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Transient transfection of mouse fibroblasts with type I interferon transgenes provides various degrees of protection against herpes simplex virus infection

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Abstract

Type I interferons (IFN) constitute one of the initial and most potent components of the innate immune response against viral infections. While there is only one IFN-β gene, there are several IFN-α genes whose products act through the same receptor calling into question the role of these gene products against viral infection. The focus of the present study was to compare the anti-viral state of cells transiently transfected with different murine type I IFN transgenes including IFN-α1, -α4, -α5, -α6, -α9, and IFN-β. Transfected cells produced biologically active IFN ranging from 6 to 46 units/ml. L929 and 3T12.3 cells transfected with the IFN-β transgene consistently showed a 2–4 fold reduction in herpes simplex virus type 1 (HSV-1) and HSV-2 viral titers compared with cells transfected with the IFN-α transgenes which were much less consistent based on HSV species and cell type. Parallel with the reduction in viral titers, cells transfected with the IFN-β transgene showed the complete absence or significant reduction in viral immediate early, early, and late gene expression. Collectively, the results suggest that the IFN-β transgene is superior to IFN-α transgenes against HSV infection in vitro in part due to a reduction in viral gene expression. These results indicate events downstream of the type I IFN receptor distinguish between the subtypes of IFN-α species relative to the activation of genes ultimately responsible for the establishment of the anti-HSV state.

Keywords: Interferon; HSV-1; HSV-2; Real time PCR

1. Introduction

Herpes simplex virus (HSV) is a member of the family of *Herpesviridae*, which has a short life cycle, but can infect a variety of cells and induce a state of latency in the host (Whitley, 1996). The

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transcription of HSV-1 DNA is characterized by the temporal and regulated expression of three gene classes including immediate early (α), early (β), and late (γ) genes (Roizman and Sears, 1996). Forty to sixty percent of individuals of lower socioeconomic status are seropositive for HSV-1 by 5 years of age, and more than 90% are seropositive by adulthood (Arvin and Prober, 1991). Relative to HSV-2, epidemiological studies show the rate of infection is approximately $1640\,000 \pm 150\,000$ cases per year (Armstrong et al., 2001) with many potential cases going undiagnosed due to misinterpretation of atypical genital and extragenital lesions (Lautenschlager and Eichmann, 2001). The success of HSV prominence in the human population lies, in part, to evasion of immune surveillance (Jennings et al., 1985; Van Strijp et al., 1988; Hill et al., 1995; Ahn et al., 1996; Nagashunmugan et al., 1998; Jerome et al., 1998). In addition, HSV-1 immediate early (infected cell protein 0, ICP0) and late ($_{\gamma 1}$34.5) gene products are known to counteract type I interferon (IFN) action by mechanisms (in the case of ICP0) that have not been fully characterized (He et al., 1997; Mossman et al., 2000; Härle et al., 2002).

IFNs are potent cytokines with known anti-viral and anti-tumor actions (Baron et al., 1991). Regarding HSV infection in vitro, IFN antagonizes immediate early and early gene expression (Mittnacht et al., 1988; Oberman and Panet, 1998) presumably by modifying the viral protein (VP)16 immediate early complex interaction (De Stasio and Taylor, 1990). In vivo, the administration of neutralizing antibody to IFN-α/β to mice infected with HSV-1 reduces cumulative survival and increases viral titers in infected tissue (Hendricks et al., 1991; Halford et al., 1997) suggesting that even in an otherwise immunocompetent host, type I IFN is central in controlling viral-mediated morbidity and mortality. With at least 12 distinct genes encoding biologically active murine IFN-α species, the question arises as to why so many different subtypes of IFN-α exist? One possible explanation may reside in the biological activity of the type I IFN species and the sensitivity of different viral pathogens to these cytokines. This selective pressure would tend to preserve the gene pool of type I IFNs and the unique traits that each gene product provides the host in controlling microbial pathogenesis. As an example, an in vivo study comparing IFN-α1, IFN-α4, and IFN-α9 transgenes administered and expressed in the tibialis anterior muscles of mice subsequently infected with cytomegalovirus (CMV) reported the IFN-α1 transgene had the greatest impact in suppressing CMV replication in comparison to the other two type I IFN transgenes (Yeow et al., 1998). In yet another in vivo study using an adenoviral vector containing the murine IFN-α2 transgene, mice transduced with the adenoviral vector were found to be highly resistant to subsequent murine hepatitis virus type 3 infection corresponding with reduced inflammation in the hepatic parenchyma (Aurisicchio et al., 2000). Since IFN may directly impact on viral replication within the infected cell or alternatively, influence additional components of the immune system including T lymphocytes, dendritic cells, and macrophages that ultimately provide surveillance and protection against the viral infection, it is difficult to determine the direct consequences of the IFN subtypes against the viral pathogen in vivo. Under such circumstances, it seems appropriate to initially compare and contrast IFN subtypes under controlled in vitro conditions. Previous in vitro studies have assessed the antiviral effect of some IFN-α species including IFN-α1, IFN-α2, and IFN-α4 (Van Heuvel et al., 1988; Mester et al., 1995) with one group (Van Heuvel et al., 1988) indicating IFN-α4 provides the greatest anti-viral effect. Another study found the genotype of the mouse cells used rather than the virus inducer influenced the subtype of IFN-α produced (Lai et al., 1994). The present study expands the repertoire of type I IFNs under comparison using HSV-1 and HSV-2 as the prototypic pathogens measuring viral titers and viral gene expression.

