurons of the CNS. With a genome containing an open reading frame encoding only five genes, VSV is a relatively simple virus. Intranasal infection of mice with VSV has been shown to lead to lethal infection of the CNS (Sabin and Olitsky, 1937), making VSV a versatile model for neurotropic viral infections (Huneycutt *et al*, 1993). Both *in vivo* and *in vitro*, proinflammatory cytokines, such as interleukin (IL)-12, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, have been found to inhibit VSV replication and promote host survival (Bi and Reiss, 1995; Reiss *et al*, 1996; Komatsu *et al*, 1996, 1997; Ireland *et al*, 1999).

IFN-γ is a biologically active, noncovalently linked, 34-kDa homodimer secreted primarily by natural killer (NK) cells, Th1 $CD4^+$ T cells, and $CD8^+$

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T cells (Farrar and Schreiber, 1993). IFN-γ treatment can reduce viral titers in infected neuronal cells *in vitro* by up to 100-fold compared with untreated cells, demonstrating its potent antiviral properties, which are mediated by nitric oxide (NO) (Komatsu *et al*, 1996). The antiviral effect is not restricted solely to VSV, but can be demonstrated in infections with other viruses, including herpes simplex virus (HSV)-1 and polio virus (Komatsu *et al*, 1996). For many viruses, the antiviral effect of IFN-γ involves the production of NO as an effector molecule (reviewed in Reiss and Komatsu, 1998).

IFN-γ signal transduction includes primary and secondary responses. The signal transducer and activator of transcription (STAT) STAT1 (Farrar and Schreiber, 1993) mediates the primary signaling response. After ligand engagement of the IFN-γ receptor, STAT1 is phosphorylated by the Janus kinases (JAKs) JAK1 and JAK2, dimerizes, and subsequently translocates to the nucleus where it induces transcription of a subset of IFN-γ–inducible genes. Included in that subset of genes are additional transcription factors, such as interferon regulatory factor (IRF)-1 and class II transactivator (CIITA), which are responsible for mediating secondary IFN-γ responses, including the up-regulation of major histocompatibility complex (MHC) I and II, expression of the chemokine IP-10, and antiviral mechanisms (Bach *et al*, 1997). Ancillary signaling pathways have also been described for IFN-γ. IFN-γ has been found to induce the phosphorylation of p42/p44 mitogen-activated protein kinase (MAPK) (ERK1/2) through Jak1 and Raf (Sakatsume *et al*, 1998); activation of Raf leads to cytoskeletal plasticity, frequently seen as morphological changes in IFN-treated cells.

Although the IFN-γ–mediated antiviral effects in neuronal infection have been widely studied (see Chesler and Reiss, 2002; Rottenberg and Kristensson, 2002 for review), the signaling pathways involved are not known. We performed this study to examine the mechanisms through which IFN-γ utilizes intracellular signaling cascades to inhibit the replication of VSV in neurons. IFN-γ treatment of neurons was found to activate multiple signaling cascades demonstrated by the phosphorylation of STAT1 and p42/p44 MAPK, and the up-regulation of IRF-1 protein expression. Although multiple signaling cascades were activated by IFN-γ, the IFN-γ–mediated inhibition of VSV replication was STAT1 dependent but required neither IRF-1 nor p42/p44 MAPK.

IFN-γ signaling through STAT1 and IRF-1 is conserved in neurons

The effects of IFN-γ following binding to its cell surface receptor are generally transduced through the activation of STAT1 and/or IRF-1 (Bach *et al*, 1997). Because neurons may not necessarily utilize the same signaling pathways as do lymphocytes or fibroblasts, we sought to determine if IFN-γ signaling pathways were conserved in neurons.

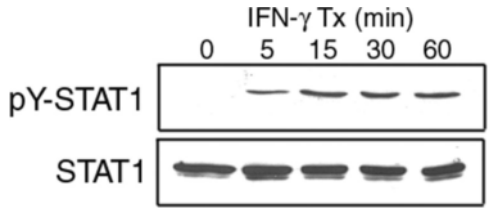

Figure 1 IFN-γ treatment induces STAT1 phosphorylation in NB41A3 cells. NB41A3 cells were treated with IFN-γ (R&D Systems; 20 ng/ml) for intervals from 0 to 60 min. Cells were lysed, equal amounts of protein resolved by 10% SDS-PAGE, and an immunoblot was performed with mAbs against phospho-STAT1 (Upstate Biotech; *top*) or STAT1α (Santa Cruz; *bottom*). Results are representative of three independent experiments.

