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Interferon Lambda Inhibits Herpes Simplex Virus Type I Infection of Human Astrocytes and Neurons

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Abstract

Herpes simplex virus type I (HSV-1) is a neurotropic virus that is capable of infecting not only neurons, but also microglia and astrocytes and can establish latent infection in the central nervous system (CNS). We investigated whether IFN lambda (IFN- λ), a newly identified member of IFN family, has the ability to inhibit HSV-1 infection of primary human astrocytes and neurons. Both astrocytes and neurons were found to be highly susceptible to HSV-1 infection. However, upon IFN- λ treatment, HSV-1 replication in both astrocytes and neurons was significantly suppressed, which was evidenced by the reduced expression of HSV-1 DNA and proteins. This IFN- λ -mediated action on HSV-1 could be partially neutralized by antibody to IFN- λ receptor. Investigation of the mechanisms showed that IFN- λ treatment of astrocytes and neurons resulted in the upregulation of endogenous IFN- α/β and several IFN-stimulated genes (ISGs). To block IFN- α/β receptor by a specific antibody could compromise the IFN- λ actions on HSV-1 inhibition and ISG induction. In addition, IFN- λ treatment induced the expression of IFN regulatory factors (IRFs) in astrocytes and neurons. Furthermore, IFN- λ treatment of astrocytes and neurons resulted in the suppression of suppressor of cytokine signaling 1 (SOCS-1), a key negative regulator of IFN pathway. These data suggest that IFN- λ possesses the anti-HSV-1 function by promoting type I IFN-mediated innate antiviral immune response in the CNS cells.

Keywords

IFN- λ ; HSV-1; IFN- α/β ; IFN regulatory factor; SOCS-1

INTRODUCTION

Interferon lambda (IFN- λ), also known as type III IFNs, is a newly discovered sub-family of IFN consists of three structurally related IFN- λ molecules called IFN- λ 1, IFN- λ 2 and IFN- λ 3 (also termed IL29, IL28A and IL28B, respectively) (Kotenko et al. 2003; Sheppard et al. 2003). Although they have low sequence homology, IFN- λ s exhibit a number of biological characteristics that are similar to IFN- α/β , including antiviral activity, antiproliferative activity and *in vivo* antitumour activity. Viral infections and Toll-like receptor (TLR) ligands (poly I:C, lipopolysaccharide or CpG) that can induce IFN- α/β expression also stimulate

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IFN- λ expression (Baccala et al. 2005; Theofilopoulos et al. 2005). Similar to type I IFNs, IFN- λ was able to inhibit replication of a number of viruses in various cell lines (Ank and Paludan 2009). IFN- λ also had antiviral effect *in vivo* in mouse models (Ank and Paludan 2009; Robek et al. 2005). Although IFN- λ was found to exert limited activity against herpes simplex virus 2 (HSV-2) *in vitro*, *in vivo* study showed that IFN- λ had the ability to reduce hepatic viral titer of HSV-2 and completely blocked HSV-2 replication in vaginal mucosa. This discrepancy between *in vivo* and *in vitro* study suggests an involvement of immune modulation in IFN- λ 's antiviral effect. In addition, IFN- λ was found to have potent antiviral activity against infection of other members of herpes virus family, such as cytomegalovirus (CMV) (Ank and Paludan 2009). However, it is unclear whether IFN- λ has the ability to inhibit HSV-1 infection of human astrocytes and neuronal cells.

HSV-1 is a common human pathogen infecting 40–80% of people worldwide (Gupta R 2007). HSV-1 is a neurotropic virus that is capable of infecting not only neurons but also microglia and astrocytes (Carr et al. 2001; LaVail et al. 2005; Lokensgard et al. 2001; Marques et al. 2006). Although primary neuron infection with HSV-1 is limited, HSV-1 can gain access to sensory neurons following primary infection of epithelial surface and spread to other neurons, resulting a lifelong latency inside central nervous system (CNS) (Becker 2002; SC. 1996). During infection, HSV-1 recognition is mainly accomplished by several TLRs, including TLR2, TLR3 and TLR9 (Kurt-Jones et al. 2004; Sergerie et al. 2007). Thus, a type I IFN-dependent mechanism is likely to be responsible for the protective effect against HSV-1 infection in the CNS. It has been reported that type I IFN proteins are crucial for HSV clearance (Alexopoulou et al. 2001; Doyle et al. 2003; Gill et al. 2006). Although studies *in vitro* and *in vivo* have highlighted the importance of type I IFNs, particularly in the restriction of viral infections in the CNS (Paul et al. 2007), there is little information about the role of IFN- λ in protecting human astrocytes and neurons from viral infections. We thus examined whether IFN- λ has antiviral activity against HSV-1 infection of primary human astrocytes and neurons. We also determined the mechanisms involved in the antiviral function of IFN- λ .