2. Material and methods

2.1. Viruses and cell lines

Vero cells (American Type Culture Collection, ATCC, Manassas, VA) were cultured in RPMI-

1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS, Life Technologies), an antibiotic/antimycotic solution (Life Technologies) and gentamicin (final concentration of 20 μg/ml culture media; Life Technologies). L929 and 3T12.3 murine fibroblasts (ATCC) were maintained in DMEM (Life Technologies) supplemented with 10% FBS, an antibiotic/antimycotic solution and gentamicin (referred to as complete medium). The cells were incubated at an atmosphere of 37 °C, 5% CO_2, and 95% humidity. HSV-1 (McKrae strain) was propagated in Vero cells (multiplicity of infection, MOI = 0.01) with stocks maintained at a concentration of 4×10^8 plaque forming units (PFU)/ml. A clinical isolate of HSV-2 was obtained from University Hospital (New Orleans, LA) and grown in Vero cells (MOI = 0.1) with stocks maintained at a concentration of 1×10^7 PFU/ml. The HSV-1 ICP0$^-$ mutant 7134 (KOS strain) was grown in the ICP0-complementing cell line, L7. The 7134 virus was used to evaluate viral gene expression.

2.2. Plasmid constructs

All murine IFN transgenes were cloned into the eukaryotic expression vector pkCMVintPolylinker (5087 bp, Vical Inc., San Diego, CA) containing a SV40 polyadenylation signal and a kanamycin resistance gene. The IFN genes (575–626 base pairs) are expressed under the control of a human CMV immediate-early enhancer/promotor. Cloning sites within the vector are as follows: IFN-α1-BamHI/BglII, IFN-α4-PstI/SalI, IFN-α5-SalI/BglII, IFN-α6-PstI/BglII, IFN-α9-XbaI/BglII, and IFN-β-SalI/XbaI. The original source and cloning procedures were as described (Kelley and Pitha, 1985; Seif and De Maeyer-Guignard, 1986; Lawson et al., 1997; Yeow et al., 1998). The plasmid constructs were transformed into the *Escherichia coli* strain INVαF′ (Invitrogen, Carlsbad, CA) and grown up in Terrific broth, containing 50 μg/ml kanamycin, followed by purification using Qiagen Maxi kits (Qiagen Inc., Valencia, CA). After each plasmid isolation, restriction enzyme digestion assays were conducted and the products were analyzed by agarose gel electrophoresis.