For STAT1 signaling, NB41A3 cells were treated for intervals from 0 to 60 min with 20 ng/ml IFN-γ (R&D Systems) and STAT1 activation was determined by probing membranes with a phospho-specific STAT1 antibody (SantaCruz). Membranes were then stripped and reprobed with an antibody recognizing STAT1 independent of its phosphorylation state (SantaCruz). IFN-γ treatment resulted in rapid phosphorylation of STAT1 in NB41A3 cells and this observed increase was not due to increased STAT1 expression (Figure 1).

Two methods were employed to examine the activation of IRF-1 in NB41A3 cells. In the first approach, NB41A3 cells were treated with IFN-γ for intervals between 0 and 120 min and IRF-1 expression was determined by immunoblotting using an IRF-1–specific antibody (Santa Cruz). We found IFN-γ treatment induced the expression of IRF-1 in NB41A3 cells in a time-dependent fashion (Figure 2A). In the second approach, NB41A3 cells were cotransfected using the Lipofectamine reagent (Gibco BRL) with pIRFLuc, a pGL3 (Promega)–based luciferase reporter construct containing the GAS elements of the murine IRF-1 gene (Rogge *et al*, 1997), and pCMVβGal, a vector expressing the β-galactosidase gene under the constitutive control of the cytomegalovirus (CMV) early promoter (Holmes *et al*, 1996). Cells were treated for 24 h with medium or 10 ng/ml IFN-γ, and lysates were prepared using the Promega Reporter Lysis Buffer. Luciferase expression was determined using the Promega Luciferase Assay system following the manufacturer's instructions. β-Galactosidase expression was measured with the Tropix Galacto-Star reagent. Both luciferase and β-galactosidase assays were measured using a Beckman LS 6000LL liquid scintillation counter using the single-photon mode. Readings were taken for each sample over 1 min at 5 s intervals (20 readings per sample).

Luciferase activity in this assay indicated transcriptional up-regulation of IRF-1. In these reporter assay experiments, IFN-γ induced three times

