



# Interferon- $\alpha$ and - $\beta$ Restrict JCV Replication in Primary Human Fetal Glial Cells

## Implications for Progressive Multifocal Leukoencephalopathy Therapy

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### Abstract

**Background:** Effective antiviral therapy for progressive multifocal leukoencephalopathy (PML) among HIV patients has not yet been found. Although highly active antiretroviral therapy (HAART) may maintain or restore cellular immunity, PML remains a serious cause of neurologic morbidity, with 50% fatality rate. PML may also arise amidst the setting of immune reconstitution inflammatory syndrome (IRIS). An apparent limitation of HAART is its inability to inhibit replication of human polyomavirus JC (JCV), the causative agent of PML. We previously demonstrated induction of genes associated with interferon (IFN) antiviral response pathway in primary human fetal glial (PHFG) cells, specifically at later stages of JCV replication (*Virology* 2006; 345:457-67). The objective of this study was to analyze specific viral events required to induce interferon-stimulated genes (ISG), and to assess the potential antiviral effects of IFN- $\alpha$  and - $\beta$  on JCV replication.

**Methods:** PHFG cells were infected with 50 HAU of JCV (Mad1) or UV-inactivated JCV, and cells were grown in the continuous presence of 100 Units/mL of IFN- $\alpha$  or - $\beta$ . To assess antiviral efficacy of IFN, at days 3, 5, 8, and 15 post-inoculation, JCV T antigen (TAG) DNA and mRNA transcripts, and ISG mRNA transcripts were analyzed by quantitative real-time-PCR and -RT-PCR, and TAG protein expression was quantitated by Western blotting.

**Results:** Treatment with both, IFN- $\alpha$  and - $\beta$  resulted in significant induction of ISG in PHFG cells at all time points. Productive JCV replication was essential for the induction of ISG in PHFG cells. JCV replication at days 5, 8, and 15 was significantly inhibited in the presence of IFN- $\alpha$  and - $\beta$ . Effect of IFN on JCV replication was reversed in the presence of neutralizing anti-IFN antibody. Furthermore, IFN- $\beta$  was found to have more potent effect on blocking JCV infection.

**Conclusions:** Our *in vitro* data demonstrates direct antiviral effect of IFN on JCV replication. It suggests that IFN- $\alpha$  and - $\beta$  may be useful as adjunct therapies to manage patients with PML. Since IFN cannot cross the blood-brain barrier, to achieve its direct antiviral effect, intrathecal administration of IFN warrants consideration of clinical trials in humans.

### Introduction

JCV is the etiologic agent of PML, a fatal, demyelinating disease of the central nervous system (CNS). PML develops in people with severely compromised immune system such as AIDS patients, as a consequence of virus reactivation and lytic infection of the myelin-producing cells of the CNS, the oligodendrocytes. Due to the lack of PML-specific therapy, AIDS-associated PML patients succumb to the disease within six months after diagnosis. Although HAART has significantly reduced PML-associated mortality, its complications include development of IRIS, and no clear survival benefit in patients with high baseline JCV load in the cerebrospinal fluid. Type 1 Interferon (IFN- $\alpha$  and - $\beta$ ) have been administered in both pre- and post-HAART era to manage PML progression, however, the outcomes have been controversial. In most clinical studies, IFN was administered systemically which results in poor access to the CNS, thereby acting as an immunomodulator rather than as an antiviral agent. Currently, there are no *in-vitro* studies evaluating the direct antiviral effect of IFN- $\alpha$  or - $\beta$  on JCV in infected target cells and such studies are warranted.