2.3. Transfection and infection of L929 and 3T12.3 cells

Prior to transfection, 5×10^5 L929 or 3T12.3 cells were plated in 6-well tissue culture plates (Intermountain Scientific, Kaysville, UT). Following an overnight (18 h) incubation, the cells were transfected with 6 μg of the pkCMV plasmid DNA alone (Vector) or plasmid vector DNA containing the indicated type I IFN transgene using a calcium phosphate protocol and reagent according to the manufacturer's instructions (Promega, Madison, WI). The medium was removed from the cultures 4 h post transfection and fresh complete medium was added to the transfected cells, followed by incubation at 37 °C, 5% CO_2, and 95% humidity. Eighteen to twenty four hour post transfection, the supernatant was collected and assayed for IFN content. The cells were then infected with HSV-1 or HSV-2 (MOI = 0.5) in 1.0 ml complete medium. One hour, post infection, the medium was removed and fresh complete medium (1.0 ml) was added. Following a 24 h incubation, the cultures were freeze–thawed and the cell-free supernatant was collected and assayed for infectious virus by plaque assay.

2.4. IFN bioassay

To quantitate biologically active amounts of IFN secreted at the end of the 24 h transfection period, supernatants from 3T12.3 or L929 transfected cell cultures were used to incubate L929 cells for 16 h in 96-well tissue culture plates. Cells were infected with vesicular stomatitis virus (VSV, originally provided by Dr Robert Fleischmann, UTMB, Galveston, TX) at a MOI = 0.05 and plates were fixed and stained with crystal violet when cytopathic effect (CPE) was maximal in untreated wells (32–37 h). A standard curve was generated using 1.0–1000 IU/ml of recombinant murine IFN-αA (rmuIFN-αA) and rmuIFN-β (PBL Biomedical Laboratories, Brunswick, NJ) in each 96-well plate. Fifty percent inhibition of CPE was typically equivalent to 1 IU/ml for both

type I IFN subtypes. The incubation of supernatants with anti-murine-IFN-α and anti-murine-IFN-β antibodies (PBL Biomedical Laboratories) for 1 h at room temperature completely neutralized the protective effect. Lowering the pH to 2 in the supernatants for 1 h with a subsequent freeze–thaw step at −20 °C and reestablishing the pH back to 7 did not reduce the protective effect in the bioassays (data not shown).

2.5. Semiquantitative real time PCR

Total cell RNA was isolated at 6 and 10 h p.i. in Ultraspect RNA isolation reagent (Biotecx Inc., Houston, TX) according to the manufacturer's protocol. Before the reverse transcription step, DNA contamination was removed using deoxyribonuclease I according to the manufacturer's protocol (Gibco-BRL, Gaithersburg, MD). First strand cDNA was synthesized using avian myoblastosis virus reverse transcriptase (Promega) and an oligonucleotide dT primer (Promega). Real-time PCR was carried out in 96-well PCR plates (Bio-Rad) using a Bio-Rad iCycler. Real-time PCR conditions for all primers included an initial denaturing step for 3 min at 95 °C followed by 30 cycles at 95 °C for 10 s. and annealing/elongation at 61 °C for 35 s. Each reaction contained 45 μl of PCR Platinum SuperMix (Gibco-BRL) and SYBRgreen I (MolecularProbes, Eugene, OR) at a final dilution of 1:100 000. $MgCl_2$ was supplemented as indicated with the primer sequences. During the optimization procedures of the primers, 1% agarose gel analysis verified the amplification of one product of the predicted size with no primer–dimer bands. The absence of primer–dimer formation for each oligonucleotide set was also validated by establishing the melt curve profile and determining a single amplicon for each primer pair. The PCR results were analyzed on the iCYCLER Software (version 2.3) and threshold-cycles were determined as follows. After subtracting the background fluorescence for each sample, the threshold fluorescence for each gene was determined at that point where the relative light units reached a level of more than 10 standard deviations above the baseline relative light units. At 40 cycles, the primers-only-control did not give a signal above the threshold. At 35 cycles the primers-with-uninfected cells did not give a signal above the threshold. The semiquantitative comparison between samples was calculated as followed: The data was normalized by subtracting the difference of the threshold cycles (C_T) between the gene of interest (GOI C_T) and the 'housekeeping' gene GAPDH C_T (GOI C_T–GAPDH $C_T = \Delta C_T$) for each sample. The ΔC_T was then compared with the expression levels of the vector control sample (sampleΔC_T − vectorΔC_T). To determine the relative enhanced expression of the gene of interest the following calculation was made: fold change = 2^[− sampleΔC_T − vectorΔC_T]. If the difference (sampleΔC_T − vectorΔC_T) was a positive value then it was calculated as: fold change = −1/2^ [− sampleΔC_T − vectorΔC_T] in order to get a negative value expressing the relative suppression of the gene of interest. Oligonucleotide sequences for the targeted genes include the following:

- *GAPDH*: 5′-GAATCTACTGGCGTCTTCACC-3′, 5′-GTCATGAGCCCTTCCACGATGC-3′ (2 mM $MgCl_2$).
- *ICP27*: 5′-TGACGCCGAGACCAGAC-3′, 5′-GGCAAAAGTGCGATAGAGG-3′ (3 mM $MgCl_2$).
- *TK*: 5′-AAACCACCACCACGCAAC-3′, 5′-ACACCCGCCAGTAAGTCATC-3′ (3 mM $MgCl_2$).
- *VP16*: 5′-GGACTCGTATTCCAGCTTCAC-3′, 5′-CGTCCTCGCCGTCTAAGTG-3′ (3 mM $MgCl_2$).

2.6. Statistics

Analysis of data was carried out by one-way ANOVA and Scheffe multiple comparison test using GBSTAT program (Dynamic Microsystems Inc., Silver Springs, MD) to determine significant ($P < 0.05$) differences between the vector and type I IFN transgene groups.

Table 1
IC_{50} of recombinant IFN-α and IFN-β against HSV-1 and HSV-2 in 3T12.3 cells[a]

Virus	L929 Cells		3T12.3 Cells	
	rIFN-αA	rIFN-β	rIFN-αA	rIFN-β
HSV-1	54.3±4.9[b]	3.7±2.3*	44.0±4.1	10.0±4.2*
HSV-2	13.9±4.9	27±7.0**	18.7±4.9**	12.9±2.6

[a] Mouse L929 or 3T12.3 cells (5×10^5 cells per ml) were incubated for 18 h with recombinant IFN-αA or IFN-β (0.1–1000.0 units/ml) and subsequently infected with HSV-1 or HSV-2 (MOI = 0.5). Twenty four hours post infection, the cells were freeze–thawed and clarified supernatants were assayed for infectious virus by plaque assay using Vero cells as the indicator cell. This figure is the summary of four to six experiments. The IC_{50} was determined by sigmoidal fit analysis using MICROCAL ORIGIN software (North Hampton, MA).

[b] Numbers are in U/ml, mean±S.E.M.

* $P < 0.05$ comparing the IC_{50} of IFN-αA and IFN-β against HSV-1 as determined by Student's T-test.

** $P < 0.05$ comparing the IC_{50} value for IFN-αA of HSV-1 vs. HSV-2 as determined by Student's t-test.

3. Results

3.1. Recombinant (r)IFN-β is superior to rIFN-αA in antagonizing HSV-1 but not HSV-2 replication

We have previously reported human rIFN-β is more efficacious against HSV-1 infection compared with human rIFN-α2 (Härle et al., 2001). In a similar fashion, murine rIFN-β was more effective than rIFN-αA in suppressing HSV-1 replication in L929 and 3T12.3 cells as determined by establishing the IC_{50} value (Table 1). However, there was no significant difference between the two type I rIFNs against HSV-2 replication (Table 1). Taken together, the results suggest that while HSV-1 is more sensitive to the anti-viral effects induced by rIFN-β in comparison to rIFN-αA, there does not appear to be any significant difference in sensitivity in HSV-2 infection.

3.2. Comparison of the sensitivity of cells transfected with mouse type I IFN transgenes against HSV-1 and HSV-2 infection

Since there are multiple IFN-α subtypes, the present findings do not address the potential difference in anti-viral efficacy within and between each subtype. We addressed this issue by using plasmid constructs containing IFN-α transgene subtypes including IFN-α1, IFN-α4, IFN-α5, IFN-6, and IFN-α9 to transfect cell lines by evaluating the anti-viral capacity of the transfected cells against HSV-1 and HSV-2 in comparison to cells transfected with the IFN-β transgene. Initially, two murine cell lines transfected with murine type I transgenes were evaluated for success of the transfection by measuring the amount of biologically active IFN. Previous results have found the success of transient transfection using the calcium phosphate approach to be less than 1% (Noisakran et al., 2000). Nevertheless, L929 and 3T12.3 transfected cells produced measurable levels of IFN ranging from 8 to 47 U/ml from L929 cells and 6–16 U/ml from 3T12.3 cells (Fig. 1). By comparison, non-transfected cells produced no measurable levels of IFN. However, plasmid vector-transfected cells secreted 1–4 U/ml IFN within 24 h post transfection in 3T12.3 and L929 cells (Fig. 1). Cells transfected with the IFN-α5 or IFN-α6 transgenes produced significantly elevated levels of biologically active IFN above plasmid vector-transfected L929 and 3T12.3 cells whereas only in the 3T12.3 cells were the levels of expression of IFN in all type I IFN transgene–transfected cells significantly above that of the plasmid vector-transfected controls (Fig. 1).