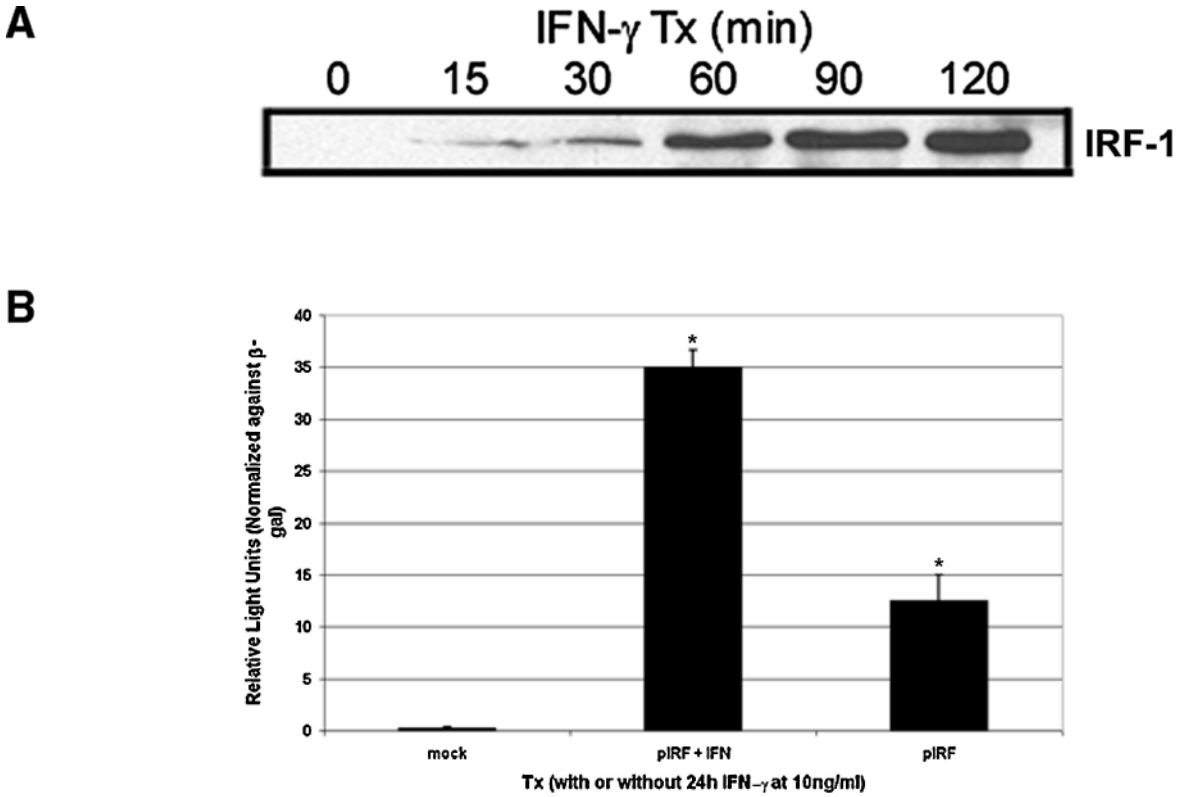

Figure 2 IFN-γ treatment induces IRF-1 expression in NB41A3 cells. (**A**) Induction of endogenous IRF-1 expression. IRF-1 expression was determined in NB41A3 cells by immunoblot. Results are representative of three independent experiments. (**B**) IRF-1 expression is STAT dependent. STAT1-dependent transcription of IRF-1 in NB41A3 cells was assessed using a luciferase assay. Data are expressed as relative light units ± SEM. Statistical analysis of the results was performed using the Mann-Whitney non-parametric test ($^*P < .05$ for pIRF versus pIRF + IFN).

more luciferase activity than medium-treated cells (Figure 2B). In other experiments, IFN-γ failed to induce luciferase activity in cells transfected with the luciferase plasmid lacking the IRF-1 GAS sequences (not shown), indicating the induction of IFN-γ–dependent, STAT-mediated IRF-1 transcription.

STAT1-dependent, IRF-1–independent inhibition of VSV replication

The role of STAT1 and IRF-1 signaling in the IFN-γ inhibition of VSV replication was examined by challenging primary neuronal cultures generated from STAT1$^{-/-}$ and IRF-1$^{-/-}$ mice with VSV following 24 h treatment with IFN-γ. C57BL/6J and IRF-1$^{-/-}$ (B6129S2*Irf*^{tmImak}) mice were obtained from Jackson Laboratories. BALB/c AnTac mice were obtained from Taconic Laboratories. STAT1$^{-/-}$mice, maintained on a BALB/c background, have been previously described (Durbin *et al*, 1996). All mice were bred and maintained in specific pathogen-free facilities at New York University in accordance with University Animal Welfare Committee guidelines.

Primary culture of murine olfactory bulb neurons was performed following a modification of published methods (Banker and Goslin, 1998; Egan *et al*, 1992; Cigola *et al*, 1998). Briefly, olfactory bulbs were dissected from P0 (<24 h old) C57BL/6J, BALB/c AnTac, STAT1$^{-/-}$, or IRF-1$^{-/-}$ mice, dissociated by enzymatic digestion (porcine trypsin 2.5%; Life Technologies, NY), and manual trituration using flame polished Pasteur pipettes. Cultures were plated on Poly-D-lysine–coated (70 to 150 kDa; Sigma) chamber slides in Dulbecco's minimal essential medium (DMEM) with 10% fetal bovine serum (FBS), 50 μg/ml gentamicin (Life Technologies, NY), 10 mM KCl, 10 μM L-glutamine, and 10 μM AraC (Sigma) for 36 h at 37°C/5% CO_2. After 36 h, the medium was replaced with Neurobasal Medium (Life Technologies, NY) with 1% N2 supplement (Life Technologies), 0.5 mM L-glutamine, 25 μM L-glutamic acid, 10 mM KCl, and 50 μg/ml gentamicin. Cultures were comprised of approximately 90% neurons determined by staining for MAP-2B (pAB, SantaCruz; not shown). The remaining 10% were comprised of cells such as activated astrocytes as determined by positive staining for glial fibrillary acidic protein (GFAP; pAb, SantaCruz; not shown). All experiments were performed on day 4 of culture.