Materials and Methods

Reagents

Recombinant human IFN- λ 1 and IFN- λ 2 were purchased from PeproTech Inc. (Rocky Hill, NJ). Goat anti-HSV-1 polyclonal antibody was purchased from Chemicon International Inc. (Temecula, CA). Donkey anti-goat IgG was from Molecular Probes (Eugene, OR). Antibody to IL-10R β was purchased from R&D Systems Inc. (Minneapolis, MN). Antibody to IFNAR was purchased from LifeSpan BioScience, Inc. (Seattle, WA).

Primary Human Astrocyte Culture

Primary human astrocyte cultures were prepared as previously described (Peterson et al. 1997). Briefly, brain tissues from 16-to-20-week-old aborted fetuses were dissociated by trypsinization (0.25%) for 30-min and plated into 75-cm² Falcon tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μ g/mL). Cell cultures were maintained at 37°C in a humidified 5% CO₂ atmosphere for three weeks with weekly medium changes. On day 21, flasks were shaken, washed and trypsinized with 0.25% trypsin in Hank's buffered salt solution (HBSS) for 30 min at 37°C. After adding FBS (final concentration 10%), centrifugation, and washing, cells were seeded into new flasks with DMEM followed by medium change after 24 h. The subculture procedure was repeated four times at a weekly interval. Astrocyte cultures were comprised of cells that were >99% glial fibrillary acidic protein (GFAP)-positive.

Primary Human Neuron Culture

Human brain tissues were obtained from the National Neurological Research Specimen Bank (Los Angeles, CA, USA). Highly enriched primary neuronal cell cultures were prepared from 16- to 18-week old human fetal brain tissues, as described previously (Hu et al. 2002). In brief, human fetal brain cortical tissue obtained under a protocol approved by the Human Subjects Research Committee of the University of Minnesota was dissociated, trypsinized and resuspended in DMEM containing 10% FBS plus penicillin (100 U/mL) and streptomycin (100 µg/mL) and plated into collagen-coated flasks. After 1 week, cells were dispersed and plated onto poly-D-lysine (PDL)-coated plates (5×10^5 , 10^6 , or 2.5×10^6 cells/well in 24-, 12-, or 6-well plates, respectively) or chamber slides (4×10^5 cells/well on Lab-Tek chamber slide). On day 5, cell cultures were treated with uridine (33.6 µg/mL) and fluorodeoxyuridine (13.6 µg/mL), followed by replacement with DMEM with 10% FBS on day 6 and every 4 days thereafter.

Virus Preparation

HSV-1 strain 17 syn⁺ was kindly provided by Dr. Jim Lokensgard (the University of Minnesota Medical School). The virus was propagated on confluent rabbit skin fibroblasts in complete DMEM. Infected fibroblast culture was harvested at 80%–100% cytopathic effect and subjected to three freeze-thaw cycles. Cellular debris was removed by low-speed centrifugation ($1000 \times g$) at 4°C. The supernatant was removed and centrifuged at $23000 \times g$ for 2 h at 4°C in 35% sucrose cushion buffer (in Tris-buffered saline; 50 mM Tris-HCl and 150 mM NaCl; pH7.4). The virus pellet was resuspended in DMEM and aliquots were stored at −80°C. Viral stocks were tittered by plaque assay on rabbit skin fibroblasts.

IFN-λ Treatment and HSV-1 Infection

The anti-HSV-1 activity of recombinant IFN-λ was assessed in primary human astrocytes and neurons. Cells were treated with IFN-λ1 (100 ng/ml) or IFN-λ2 (100 ng/ml) for 24 h prior to HSV-1 infection (MOI=0.01) for 90 min. The cells were then washed with plain medium without FBS and maintained in fresh complete medium for up to 72 h. HSV-1 replication was analyzed by the real-time PCR detection of HSV-1 glycoprotein D (gD) DNA (GenBank accession No.x14112) expression.