L929 cells transfected with the IFN-β transgene were less sensitive to HSV-1 infection with a 2-fold reduction in viral titer (Fig. 2). By comparison, none of the IFN-α transgene subtypes significantly reduced HSV-1 replication in L929 cells (Fig. 2). Unlike the L929 cell line, 3T12.3 cells tended to be more receptive to induction of an anti-viral state following transfection with type I IFN transgenes. Specifically, most of the type I IFN transgenes (exception being IFN-α4 and IFN-α9) suppressed HSV-1 replication 2–3 fold in the 3T12.3 cells (Fig. 3). Consistent with the results using recombinant IFN protein, HSV-2 was more sensitive to the anti-viral effects of the IFN transgenes compared with HSV-1 in 3T12.3 cells. With the exception of the IFN-α5 transgene, all type I IFN transgenes tested significantly reduced HSV-2 replication in transfected L929 cells (Fig. 2).

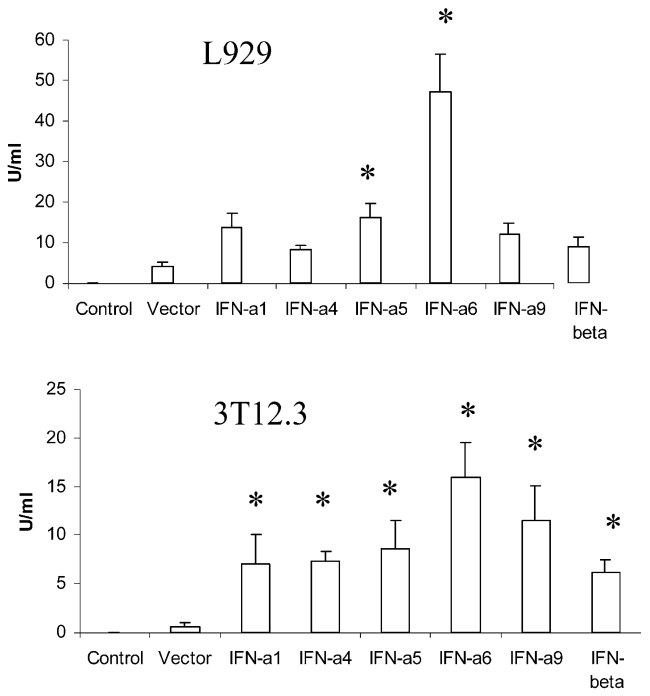

Fig. 1. Transfected mouse cell lines produced biologically active IFN. L929 or 3T12.3 (5×10^5) cells were transfected with 6 μg of the pkCMV plasmid DNA alone (Vector) or plasmid vector DNA containing the indicated type I IFN transgene using a calcium phosphate protocol and reagent according to the manufacturer's instructions (Promega). Medium was removed from the cultures 4 h post transfection and fresh medium was added to the transfected cells, followed by incubation at 37 °C, 5% CO_2, and 95% humidity. Twenty-four-hours later, the supernatant was collected and assayed for IFN content by plaque reduction assay using vesicular stomatitis virus (MOI = 0.01) and L cells. Non-transfected cells were used to establish basal levels of IFN secretion (Control). This figure is a representative of four experiments for each transgene using each cell line and virus. *, $P < 0.05$ comparing the IFN content of the type I IFN transgene–transfected cells to the plasmid vector transfected cells as determined by ANOVA and Scheffe multiple comparison test. Bars represent the mean ± S.E.M.