Infectious virus was determined using a standard plaque assay on Chinese Hamster Overian (CHO) cell monolayers (Komatsu *et al*, 1996). Briefly, confluent monolayers of CHO cells on 24-well plates (Nunc) were infected with 0.1-ml serial dilutions of experimental samples at 37°C for 30 min. Following infection, virus was removed, 1 ml of a mixture of equal volumes of 1.8% agar and 2× Joklik's growth medium added to each well, and the wells were then incubated at 37°C for 20 h. Plaques were fixed with 10% buffered formalin for 45 min and stained with 0.5% methylene blue.

The antiviral effects of IFN-γ were absent in STAT1$^{-/-}$ cultures, which were also found to be more permissive to VSV infection (Figure 3A) Though IFN-γ induction of IRF-1 is present in neurons (Figure 2A), IRF-1 is not required for the IFN-γ–induced inhibition of VSV replication. IFN-γ treatment effectively inhibited VSV replication in both wild-type and IRF-1$^{-/-}$ cultures (Figure 3B).

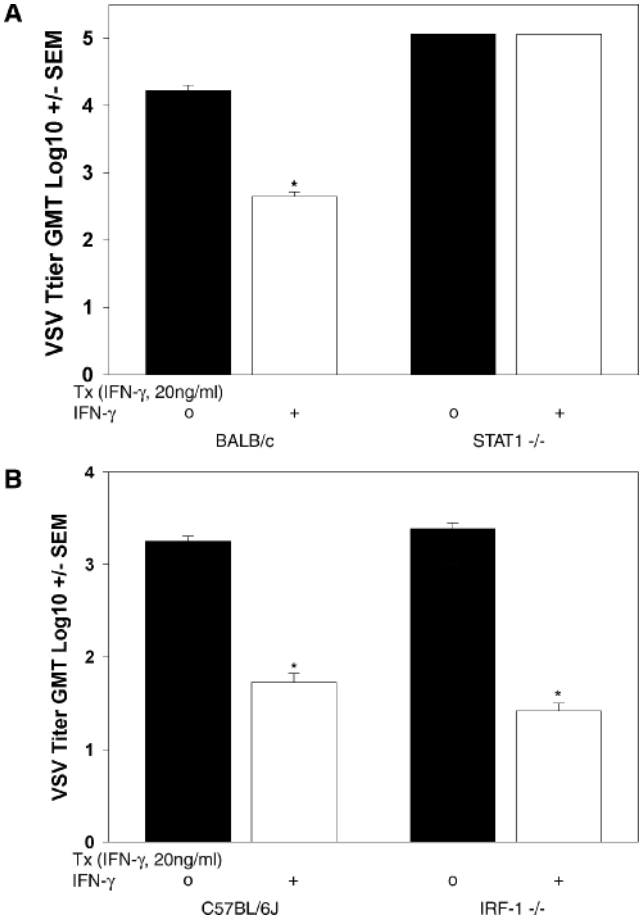

Figure 3 STAT1-dependent, IRF-1–independent inhibition of VSV replication in neurons. Olfactory bulb–enriched primary neuronal cultures generated from P0 BALB/c AnTac and STAT1$^{-/-}$ (**A**) or C57BL/6J and IRF-1$^{-/-}$ (**B**) mice were treated with medium or 20 ng/ml IFN-γ for 24 h and infected with 1×10^3 pfu VSV (~m.o.i. of 0.01). Eight hours post infection, viral supernatants were collected and viral titers determined by plaque assay on CHO cell monolayers. Data are represented as mean titers ± SEM. Statistical analysis of the results was performed using the Mann-Whitney nonparametric test ($^*P < .05$ versus medium treated). Results are representative of two independent experiments.

p42/p42 MAPK (ERK1/2) is phosphorylated in response to IFN-γ *in vitro* but is not required for observed antiviral effect