RNA/DNA Extraction and Real-Time PCR

Total RNA was extracted from cell culture with Tri-Reagent (Molecular Research Center, Cincinnati, OH), following the manufacturer's instructions. RNA samples were treated with deoxyribonuclease I (DNase I; Invitrogen Life Technology) according to the manufacturer's instruction. Cellular DNA were extracted from cells lysed in a buffer containing 100 mM KCl, 20 mM Tris, pH 8.4, 500 µg/ml proteinase K (BDH, Poole, UK), and 0.2% (v/v) NP-40 (BDH). Lysates were incubated at 60°C for 2 h followed by 100°C for 15 min. The real time PCR was described previously (Guo et al. 2004). The primer sequences (Table 1) for HSV-1 gD, IFN-α/β, IFN-regulatory factors (IRFs), myxovirus resistance A (MxA), 2'-5'-oligoadenylate synthetase 1 (OAS-1), protein kinase (PKR), and IFN-stimulated gene 56 (ISG56) were synthesized by Integrated DNA Technologies Inc. (IDT; Coralville, IA). PCR was performed with the Brilliant SYBR Green Master Mix (Bio-Rad Laboratories, Hercules, CA). A melting curve analysis was performed to assess primer specificity and product quality by step-wise denaturation of the PCR product at a rate of 0.1°C/sec to 95°C. All values were normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) level. The relative expression levels of other genes were analyzed using the $2^{-\Delta\Delta C_t}$ method.

Immunofluorescence Assay

Primary astrocytes or neurons were cultured on chamber slides or glass coverslips in 24-well plate. After treatment and infection, cells were washed with $1\times$ ice-cold PBS (with Ca^{2+} and Mg^{2+}) twice, then fixed at 4°C in 4% paraformaldehyde plus 4% sucrose in PBS for 30 min. Subsequently, the cells are permeated with 0.2% Triton X-100 in PBS on ice for additional 10 min. Cells were blocked in Block Solution (Pierce, Rockford, IL) for 1 h at room temperature. The cells were then incubated at room temperature with goat polyclonal antibody against HSV-1 gD (1:200) for 1 h. After three times wash with PBS, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated donkey anti-goat IgG (1:250) for 1 h. For additional Hoechst staining, $1\mu\text{g}/\text{ml}$ of Hoechst was used to incubate the cells for 1 min. Cells were then viewed under a fluorescence microscope (Olympus IX71, Japan).

Data Analysis

Where appropriate, data were expressed as mean $\pm$ SD. For comparison of the mean of the multiple groups, statistical significance was measured by one way analysis of variance (ANOVA) and Newman Keul's posttest. Statistical significance was defined as $P < 0.05$.

RESULTS

HSV-1 Infection of Primary Human Astrocytes and Neurons

HSV-1 is a neurotropic virus that has the ability to establish latent infection in the nervous system, following productive infection of permissive cells at the periphery (Shimeld et al. 2001). To determine whether HSV-1 can infect primary human astrocytes and neurons, we incubated the cells with HSV-1 17 syn⁺, a laboratory-adopted strain. As shown in Fig. 1, there was a gradual increase in HSV-1 gD gene expression in both astrocytes and neurons during the course of infection. In addition, following HSV-1 infection, astrocytes became rounding and detached, presenting typical cytopathic effects of HSV-1 infection (Fig. 1A insert), while neuronal cells showed significant morphologic change, including loss of axon terminal and dendrite, expansion of cell body, compared with uninfected control (Fig. 1B insert).

IFN- λ Inhibits HSV-1 Infection of Astrocytes and Neurons

We next examined the ability of IFN- λ to inhibit HSV-1 replication in astrocytes and neurons. Both IFN- $\lambda 1$ and IFN- $\lambda 2$ significantly inhibited HSV-1 gD DNA synthesis in astrocytes (Fig. 2A and 2C) and neurons (Fig. 2B and 2D). This inhibition on HSV-1 gD DNA synthesis by IFN- λ was dose- and time-dependent (Fig. 2). The inhibitory effect of IFN- λ on HSV-1 was not due to cytotoxicity to astrocytes and neurons, since IFN- λ at the concentration of 1000 ng/ml or lower did not induce the cell death (data not shown). IFN- λ -mediated inhibition of HSV-1 was also evidenced by the observation that IFN- $\lambda 1$ or IFN- $\lambda 2$ -treated cells expressed lower levels of HSV-1 proteins than untreated control cells (Fig. 3).