With the exception of the IFN-α5 transgene, all type I IFN trangenes tested significantly reduced HSV-2 replication in transfected L929 cells. Specifically, transfected 3T12.3 cells showed a 2–4 fold reduction in viral titer to HSV-2 infection regardless of the transgene employed (Fig. 3). Without exception of cell line or HSV type, the results clearly show that cells transfected with the plasmid containing the IFN-β transgene consistently displayed the greatest reduction in viral yield relative to the plasmid vector-transfected controls even though the transfected cells secreted modest levels of biologically active IFN-β. In determining the rank order of potency for each type I IFN, cells transfected with the IFN-β transgene were the most resistance to HSV-1 and HSV-2 infection in both L929 and 3T12.3 cell lines tested (Table 2). Consistent with these findings, L929 cells transfected with the IFN-β transgene were also found to express modest to no measurable level of HSV-1 immediate early, early, or late genes as measured by real time PCR (Fig. 4). Cells transfected with the IFN-α transgenes displayed various levels of viral gene expression, none of which reached the degree of suppression found in the IFN-β transgene–transfected group (Fig. 4).

4. Discussion

The present findings illustrate the anti-viral state established in cells transfected with the plasmid containing the IFN-β transgene is superior to that using plasmids containing IFN-α transgenes. Gi-

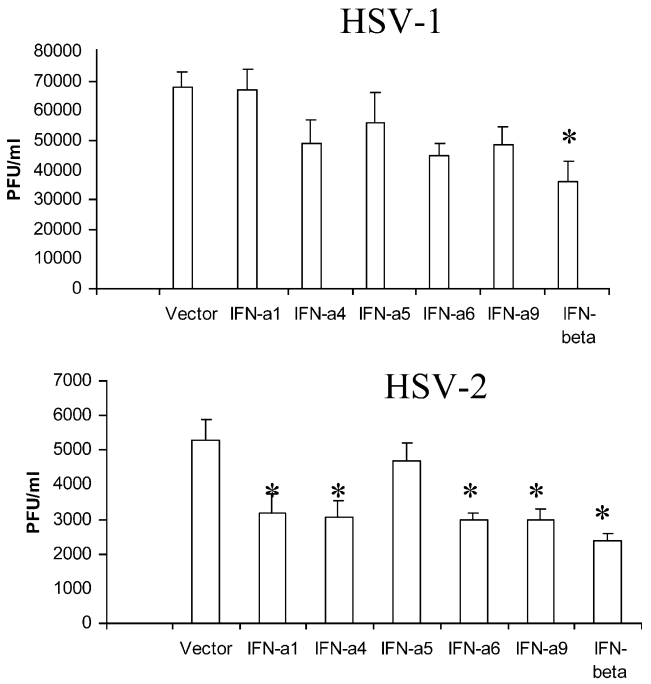

Fig. 2. L929 cells transfected with the IFN-β transgene are less sensitive to HSV-1 and HSV-2. Cells were transfected as described in Fig. 1 legend. Eighteen hours post transfection, the supernatant was removed and cells were infected with HSV-1 (McKrae strain) or HSV-2 (clinical isolate, Charity Hospital, New Orleans, LA) (MOI = 0.5) in 1 ml medium. One hour post infection, the medium was replaced and the cells were incubated an additional 24 h at 37 °C, 5% CO_2, and 95% humidity. Supernatants were subsequently collected and assayed for infectious virus by plaque assay using Vero cells. This figure is a summary of four experiments conducted in triplicate for each transgene. *, $P < 0.05$ comparing the viral titer for the indicated transgene to that of the vector-transfected group as determined by ANOVA and Scheffe multiple comparison test. Bars represent mean ± S.E.M.

ven that a modest amount of biologically active IFN was generated from transfected cells, it is not surprising that the IFN-β transgene suppressed viral replication in the transfected L929 cells since the IC_{50} for IFN-β against HSV is 4–15 fold lower compared with the IFN-α subtypes tested.

However, the IFN-α6 transgene had no significant impact on HSV-1 yield in L929 cells even though levels of biologically active cytokine secreted from the transfected cells reached levels within the IC_{50} for IFN-α. Therefore, we conclude the IFN-α6 transgene is not as efficient in establishing an anti-viral state in L929 cells compared with IFN-β transgene, an observation that is substantiated comparing viral yield and viral gene expression of these two groups of transfected cells.