Several groups have demonstrated the activation of the p42/p44 MAPK (ERK1/2) by IFN-γ through a JAK1-dependent pathway. ERK1/2 have been demonstrated to have antiapoptotic properties in several systems (Stadheim and Kucera, 1998; Anderson and Tolkovsky, 1999). To determine if IFN-γ could activate ERK1/2 in neurons, NB41A3 cells were treated for intervals from 0 to 60 min with IFN-γ, or TNF-α as a positive control, and whole cell lysates were collected. Equal amounts of these lysates were resolved by (SDS-PAGE) and ERK1/2 activation was determined by immunoblot with a phospho-specific ERK1/2 antibody (Promega) or with an antibody recognizing ERK1/2-independent of its phosphorylation state (Promega). Increases in phosphorylated ERK above basal levels in as little as 15 min were detected. These changes were not associated with alteration in Erk expression levels by IFN-γ (Figure 4A).

To determine if the activation of ERK1/2 played a role in IFN-γ–mediated inhibition of VSV replication, the MEK inhibitor PD98059 (Calbiochem), which has been shown to block the activation of ERK1/2 (Waters *et al*, 1995), was utilized. Cells were treated with IFN-γ or medium for 24 h in the presence or absence of PD98059 and infected with VSV at a multiplicity of infection (m.o.i.) of 0.01. At 8 h post infection, viral supernatants were collected and viral titers determined as using a standard plaque assay as previously described (Komatsu *et al*, 1996). No difference was found in VSV titers with Erk blockade through treatment with PD98059 (Figure 4B). As determined by immunoblot, the dosage of PD98059 used effectively blocked the activation of ERK1/2 in NB41A3 cells (not shown). Thus, the activation of MAPK by IFN-γ was not necessary for the inhibition of VSV replication.

In this study, we examined the signaling pathways engaged by IFN-γ in neurons and their respective contribution to the inhibition of VSV replication following experimental infection. IFN-γ signaling can be divided into primary and secondary events. Initial signaling from the IFN-γ receptor takes place through Janus kinases and STAT1 (Levy, 1997; Bach *et al*, 1997). Activated STAT1 can in turn translocate to the nucleus, resulting in the transcription of IFN-inducible genes, including secondary signaling components, such as IRF-1, which is itself a transcription factor (Taniguchi *et al*, 2001). In addition, IFN-γ has been shown to