Anti-HSV-1 Effect of IFN- λ is Mediated through Its Receptor

IFN- λ exerts its biological function through a heterodimeric receptor complex, composed of IL-28R α and IL-10R β . We have previously demonstrated the expression of both IL-28R α and IL-10R β in human macrophages (Zhou et al. 2009), brain tissue and neuronal cells (Zhou et al. 2009). To examine the functional relevance of IFN- λ receptor for IFN- λ -mediated anti-HSV-1 effect, we incubated astrocytes and neurons with antibody against the extracellular domain of IL-10R β prior to IFN- λ treatment for 2 h, followed by HSV-1 infection. As shown in Fig. 4, antibody to IL-10R β partially blocked the ability of both IFN- $\lambda 1$ and IFN- $\lambda 2$ to inhibit HSV-1 replication in astrocytes (Fig. 4A) and neurons (Fig. 4B), while the antibody alone or control IgG had little effect.

IFN- λ Activates Type I IFN Pathway

IFN- α/β system plays an essential role in the innate immunity of host cells against viral infections. We and others have shown that human astrocytes and neurons express type I IFNs and IFN-associated antiviral factors (Paul et al. 2007; Wan et al. 2008; Wang et al. 2009). We thus examined whether IFN- λ has the ability to activate type I IFN pathways, inducing the expression of IFN- α/β and IFN-stimulated genes (ISGs) in astrocytes and neurons. We found that IFN- λ treatment of astrocytes selectively induced endogenous IFN- α and IFN- β expression (Fig. 5A), while in neurons, IFN- λ only induced IFN- α expression (Fig. 5C). We next evaluated the ability of IFN- λ to induce the expression of ISGs in astrocytes and neurons. We showed that IFN- λ treatment of both astrocytes and neurons induced the expression of ISGs, including ISG56, MxA, and OAS-1 (Fig. 5B and 5D). In order to determine the role of endogenous IFN- α/β in the IFN- λ action on HSV-1, we used antibody to IFN- α/β receptor (IFNAR) to treat astrocytes and neurons prior to IFN- λ treatment. As shown in Fig. 6A and 6B, to block IFN- α/β receptor compromised the anti-HSV-1 effect of IFN- λ in astrocytes and neurons. We also examined whether the induction of ISGs by IFN- λ treatment is a direct result of IFN- λ -induced IFN- α/β . It was found that antibody to IFN- α/β receptor compromised IFN- λ -mediated ISG induction in astrocytes and neurons.

IFN- λ Modulates the Key Regulators of IFN- α/β

To determine the mechanisms involved in the IFN- λ action on endogenous IFN- α/β expression in neurons and astrocytes, we investigated whether IFN- λ has the ability to induce the expression of IFN regulatory factors (IRFs), the key positive regulators of type I IFNs. It was observed that IFN- λ treatment of astrocytes induced the expression of IRF, IRF5 and IRF7 (Fig. 7A). However, IFN- λ treatment of neurons only induced IRF7 expression (Fig. 7B). To further determine the mechanism involved in IFN- λ -mediated induction of IFN- α/β , we examined the impact of IFN- λ on the expression of suppressor of cytokine signaling (SOCS) proteins, the key negative regulators in IFN signaling pathway, showing that IFN- λ treatment selectively downregulated the SOCS-1 expression in astrocytes (Fig. 7C) and neurons (Fig. 7D).

DISCUSSION

In the present study, we examined whether IFN- λ , a newly identified member of IFN family, has the ability to inhibit HSV-1 infection of human astrocytes and neurons. In agreement with the study (Cheeran et al. 2000), we demonstrated that both astrocytes and neurons could be efficiently infected by HSV-1, which was evidenced by the detection of HSV-1 DNA and protein expression in these cells (Figs. 1 and 3). In the presence of IFN- λ , HSV-1 replication, however, was suppressed in these cells (Fig. 2). This finding is supported by a recent report (Melchjorsen et al. 2006) showing IFN- λ 1 exerts potential antiviral activity against HSV-1 replication in human macrophages and dendritic cells by repressing HSV-1 ICP27 gene transcription. The anti-HSV-1 effect by IFN- λ is IFN- λ receptor (IL-10R β) mediated, as antibody to IL-10R β could block the IFN- λ action on HSV-1. This receptor-mediated ability to respond to IFN- λ treatment by human astrocytes and neurons supports our earlier study showing that human neurons express functional IFN- λ receptors, IL-28R α and IL-10R β (Zhou et al. 2009).