Within the limits of our assay, we conclude that HSV-2 is more sensitive than HSV-1 to the anti-viral effects of IFN reflected in both type I IFN transgene-transfected cell lines. However, the confines of the in vitro system reported herein may not illustrate the true virulence of HSV-1 and HSV-2 in the host. For example, HSV-2 is reportedly more neurovirulent with more frequent recurrences compared with HSV-1 (Stanberry et al., 1997; Sucato et al., 1998). By way of explanation, the HSV-2 virion host shutoff (vhs) protein is thought to work 40% faster than the vhs protein of HSV-1 in polysomal disaggregation and cytoplasmic degradation of cellular mRNAs (Fenwick and Everett, 1990; Everly and Read, 1997). Likewise, HSV-2 but not HSV-1 suppresses Fas ligand expression, a molecule associated with inducing events leading to apoptosis (Sieg et al., 1996). However, the present study investigated only one clinical isolate of HSV-2 in comparison to the highly neurovirulent McKrae strain of HSV-1

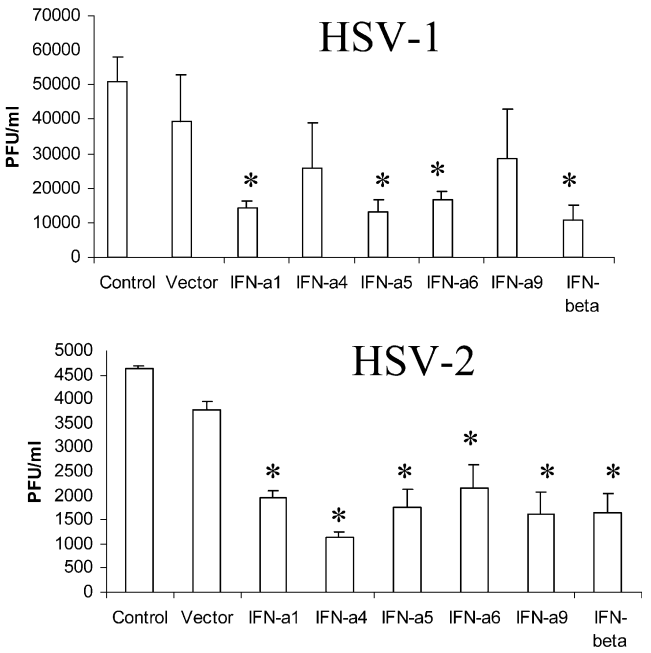

Fig. 3. 3T12.3 cells transfected with the type I IFN transgenes are less sensitive to HSV infection compared with plasmid vector-transfected control cells. Cells were transfected as described in Fig. 1 legend. Eighteen hours post transfection, the supernatant was removed and cells were infected with HSV-1 (McKrae strain) or HSV-2 (clinical isolate, Charity Hospital, New Orleans, LA) (MOI = 0.5) in 1 ml medium. One hour post infection, the medium was replaced and the cells were incubated an additional 24 h at 37 °C, 5% CO_2, and 95% humidity. Supernatants were subsequently collected and assayed for infectious virus by plaque assay using Vero cells. This figure is a summary of three to four experiments conducted in triplicate for each transgene. *, $P < 0.05$ comparing the viral titer for the indicated transgene to that of the vector-transfected group as determined by ANOVA and Scheffe multiple comparison test. Bars represent mean ± S.E.M.

whereas another study illustrated the strain-specific nature of HSV-1 and HSV-2 to the anti-viral action of type I and II IFNs (Arao et al., 1997). Accordingly, additional studies are required to address the sensitivity of HSV-1 and HSV-2 to the anti-viral effects of the type I IFN transgenes using techniques that generate levels of the IFN-α subtypes from the transgene–transfected cells above the established IC_{50} value of rIFN-α.