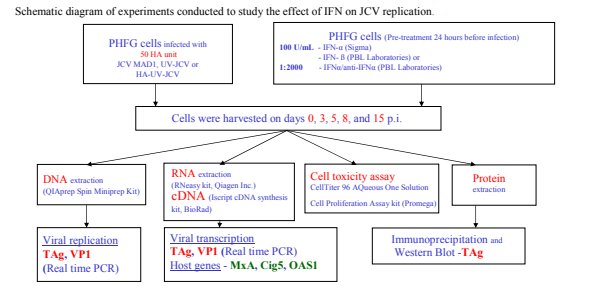
### Hypothesis and Objectives

Using JCV-permissive PHFG cells, we previously demonstrated (*Virology* 2006; 345:457-67) marked up-regulation of several interferon-stimulating genes (ISG). We hypothesized that antiviral products along with JCV early proteins may modulate severity of the infection, and limit spread of JCV, thereby explaining the slow replication and subtle cytopathogenic effects in PHFG cells. Since IFN- $\alpha$  and - $\beta$  are potent inducers of ISG, and are common therapeutic options for viral diseases, the present study was designed to analyze the specific viral events associated with the induction of ISG, and to characterize the effect of IFN- $\alpha$  and - $\beta$  on JCV replication in PHFG cells.

### Rationale

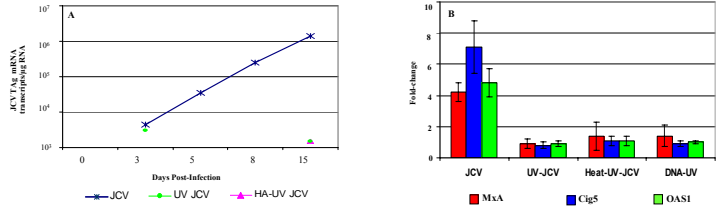
Understanding of host factors contributing to suppression of JCV replication will aid in the development of better therapeutic strategies for PML patients.

### Materials and Methods



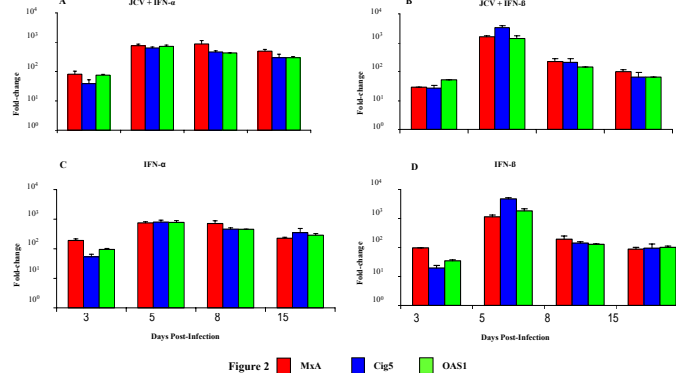
### Results

**JCV, but not UV-JCV or UV-HA-JCV infects and replicates in PHFG cells, and induces ISG.** To confirm virus inactivation by UV, transcription of viral T antigen gene was analyzed on days 3, 5, 8, and 15 p.i. in PHFG cells infected with JCV, UV-JCV or HA-UV-JCV. Robust replication of JCV T antigen transcripts was observed in PHFG cells infected with JCV, from day 5 p.i., whereas in UV-JCV and HA-UV-JCV TAG was absent or below the detection cutoff at all time points (Figure 1A). With the threshold for significant change in ISG mRNA expression set at 2-fold, cells infected with JCV showed increase in transcripts of ISG identified previously (MxA, *cig5* and OAS1) at day 15 p.i. On the other hand, UV-JCV, HA-UV-JCV or transfection with non specific DNA did not show significant up-regulation of any ISG at all time points (Figure 1B).

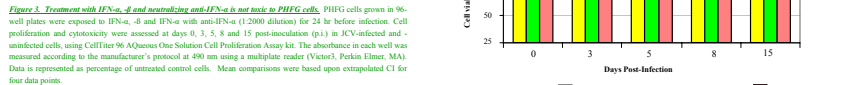


**Figure 1. JCV, but not UV-JCV or UV-HA-JCV replicates and induces ISG in PHFG cells.** PHFG cells were obtained and presented as described previously (*Virology Journal* 2006:3). and were infected with JCV (Mad1), UV-inactivated JCV (UV-JCV), heat and UV-inactivated JCV (HA-UV-JCV) or transfected with UV-inactivated non-JCV plasmid (pUC8) DNA (UV-DNA) (A) at days 3, 5, 8, and 15 p.i. and JCV mRNA transcripts were extracted. One microgram of total RNA from each sample was used as template for cDNA synthesis and real-time reverse transcriptase PCR (qRT-PCR) was performed on Cycler (Bio-Rad). JCV TAG and VPI cDNA transcripts were amplified using specific primers and conditions as described previously (*Virology Journal* 2006:3). (B) cDNA from RNA extracted from PHFG cells of JCV, UV-JCV, HA-UV-JCV, and UV-DNA at day 15 p.i., were subjected to qRT-PCR using MxA, *cig5* and OAS1 specific primers as described previously (*Virology* 2006; 345:457-67). Changes in the levels of each ISG transcript as compared to uninfected control were first normalized to GAPDH and then the fold-change was calculated using Pfaffl method. Data are reported as mean  $\pm$  SD of triplicate experiments.