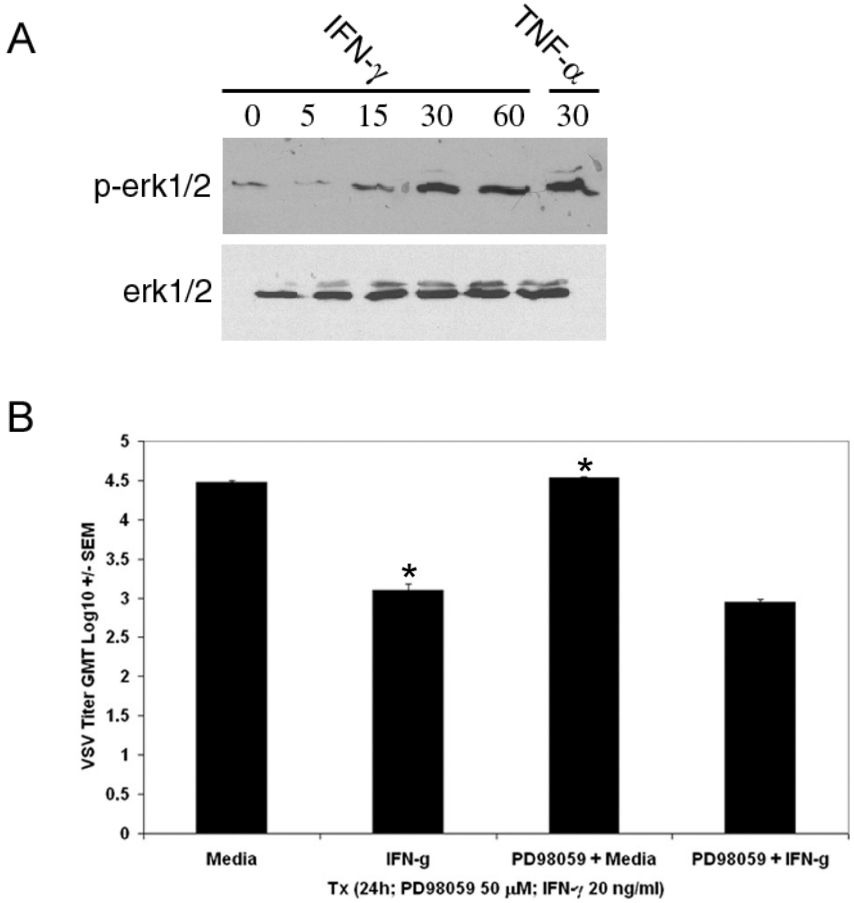

Figure 4 IFN-γ–induced phosphorylation of p42/p44 MAPK is not required for the inhibition of VSV replication. (**A**) p42/p44 MAPK activation was examined by immunoblot in IFN-γ and untreated NB41A3 cells. Results are representative of two independent experiments. (**B**) The contribution of p42/p44 MAPK in IFN-γ–induced inhibition of VSV in NB41A3 cells were determined by viral plaque assay. Data are represented as mean titers ± SEM. Statistical analysis was performed using the one-way ANOVA in conjunction with a Tukey's post hoc test for differences between groups ($^*P < .001$ versus medium treated). Results are representative of two independent experiments.

activate ERK1/2 through a Jak1-dependent mechanism and CCAAT/enhancer-binding-protein (Nishiya *et al*, 1997; Roy *et al*, 2000; Gil *et al*, 2001; Hu *et al*, 2001).

Consistent with the results of others examining other cell types, IFN-γ induces the rapid phosphorylation of both STAT1 (Figure 1) and ERK (Figure 4A) in NB41A3 neuroblastoma cells. IFN-γ induction of IRF-1 has been previously described in neuronal and glial cultures (Thomas *et al*, 1997). Increases in IRF-1 protein expression (Figure 2A) and STAT1-dependent transcription, measured through the use of a luciferase reporter construct containing the GAS sequences from the IRF-1 promoter, were found (Figure 2B) (Coccia *et al*, 1999).

The general action of IFN-γ requires the *de novo* synthesis of target gene mRNA and subsequent protein translation. In the case of NO synthase (NOS)-2, IFN-γ has been shown to induce its expression through IRF-1 and its subsequent activation of the NOS-2 promoter (Faure *et al*, 1999). Sequence analysis of available murine NOS-1 promoter sequences has revealed several putative GAS and IRF-1 binding sequences, though the contribution of these elements to the regulation of NOS-1 by IFN-γ appears unlikely, given the absence of changes in NOS-1 mRNA levels (Chesler and Reiss, 2002; Chesler *et al*, 2004; Sasaki *et al*, 2000). Consequently, in experiments using primary neuronal cultures, IRF-1 was not found to be essential for the IFN-γ–induced antiviral response to VSV infection (Figure 3). *In vivo*, STAT1 is required for the host immune response following systemic infection with VSV (Fernandez-Sesma *et al*, 1998), though this might not be the case in the CNS. IFN-γ–induced antiviral responses independent of STAT1 have been described. STAT1$^{-/-}$ mice challenged with Sendai virus or murine cytomegalovirus virus (MCMV) were only slightly more susceptible than their wild-type controls (Gil *et al*, 2001). This is in contrast to mice deficient in both the the IFN-α/β receptor and IFN-γ receptor, which were found to be exquisitely sensitive to challenge with both Sendai virus and MCMV (Gil *et al*, 2001). STAT1 expression

in primary neurons is required for the IFN-γ–induced antiviral effect against VSV (Figure 3A). In contrast to findings with Sendai or MCMV, neurons deficient in STAT1 were 10-fold more susceptible to VSV infection compared with wild-type primary neuronal cultures.