Host cell innate immune responses play a vital role in suppressing HSV-1 infection and even infected, maintaining HSV-1 in a quiescent status. Among these responses, the production of type I IFNs is of the most importance in controlling viral replication (Hochrein et al. 2004). It has been known for several decades that IFN- α/β , secreted by blood cells and fibroblasts, have potent anti-HSV activity (Koelle and Corey 2008). The importance of type I IFN in the

host response to HSV infection is highlighted by the findings that there is enhanced virulence of HSV in type I IFN-receptor-deficient mice (Leib 2002), and that several HSV proteins have the specific type I IFN evasion functions. Type I IFN-mediated innate immunity also has a key role in restricting viral infections in the CNS (Paul et al. 2007). It has been demonstrated that type I IFNs can inhibit transmission of HSV-1 from neuronal axon to epidermal cells and subsequent spread in these cells (Mikloska and Cunningham 2001). Thus, the induction of intracellular IFN- α/β expression by IFN- λ provides a plausible mechanism for the anti-HSV-1 action of IFN- λ in astrocytes and neurons. The role of type I IFNs in IFN- λ -mediated action on HSV-1 inhibition was confirmed by the observation that antibody to IFN- α/β receptor could compromise the IFN- λ action in astrocytes (Fig. 6A) and neurons (Fig. 6B).

To further determine the underlying mechanism of anti-HSV-1 effect by IFN- λ , we examined whether IFN- λ has the ability to activate IFN pathways in astrocytes and neurons. We found that IFN- λ treatment of astrocytes and neurons induced the expression of ISGs (MxA, OAS-1, and ISG56), which could be compromised by antibody to IFN- α/β receptor (Fig. 6). This finding supports the role of IFN- α/β in the IFN- λ actions on ISGs. However, because of the lack of a complete blockage of the IFN- λ action by the antibody, it is likely that other mechanism(s) are also involved in the IFN- λ action. One possibility is that IFN- λ has the ability to directly activate ISGs and other antiviral factors (Brand et al. 2005; Li et al. 2009). It has been shown that although IFN- λ use the receptor complex different from that of type I IFNs, the activation of IFN- λ receptor leads to the expression of type I IFN-induced antiviral genes (Dumoutier et al. 2004). The investigation from several group showed that IFN- λ -mediated antiviral activity is linked to its ability to activate ISG3 and several antiviral genes in human hepatocytes (Doyle et al. 2006) and several carcinoma cell lines (Dumoutier et al. 2004). Taken together, these findings observed in different model systems support the notion that IFN- λ functionally resembles type I IFNs, inducing the expression of ISGs and resulting in the establishment of an antiviral state.

Type I IFN gene expression is tightly regulated by the transcription factors such as IFN-regulatory factors (IRFs). Therefore, we examined the effect of IFN- λ on the induction of IRFs in astrocytes and neurons. Our data that the selective induction of IRF7 expression by IFN- λ in neurons provides a sound mechanism for the positive action of IFN- λ on IFN- α expression, as IRF7 is a master regulator of IFN- α (Honda et al. 2005). Whereas in astrocytes, in addition to IRF7, the upregulation of IRF3 and IRF5 by IFN- λ treatment was also observed. This finding was in parallel with the observation that IFN- λ induced not only IFN- α but also IFN- β expression in astrocytes, as IRF3 is a key positive regulator of IFN- β . We also determined the impact of IFN- λ on the expression of the key negative regulators in IFN- α/β signaling pathway, as IFN- λ has the ability to activate JAK-STAT pathway (Dumoutier et al. 2004; Kotenko et al. 2003; Sheppard et al. 2003), which is highly regulated by type I IFNs. The suppressor of cytokine signaling (SOCS) is a group of key negative regulators of the IFN- α -triggered STAT transactivation. It has been reported that SOCS-1 and SOCS-3 could be induced by HSV-1 infection, leading to a high preference of viral replication (Yokota et al. 2005). Therefore, we investigated whether IFN- λ treatment could inhibit SOCS expression. The finding that IFN- λ treatment selectively inhibited SOCS-1 expression provides an additional mechanism responsible for the IFN- λ action on the induction of IFN- α/β and ISGs in astrocytes and neurons.