**PHFG cells exhibit a biological response to IFN- $\alpha$  and - $\beta$ .** IFN- $\alpha$  and - $\beta$  induced significant expression of ISG (MxA, *cig5* and OAS1) in the range of 100-1,000-fold at all time points in JCV-infected (Figures 2A, B) and -uninfected (Figures 2C, D) cultures indicating that response to IFN- $\alpha$  and - $\beta$  was maintained throughout the experimental time course and is not eliminated by JCV infection. Cell viability assays were performed to assess IFN toxicity on IFN- $\alpha$  and - $\beta$  treated cells, and at all time points no significant change as compared to untreated controls was observed in both, JCV-infected and -uninfected PHFG cells (Figure 3).

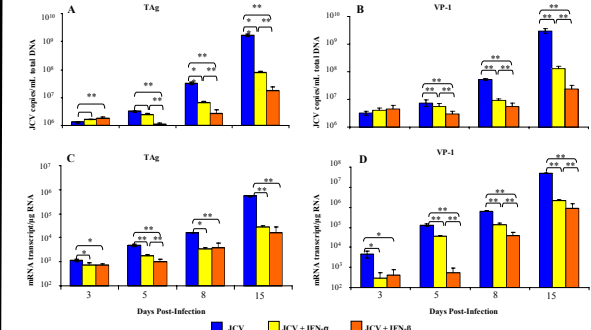


**Figure 2. IFN- $\alpha$  and - $\beta$  induces transcription of ISG in PHFG cells.** Native PHFG cells or cells infected with JCV, were exposed to 100 U/mL of IFN- $\alpha$  or - $\beta$  for 15 consecutive days and at days 3, 5, 8, and 15 p.i., MxA, *cig5* and OAS1 expression levels were measured as described above in JCV-infected, IFN- $\alpha$  (A) and - $\beta$  (B), JCV-uninfected, IFN- $\alpha$  (C) and - $\beta$  (D) treated PHFG cells. Data are represented as mean, and error bars represent the SD from three independent experiments.



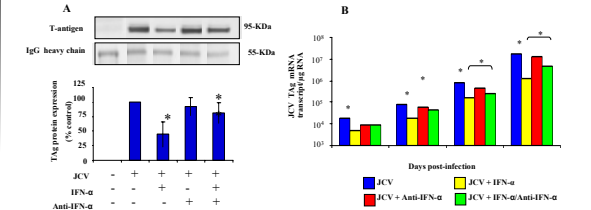
**Figure 3. Treatment with IFN- $\alpha$  and neutralizing anti-IFN- $\alpha$  is not toxic to PHFG cells.** PHFG cells grown in 96-well plates were exposed to IFN- $\alpha$ , - $\beta$  and IFN- $\alpha$  with anti-IFN- $\alpha$  (1:2000 dilution) for 24 hr before infection. Cell proliferation and cytotoxicity were assessed at days 0, 3, 5, 8 and 15 post-inoculation (p.i.) in JCV-infected and -uninfected cells, using CellTiter 96 AQueous One Solution Cell Proliferation Assay kit. The absorbance in each well was measured according to the manufacturer's protocol at 490 nm using a multiple reader (Victor), Perkin Elmer, MA. Data is represented as percentage of untreated control cells. Mean comparisons were based upon extrapolated CI for four data points.