These studies demonstrate that IFN-γ–induced signaling is conserved in CNS neurons compared with peripheral cell types. STAT1 and p42/p44 MAPK phosphorylation (Figures 1 and 4A) and induction of IRF-1 protein expression (Figure 2) are observed following IFN-γ treatment. The ability of IFN-γ to inhibit VSV replication in neurons is STAT1 dependent (Figure 3A), but independent of both IRF-1 (Figure 3B) and p42/p44 MAPK (Figure 4B).

References

Anderson CN, Tolkovsky AM (1999). A role for MAPK/ERK in sympathetic neuron survival: protection against a p53-dependent, JNK-independent induction of apoptosis by cytosine arabinoside. *J Neurosci* **19:** 664–673.

Bach EA, Aguet M, Schreiber RD (1997). The IFN gamma receptor: a paradigm for cytokine receptor signaling. *Annu Rev Immunol* **15:** 563–591.

Banker G, Goslin K (1998). *Culturing nerve cells. 665*. Cambridge, MA: the MIT Press.

Bi Z, Barna M, Komatsu T, Reiss CS (1995). Vesicular stomatitis virus infection of the central nervous system activates both innate and acquired immunity. *J Virol* **69:** 6466–6472.

Bi Z, Reiss CS (1995). Inhibition of vesicular stomatitis virus infection by nitric oxide. *J Virol* **69:** 2208–2013.

Chesler DA, Reiss CS (2002). The role of IFN-gamma in immune responses to viral infections of the central nervous system. *Cytokine Growth Factor Rev* **13:** 441–454.

Chesler DA, McCutcheon JA, Reiss CS (2004). Post-transcriptional regulation of neuronal nitric oxide synthase by Interferon-gamma. *Journal of Interferon and Cytokine research* in press.

Christian AY, Barna M, Bi Z, Reiss CS (1996). Host immune response to vesicular stomatitis virus infection of the central nervous system in C57BL/6 mice. *Viral Immunol* **9:** 195–205.

Cigola E, Volpe BT, Lee JW, Franzen L, Baker H (1998). Tyrosine hydroxylase expression in primary cultures of olfactory bulb: role of L-type calcium channels. *J Neurosci* **18:** 7638–7649.

Coccia EM, Passini N, Battistini A, Pini C, Sinigaglia F, Rogge L (1999). Interleukin-12 induces expression of interferon regulatory factor-1 via signal transducer and activator of transcription-4 in human T helper type 1 cells. *J Biol Chem* **274:** 6698–6703.

Durbin JE, Hackenmiller R, Simon MC, Levy DE (1996). Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell* **84:** 443–450.

Egan TM, Dagan D, Kupper J, Levitan IB (1992). Properties and rundown of sodium-activated potassium channels in rat olfactory bulb neurons. *J Neurosci* **12:** 1964–1976.

Farrar MA, Schreiber RD (1993). The molecular cell biology of interferon-gamma and its receptor. *Annu Rev Immunol* **11:** 571–611.

Faure V, Hecquet C, Courtois Y, Goureau O (1999). Role of interferon regulatory factor-1 and mitogen-activated protein kinase pathways in the induction of nitric oxide synthase-2 in retinal pigmented epithelial cells. *J Biol Chem* **274:** 4794–4800.

Fernandez-Sesma A, Peluso RW, Bai X, Schulman JL, Levy DE, Moran TM (1998). Superantigen-activated T cells redirected by a bispecific antibody inhibit vesicular stomatitis virus replication in vitro and in vivo. *J Immunol* **160:** 1841–1849.

Gil MP, Bohn E, O'Guin AK, Ramana CV, Levine B, Stark GR, Virgin HW, Schreiber RD (2001). Biologic consequences of Stat1-independent IFN signaling. *Proc Natl Acad Sci U S A* **98:** 6680–6685.

Guidotti LG, Chisari FV (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* **19:** 65–91.

Holmes TC, Fadool DA, Levitan IB (1996). Tyrosine phosphorylation of the Kv1.3 potassium channel. *J Neurosci* **16:** 1581–1590.

Hu J, Roy SK, Shapiro PS, Rodig SR, Reddy SP, Platanias LC, Schreiber RD, Kalvakolanu DV (2001). ERK1 and ERK2 activate CCAAAT/enhancer-binding protein-beta-dependent gene transcription in response to interferon-gamma. *J Biol Chem* **276:** 287–297.

