Investigating the Effects of the Interferon-Induced Proteins IFI44 and IFI44L on Human Cytomegalovirus Replication

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Abstract

Interferon (IFN) secretion by virus-infected cells leads to the expression of Interferon Stimulated Genes (ISG) in neighboring cells. Interferon-Induced protein 44 and 44-like (IFI44 and IFI44L) are two such genes. Previous research has shown that the IFI44 and IFI44L proteins have proviral effects in human kidney and lung epithelial cancer cells infected with different single-stranded RNA (ssRNA) viruses [3,5]. In contrast, another study showed that transcription of both IFI44 and IFI44L genes is reduced in myeloid cells infected with the human cytomegalovirus (CMV), a double-stranded DNA virus, suggesting that their products may exert antiviral activities [4]. This study aimed at further investigating the anti- or proviral properties of IFI44 and IFI44L in CMV-infected human foreskin fibroblasts (HFF) and retinal epithelial cells (ARPE-19). To this end, both proteins were overexpressed prior to infection by transduction of each cell type with retroviral particles, and viral progeny was then quantified by titration. We report that overexpression of the IFI44 and IFI44L proteins has minimal to no effect on CMV replication in both HFF and ARPE-19 cells, suggesting that these proteins do not exert antiviral functions. More research is needed to determine if they may be supporting CMV infection instead, as well as to establish if their functions are specific to select virus families. The data obtained from this and other studies can increase our understanding of the role played by ISG-encoded proteins and may provide novel host targets to treat infection by specific viruses.

Introduction

Virus-infected mammalian cells secrete IFNs to alert bystander cells of the pathogen's presence, prompting initiation of antiviral defenses [1]. Upon binding to specific cell surface receptors, IFNs induce the expression of a broad number of ISGs, most of which exert antiviral functions (figure 1).

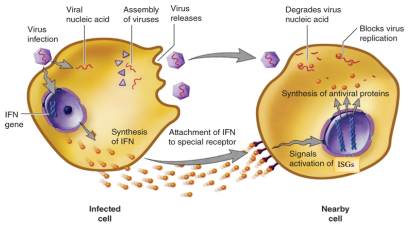


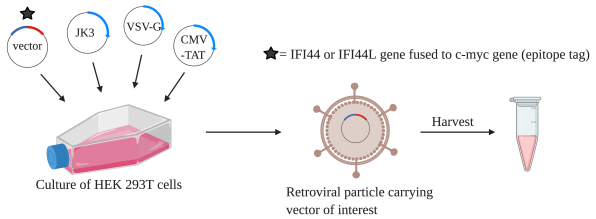
Figure 1. A schematic of the IFN and ISG action [2].

IFI44 and IFI44L are two paralogous ISGs whose transcription was found to be downregulated in myeloid cells infected with CMV, suggesting that they may exert antiviral functions and hence be targeted by CMV's immune evasion mechanisms acting on gene transcription [3,4]. In contrast, other studies reported that IFI44 and IFI44L may have proviral properties during replication of ssRNA viruses and may negatively modulate innate immune responses [3,5].

We sought to investigate if the IFI44 and IFI44L proteins exert negative or positive effects on CMV replication in HFF and ARPE-19 cells to determine if these two gene products might be useful targets to control CMV infection, which is a main cause of disease in newborns and immunocompromised individuals [6,7].

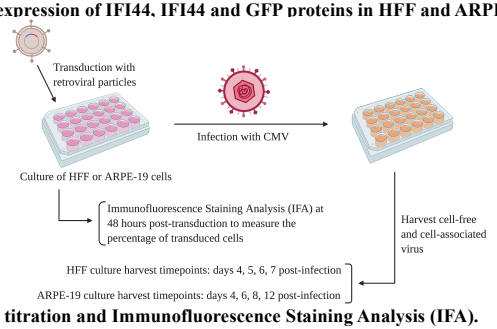
Methods

A. Production of replication-incompetent VSV-G pseudotyped retroviral particles containing expression vectors for the IFI44 and IFI44L proteins.

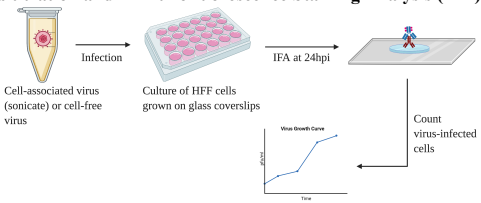


Methods Continued

B. Overexpression of IFI44, IFI44 and GFP proteins in HFF and ARPE-19 cells.



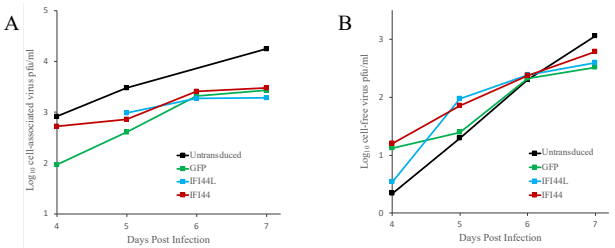
C. Virus titration and Immunofluorescence Staining Analysis (IFA).



Results

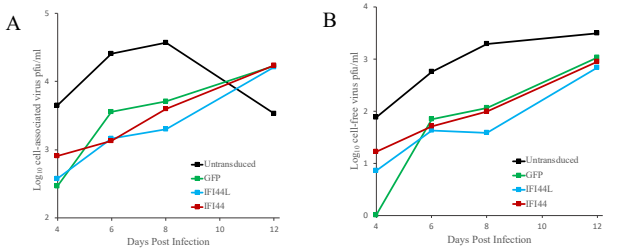
	Percentage of Transduced Cells	
Overexpressed Protein	HFF	ARPE-19
GFP	86.5	74.0
IFI44	89.4	80.6
IFI44L	94.6	88.1

Table 1. Percentage of HFF (average of two experiments) and ARPE-19 cells (one experiment) overexpressing GFP, IFI44, or IFI44L.



Effects of IFI44 and IFI44L overexpression on CMV strain TB40/E growth in HFF. Cells were left untransduced or were exposed to retroviruses for expression of the IFI44, IFI44L or of the green fluorescent proteins (GFP) for 48 hours prior to infection with CMV at a multiplicity of infection (MOI) of 0.01pfu/ml. The proportion of cells expressing each protein, as determined by IFA, are shown in table 1. The amounts of cell-associated progeny from one experiment (A) and of cell-free progeny from two independent experiments (averages) (B) are shown.

Results Continued



Effects of IFI44 and IFI44L overexpression on CMV strain TB40/E (stock SE2) growth in ARPE-19 cells. Cells were left untransduced or were exposed to retroviruses for expression of the IFI44, IFI44L or of the green fluorescent proteins (GFP) for 48 hours prior to infection with CMV at an MOI of 0.1pfu/ml. The proportion of cells expressing each protein, as determined by IFA, are shown in table 1. The amounts of cell-associated (A) and of cell-free (B) progeny from one experiments are shown.

Discussion

- No significant differences (10-fold or more) were observed between CMV-infected cells overexpressing GFP (control) and cells overexpressing the IFI44 or IFI44L proteins. Consequently, it is unlikely that these two ISG products exert antiviral functions, as was initially hypothesized.
- Inhibition of these genes' expression in CMV-infected myeloid cells may thus be part of a more general process of CMV-induced transcriptional downregulation of genes with non-essential functions during viral replication.
- Additional research using cells where expression of the IFI44 and IFI44L proteins has been reduced or eliminated is needed to determine if they may play pro-viral roles instead.

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