Collectively, although the accurate mechanisms for IFN- λ -mediated suppression on HSV-1 replication in astrocytes and neurons remain to be determined, our data that IFN- λ has the ability to activate type I IFN pathway, leading to the induction of IFN- α/β and IFN-stimulated antiviral genes provide a sound mechanism responsible for the IFN- λ action on HSV-1 inhibition. Future animal and *in vivo* studies are necessary to determine whether

IFN- λ -mediated antiviral function is beneficial for human therapy against HSV-1 infections in the CNS. In addition, further research into the fundamental and biological functions of IFN- λ also is a key to address the role of IFN- λ in the host response to viral infections and to understand the mechanisms of IFN- λ actions at both the molecular and cellular levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. HSV-1 infection of astrocytes and neurons

Primary human astrocytes (A) and neurons (B) cultured in 24-well plates (5×10^5 cells/well) were infected with HSV-1 17 syn⁺ strain (MOI=0.01) for 90 min. At the indicated time points postinfection, genomic DNA was extracted and subjected to the quantitative real-time PCR for HSV-1 gD gene expression. Data were expressed as HSV-1 gD DNA copies per 1E+4 GAPDH copies. Representative data of three independent experiments are shown. Inserts are the morphological view of astrocytes and neurons infected with either UV-inactivated HSV-1 (mock) or alive HSV-1, which was observed at 72 h postinfection (magnification: $\times 100$).

Fig. 2. Antiviral activity of IFN-λ to HSV-1 replication

A and B: Dose-dependent anti-HSV-1 effect. Primary human astrocytes (A) and neurons (B) were cultured in 24-well plates at 5×10^5 cells per well and pretreated with IFN-λ1 or IFN-λ2 at indicated concentrations for 24 h followed with HSV-1 17 syn⁺ (MOI=0.01) infection for 90 min. At 72 h postinfection, genomic DNA was extracted and subjected to the real-time PCR for HSV-1 gD gene expression. C and D: Time-dependent anti-HSV-1 effect.

Astrocytes (C) and neurons (D) were pretreated with IFN-λ1 (100 ng/ml) or IFN-λ2 (100 ng/ml) for 24 h followed with infection with HSV-1 17 syn⁺ (MOI=0.01) for 90 min. At indicated time points postinfection, genomic DNA was extracted and subjected to the real-time PCR for HSV-1 gD gene expression. HSV-1 replication in treated cultures was expressed as percentage of HSV-1 gD levels relative to control (without treatment, which is defined as 100%). Data are expressed as mean $\pm$ SD of three different experiments. * $P < 0.05$; ** $P < 0.01$.

Fig. 3. IFN- λ inhibits HSV-1 protein expression

Primary human astrocytes (A) and neurons (B) pretreated with or without IFN- λ 1 (100 ng/ml) or IFN- λ 2 (100 ng/ml) for 24 h were infected with HSV-1 17 syn+ strain (MOI=0.01) for 90 min. UV-inactivated HSV-1 (mock)-infected cells were used as control. At 72 h postinfection, cells were fixed and the expression of HSV-1 proteins was determined by immunocytochemistry using a polyclonal anti-HSV-1 antibody. Representative data from three independent experiments were shown (magnification: $\times 100$).

Fig. 4. Effect of antibody against IL-10Rβ on IFN-λ-mediated anti-HSV-1 activity

Primary human astrocytes (A) and neurons (B) were pretreated with antibody to IL-10Rβ (5 μg/ml) or control IgG for 1 h followed with IFN-λ1 (100 ng/ml) or IFN-λ2 (100 ng/ml) treatment for 24 h prior to HSV-1 infection (MOI=0.01) for 90 min. HSV-1 replication was measured by the real-time PCR for HSV-1 gD gene expression at 72 h postinfection. Value is mean ± SD of three different cultures. * $P < 0.05$.