The rank order of potency comparing cells transfected with the type I IFN transgenes consistently showed the IFN-β transgene provided the greatest efficacy against HSV-1 and HSV-2 infection in comparison to the IFN-α transgenes. Likewise, cells transfected with the IFN-α4 transgene were also found to possess a high degree of resistance to HSV infection. It is thought that IFN-α4 or IFN-β are the rapid responding IFNs to viral infection that subsequently induce the expression of other IFN-α species through the type I IFN receptor resulting in the stimulation of the Jak–Stat pathway (Marie et al., 1998). Therefore, it is likely the efficacy of the IFN-β and IFN-α4 transgenes is, in part, due to their ability to coordinately induce other type I IFNs following transfection. The superior anti-viral effect elicited in cells transfected with the IFN-β in comparison to the IFN-α species may reside in the strength of induction of the Jak–Stat pathway (Grumbach et al., 1999) or selective IFN-stimulatory genes (Der et al., 1998). Alternatively, the stability of each type I IFN species secreted into the medium must also be taken under consideration as the rate of decay may be different and impact on the degree of

Table 2
Rank order of potency of type I IFN transgenes against HSV-1 and HSV-2[a]

	L929 Cells		3T12.3 Cells	
Transgene	HSV-1	HSV-2	HSV-1	HSV-2
IFN-α1	0.14[b]	2.90	9.01	6.76
IFN-α4	3.40	5.06	4.65	9.09
IFN-α5	1.11	0.68	7.79	6.16
IFN-α6	0.72	0.93	3.56	2.69
IFN-α9	2.41	3.58	2.35	4.96
IFN-β	5.22	6.11	11.77	9.03

[a] 5×10^5 L929 or 3T12.3 cells were transfected with 6 μg of the pkCMV plasmid DNA alone (Vector) or plasmid vector DNA containing the indicated type I IFN transgene using a calcium phosphate protocol and reagent according to the manufacturer's instructions (Promega). Medium was removed from the cultures 4 h post transfection and fresh medium was added to the transfected cells, followed by incubation at 37 °C, 5% CO_2, and 95% humidity. Twenty-four hours later, the supernatant was collected and assayed for IFN content by plaque reduction assay using vesicular stomatitis virus (MOI = 0.01) and L cells. The cells were infected with HSV-1 (McKrae strain) or HSV-2 (clinical isolate, Charity Hospital, New Orleans, LA) (MOI = 0.5) in one ml medium. One hour post infection, the medium was replaced and the cells were incubated an additional 24 h at 37 °C, 5% CO_2, and 95% humidity. Supernatants were subsequently collected and assayed for infectious virus by plaque assay using Vero cells. The table summarizes the outcome of four experiments.

[b] The numbers represent the percent inhibition in viral titer compared with virus recovered from plasmid vector-transfected cells divided by the amount of biologically active IFN produced by the transfected cells. The larger the number, the higher the rank order of potency.

induction of the anti-viral state of the cells. Additional work is warranted to substantiate these issues relative to HSV infection.

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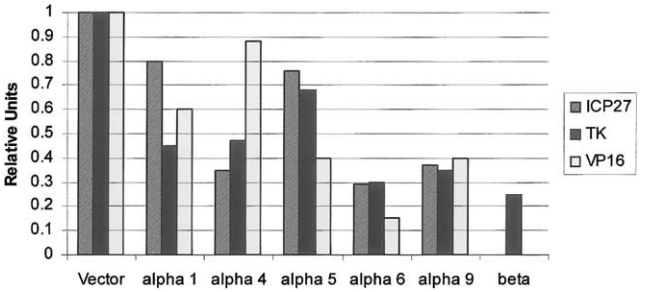

Fig. 4. L929 cells transfected with the IFN-β transgene express less HSV-1 viral transcripts compared with plasmid-vector transfected cells. L929 cells were transfected as described in Fig. 1 legend. Following an overnight incubation, the supernatant was removed and the cells were infected with HSV-1 (KOS strain mutant, 7134) at an MOI of 0.5. At 10 h post infection, the cells were collected and RNA was harvested and used to generate cDNA using reverse transcriptase (Promega). Using oligonucleotide primers specific for the immediate early gene ICP27, the early gene TK, and the late gene VP16 the relative amounts of each HSV-1 lytic gene transcript was determined by real time PCR using an icycler apparatus (BioRad, Hercules, CA). The change in viral gene expression was calculated from the relative level of expression for each type I IFN transgene group normalized to the relative value of GAPDH for each group. The plasmid vector-transfected cells were assigned a value of 1 in order to compare the levels of expression for each viral gene to that expressed in the type I IFN transgene-treated groups. This figure is a representative of two experiments with similar results.

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