**IFN- $\alpha$  and - $\beta$  Restrict JCV Replication, Transcription, and Translation.** Presence of 100 U/mL IFN- $\alpha$  and - $\beta$  in the media significantly reduced JCV replication (Figures 4A, B), and early (T antigen) and late viral protein-1 (VPI) transcripts, (Figures 4C, D), as measured by qPCR and qRT-PCR, respectively, at days 3, 5, 8, and 15 p.i. (p<0.001). The decrease in T antigen and VPI copy numbers between 1-2 logs, which correspond to 90-95% restriction of JCV replication. Compared to IFN- $\alpha$ , IFN- $\beta$  had more potent inhibitory effect on JCV replication. At the post-translational level, the presence of IFN- $\alpha$  reduced the T antigen protein accumulation by 55% (Figure 5A) at day 15 p.i.



**Figure 4. IFN- $\alpha$  and - $\beta$  inhibit JCV replication and transcription.** PHFG cells were treated with IFN- $\alpha$  or - $\beta$  as described previously, and JCV T antigen and VPI DNA copies (A and B) and mRNA transcripts (C and D) were measured in untreated, IFN- $\alpha$  treated, or IFN- $\beta$  treated JCV-infected PHFG cells. Analysis of variance with log transformations was done using SAS software package to test for significant differences between treatments. The figure represents data from experiments on four independent biological samples. \*p value < 0.05, \*\*p value < 0.001 compared to JCV-infected samples at the same time point.

**Inhibitory effect of IFN- $\alpha$  on JCV replication is reversed in the presence of neutralizing antibody.** The T antigen protein profile indicates that anti-IFN- $\alpha$  rescued the inhibitory effect of IFN- $\alpha$  on JCV protein accumulation at day 15 p.i. (Figure 5A). Further verification of specificity of IFN- $\alpha$  in restricting JCV replication in presence of anti-IFN- $\alpha$  indicated significant restoration of JCV replication in presence of anti-IFN- $\alpha$  in IFN-treated PHFG cells at all time points (p<0.001, Figure 5B).



**Figure 5. Anti-IFN- $\alpha$  reverses the inhibitory effect of IFN- $\alpha$  on JCV replication in PHFG cells.** (A) PHFG cells were exposed to anti-IFN- $\alpha$  prior to IFN- $\alpha$  treatment and one million of total protein from day 15 JCV-infected PHFG cells with and without IFN- $\alpha$  treatment were subjected for immunoprecipitation using a mouse anti-SV40 large T antigen (DPO2, Calbiochem), separated on SDS-PAGE and immunoblotted with SV40 large T antigen antibody. The figure represents data from experiments performed on three independent biological samples. The blots were scanned using a PhosphorImager and analyzed using Quantity one program. Data is represented as percentage of JCV-infected, untreated cells. Mean comparisons were based upon extrapolated CI for three data points. \*\*p<0.05, compared to JCV-infected cells, \*\*p<0.05, compared to IFN- $\alpha$  treated JCV-infected cells. (B) JCV T antigen mRNA expression was measured by qRT-PCR in untreated, IFN- $\alpha$  treated, anti-IFN- $\alpha$  alone, and IFN- $\alpha$  with anti-IFN- $\alpha$  treated infected PHFG cells. Analysis of variance with log transformations was done using SAS software package to test for significant differences between treatments (\*p value < 0.001).

### Conclusions

- The induction of ISG by JCV only and not by UV-JCV, HA-UV-JCV and UV-DNA in PHFG cells, suggest that active virus transcription, not just virus-receptor binding, or penetration in the host cells, is essential for induction of a strong antiviral response to JCV infection.
- The robust (10-1,000 fold) up-regulation of ISG at all time points in IFN-treated PHFG cells suggest that both oligodendrocytes and astrocytes were responsive to IFN- $\alpha$  and - $\beta$  treatment at the non-toxic dose of 100 U/mL.
- Our *in-vitro* data for the first time demonstrate statistically significant inhibition of JCV gene and protein expression, in IFN- $\alpha$  and - $\beta$  pre-treated vs. untreated PHFG cells.
- In this study, we cannot ascertain at which stage the infection was most compromised though it seems likely that products of IFN-induced ISG are targeting multiple stages of replication, as viral DNA, RNA and protein synthesis were inhibited in PHFG cells.
- Based on our results and available literature on systemic administration of IFN in clinical studies, a route of administration known to result in poor access to the CNS, we propose that for direct inhibition of JCV replication, intrathecal infusion of IFN should be considered as an adjunct therapy for PML.

### Acknowledgements

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