Huneycutt BS, Bi Z, Aoki CJ, Reiss CS (1993). Central neuropathogenesis of vesicular stomatitis virus infection of immunodeficient mice. *J Virol* **67:** 6698–6706.

Ireland DD, Bang T, Komatsu T, Reiss CS (1999). Delayed administration of interleukin-12 is efficacious in promoting recovery from lethal viral encephalitis. *Viral Immunol* **12:** 35–40.

Komatsu T, Barna M, Reiss CS (1997). Interleukin-12 promotes recovery from viral encephalitis. *Viral Immunol* **10:** 35–47.

Komatsu T, Bi Z, Reiss CS (1996). Interferon-gamma induced type I nitric oxide synthase activity inhibits viral replication in neurons. *J Neuroimmunol* **68:** 101–108.

Levy DE (1997). The house that Jak/Stat built. *Cytokine Growth Factor Rev* **8:** 81–90.

Nishiya T, Uehara T, Edamatsu H, Kaziro Y, Itoh H, Nomura Y (1997). Activation of Stat1 and subsequent transcription of inducible nitric oxide synthase gene in C6 glioma cells is independent of interferon-gamma-induced MAPK activation that is mediated by p21ras. *FEBS Lett* **408:** 33–38.

Plakhov IV, Arlund EE, Aoki C, Reiss CS (1995). The earliest events in vesicular stomatitis virus infection of the murine olfactory neuroepithelium and entry of the central nervous system. *Virology* **209:** 257–262.

Reiss CS, Komatsu T (1998). Does nitric oxide play a critical role in viral infections? *J Virol* **72:** 4547–4551.

Reiss CS, Komatsu T, Barna M, Bi Z (1996). Interleukin-12 promotes enhanced recovery from viral infection of neurons in the central nervous system. *Ann N Y Acad Sci* **795:** 257–265.

Rogge L, Barberis-Maino L, Biffi M, Passini N, Presky DH, Gubler U, Sinigaglia F (1997). Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J Exp Med* **185:** 825–831.

Rottenberg M, Kristensson K (2002). Effects of interferon-gamma on neuronal infections. *Viral Immunol* **15:** 247–260.

Roy SK, Wachira SJ, Weihua X, Hu J, Kalvakolanu DV (2000). CCAAT/enhancer-binding protein-beta regulates interferon-induced transcription through a novel element. *J Biol Chem* **275:** 12626–12632.

Sabin AB, Olitsky PK (1937). Influence of host factors on the neuroinvasiveness of vesicular stomatitis virus. Effect of age on the invasion of the brain by virus instilled in the nose. *J Exp Med* **66:** 15.

Sakatsume M, Stancato LF, David M, Silvennoinen O, Saharinen P, Pierce J, Larner AC, Finbloom DS (1998). Interferon gamma activation of Raf-1 is Jak1-dependent and p21ras-independent. *J Biol Chem* **273:** 3021–3026.

Sasaki M, Gonzalez-Zulueta M, Huang H, Herring WJ, Ahn S, Ginty DD, Dawson VL, Dawson TM (2000). Dynamic regulation of neuronal NO synthase transcription by calcium influx through a CREB family transcription factor-dependent mechanism. *Proc Natl Acad Sci U S A* **97:** 8617–8622.

Stadheim TA, Kucera GL (1998). Extracellular signal-regulated kinase (ERK) activity is required for TPA-mediated inhibition of drug-induced apoptosis. *Biochem Biophys Res Commun* **245:** 266–271.

Taniguchi T, Ogasawara K, Takaoka A, Tanaka N (2001). IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol* **19:** 623–655.

Thomas HE, Dutton R, Bartlett PF, Kay TW (1997). Interferon regulatory factor 1 is induced by interferon-gamma equally in neurons and glial cells. *J Neuroimmunol* **78:** 132–137.

Waters SB, Holt KH, Ross SE, Syu LJ, Guan KL, Saltiel AR, Koretzky GA, Pessin JE (1995). Desensitization of Ras activation by a feedback disassociation of the SOS-Grb2 complex. *J Biol Chem* **270:** 20883–20886.