Fig. 5. Effect of IFN-λ on the expression of type I IFNs and IFN-stimulated genes (ISGs)
 A and C: Effect of IFN-λ on type I IFN expression. Primary human astrocytes (A) and neurons (C) were treated with or without IFN-λ1 or IFN-λ2 at indicated concentrations for 24 h and RNA was extracted for type I IFN expression. B and D: Effect of IFN-λ on ISG expression. Primary human astrocytes (B) and neurons (D) were treated with or without IFN-λ1 or IFN-λ2 at 100 ng/ml for 24 h and RNA was extracted for real-time PCR of ISG expression. Data shown were normalized based on the GAPDH expression and expressed as mean ± SD. * $p < 0.05$; ** $p < 0.01$.

Fig. 6. Effect of antibody to IFN- α/β receptor on the IFN- λ actions on HSV-1 and ISGs
 A and B: Primary human astrocytes (A) and neurons (B) were pretreated with antibody to IFNAR (5 μ g/ml) or control IgG for 1 h followed with IFN- λ treatment for 24 h prior to HSV-1 infection (MOI=0.01) for 90 min. HSV-1 replication was measured by the real-time PCR for HSV-1 gD gene expression at 72 h postinfection. C-H: Primary human astrocytes (C, E, G) and neurons (D, F, H) were pretreated with antibody against IFNAR (5 μ g/ml) or control IgG for 1 h prior to the addition of IFN- λ to the cultures. After 24 h treatment, RNA was extracted and ISG mRNA expression was examined. Data shown were normalized based on the GAPDH expression and expressed as mean $\pm$ SD. * p <0.05.

Fig. 7. Effect of IFN-λ on the key regulators of type I IFNs

Primary astrocytes and neurons were treated with or without IFN-λ1 or IFN-λ2 (100 ng/ml) for 24 h. RNA was then extracted from the cells and subjected to the RT-PCR analysis of IRFs and SOCSs as indicated; Data shown were normalized based on the GAPDH expression and expressed as mean $\pm$ SD. * p <0.05; ** p <0.01.

Table 1

Primers for Real-Time PCR

Primer	Sense	Antisense
GAPDH	5' - GGTGGTCTCTCTGACTTCAACA -3'	5' - GTTGCTGTAGCCAAATTCGTTGT -3'
HSV-1 gD	5' - ATCCGAACGCAGCCCCGC -3'	5' - TCCTCCGTCCAGTCGTTTAT -3'
IFN-α	5' - TTTCTCTGCTGAAAGGACAG -3'	5' - GCTCATGATTTCTGCTCTGACA -3'
IFN-β	5' - AAAGAAAGCAGCAAATTTTCAGC -3'	5' - CCTTGGCCTTCAGGTAATGCA -3'
IRF1	5' - TGAAGCTACAAACAGATGAGG -3'	5' - AGTAGGTACCCCTTCCCATC -3'
IRF3	5' - ACCAGCCGTGGACCAAGAG -3'	5' - TACCAAGGCCCTGAGGCAC -3'
IRF5	5' - AAGCCGATCCGGCCAA -3'	5' - GGAAAGTCCCGGCTCTTGTAA -3'
IRF7	5' - TGGTCTCTGGTGAAGCTGAA -3'	5' - GATGTCGTCA TAGAGCTGTGTGG -3'
IRF8	5' - GGAGTGGGTCGCTCTGAAA -3'	5' - GTCGTAGGTGGTGTACCCCGTCA -3'
IRF9	5' - GCATCAGGCAGGGCACGCTGCACC -3'	5' - GCCTGCA TGT TTCCAGGGAATCCG -3'
MxA	5' - GCCGGCTGTGGATATGCTA -3'	5' - TTTATCGAAACATCTGTGAAAAGCAA -3'
OAS-1	5' - AGAAGGCAGCTCAGGAAACC -3'	5' - CCACCACCAAGTTTCTGTGA -3'
PKR	5' - AGAGTAACCGTTGGTGACATAACCT -3'	5' - GCAGCCTCTGCAGGCTCTATGTT -3'
ISG56	5' - TTCGGAGAAAGGCATTAGA -3'	5' - TCCAGGGCTTCATTTCATAT -3'
SOCs-1	5' - GAC GCC TGC GGA TTC TAC TG -3'	5' - GGC CAT CTT CAC GCT AAG GG -3'
SOCs-3	5' - TGC GCC TCA AG A CCT TCA GC -3'	5' - GAT GCG CAG GTT CTT GGT CC -3'