Interferon-Gamma Prolongs Survival of Varicella Zoster Virus-Infected Human Neurons In Vitro

Nicholas L. Baird,a Jacqueline L. Bowlin,a Taylor J. Hotz,a Randall J. Cohrs,a,b Don Gilden,a,b,#

Departments of Neurologya and Immunology & Microbiologyb, University of Colorado School of Medicine, Aurora, Colorado, USA

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# Corresponding footnote:
Address correspondence to Don Gilden, don.gilden@ucdenver.edu.
ABSTRACT

Infection of human neurons in vitro with varicella-zoster virus (VZV) at low multiplicity of infection does not result in a cytopathic effect (CPE) within 14 days post-infection (dpi), despite production of infectious virus. We showed that by 28 dpi a CPE ultimately developed in infected neurons, and that interferon gamma not only inhibited the CPE, but also VZV DNA accumulation, transcription and virus production, thereby prolonging the life of VZV-infected neurons.
Varicella zoster virus (VZV) is a neurotropic alphaherpesvirus that causes varicella (chickenpox), after which virus becomes latent in ganglionic neurons along the entire neuraxis. As VZV-specific cell mediated immunity declines with age and in immunocompromised individuals, VZV reactivates to cause zoster (shingles) and other neurologic diseases (1, 2).

Interferon gamma (IFNγ) is a potent cytokine produced following primary VZV infection (3, 4). Non-human primates experimentally infected with simian varicella virus (SVV), the counterpart of VZV, produce IFNγ 7-11 dpi (5, 6). IFNγ also inhibits herpes simplex virus type-1 (HSV-1) infection in vitro (7) and in vivo (8-10). Furthermore, VZV reactivation correlates with a decline of VZV-specific IFNγ-producing immune cells (11).

VZV infection of human neurons in vitro is productive, although infected cells appear healthy 2 weeks later (12-14). Herein, we tested whether a cytopathic effect (CPE) eventually develops in VZV-infected neurons, and if so, whether IFNγ treatment suppresses viral growth and promotes neuronal survival after VZV infection.

Like ganglionic sensory neurons which express the IFNγ receptor on the cell surface (15), immunofluorescent staining revealed that the iPSC-derived neurons used in this study also expressed the IFNγ receptor (data not shown). To analyze the antiviral effects of IFNγ, cultures were either pretreated with tissue culture medium containing 10 ng/ml IFNγ or left untreated, followed by infection the next day with VZV at low MOI (0.001 to 0.0001; Zostavax, Merck) (14, 16, 17) for 3 hr in the absence of IFNγ. After infection, the inoculum was removed and replenished twice weekly for 14 days with medium containing or lacking 10 ng/ml IFNγ. Immunofluorescence analysis for VZV
immediate early (ORF63) and late (ORF68, gE) proteins at 14 dpi revealed that IFNγ greatly reduced VZV spread in neurons (Fig. 1).

Neurons infected with VZV at a low multiplicity of infection (MOI < 0.001) do not develop a CPE 14 days post-infection (dpi) and are indistinguishable from uninfected neurons (12, 14), despite extensive viral transcription (16), translation (12, 14) and production of infectious virus (17). However, by 28 dpi, a CPE did develop as evidenced by rounded cell bodies, and retracted and fragmented neurites resembling a string of beads (Fig. 2, top panel). In contrast, cells that were pretreated with IFNγ, infected and maintained in the presence of IFNγ for 28 days retained the healthy appearance of plump cell bodies and extended neurites (Fig. 2, bottom panel), features of uninfected neurons (14). Uninfected neurons treated with IFNγ for 28 days also maintained a healthy appearance and were indistinguishable from untreated cultures (data not shown).

The effects of IFNγ treatment on VZV replication and transcription were examined. Untreated and treated cells were infected, maintained as described above, and harvested at 28 dpi. DNA was extracted (GenElute; Sigma, St. Louis, MO) for quantification of viral genomes in 10 ng of total DNA using TaqMan-based qPCR targeted to a region within VZV ORF29 (Fig. 3, left panel) as described (17). At 28 dpi, IFNγ-treated VZV-infected neurons contained 4.4-fold fewer copies of VZV DNA than untreated neurons. To analyze VZV transcripts, total RNA was harvested (mirVana; Ambion, Foster City, CA) and mRNA from 100 ng total RNA was bound to oligo[dT] beads (µMACS; Miltenyi, Bergisch Gladbach, Germany), DNase-treated (DNasel; Ambion), eluted and reverse-transcribed (Transcriptor First cDNA synthesis kit; Roche,
Basel, Switzerland). Compared to untreated cultures, IFNγ treatment reduced the abundance of VZV transcripts: ORF62 (5.4-fold), ORF63 (11.1-fold), ORF29 (5.6-fold) and ORF68 (7.3-fold), encompassing immediate early, early and late kinetic classes (Fig. 3, right panel).

Production of infectious virus after IFNγ treatment was examined by releasing treated and untreated VZV-infected neurons with trypsin at 28 dpi. Neurons were then co-cultivated with uninfected fibroblasts and observed for plaque formation. At 28 dpi, when a CPE developed in untreated neurons, 69±1 plaque-forming units (PFU) were found compared to 31±5 PFU in IFNγ-treated cultures (Fig. 4).

Overall, IFNγ prolonged the life of VZV-infected neurons by inhibiting viral growth, reducing VZV genome content and transcript abundance and decreasing production of infectious virus. To develop an in vitro model of VZV latency, future studies will examine the effects of other interferons (α and β) as well as additional cytokines on VZV-infected neurons.
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REFERENCES


FIGURE LEGENDS

**FIG 1** Inhibition of VZV spread in human neurons by IFNγ. VZV-infected neurons were untreated (top) or treated with IFNγ (bottom) for 14 dpi, fixed and immunostained for an immediate early protein (VZV ORF63, green) and a late protein (VZV ORF68, red); DAPI staining is blue.

**FIG 2** Prevention of VZV-induced CPE in human neurons by IFNγ. (Top) At 28 dpi, CPE developed in VZV-infected neurons (top panel) characterized by rounding of cell bodies (dashed arrows) with retraction and fragmentation of neurites resembling a string of beads (solid arrows). VZV-infected neurons cultured in the presence of IFNγ remained healthy at 28 dpi (bottom panel) exhibiting large cell bodies (dashed arrows) and extensive neurite outgrowth that forms a mesh throughout the culture (solid arrows).

**FIG 3** Inhibition of VZV DNA accumulation and viral transcription in VZV-infected human neurons by IFNγ. VZV-infected human neurons were either untreated (black bars) or treated with IFNγ (grey bars) for 28 days, when the abundance of viral DNA (left panel) and transcripts (right panel) corresponding to VZV ORFs 62, 63, 29 and 68 was determined. Ct, cycle threshold. Data are mean ±SD Ct values from 2 independent cultures.

**FIG 4** Reduced production of infectious VZV in human neurons by IFNγ. VZV-infected human neurons were either untreated (black bar) or IFNγ-treated (grey bar) for 28 days.
At 28 dpi, when a CPE developed in untreated neurons, plaque-forming units (PFU) were determined. Data are mean ± SD from 2 independent cultures.