THE RELATIVE ANTIVIRAL ACTIVITY OF HUMAN INTERFERON ALPHAS ON PRIMATE CELL LINES, AND MOUSE ALPHA INTERFERONS ON HAMSTER AND RAT CELL LINES.

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ABSTRACT

The Type I IFN subtypes are often relatively species-selective, which makes cross-species experiments difficult to interpret. The Syrian golden hamster and Norwegian rat are common rodent models, and two macaques, rhesus and cynomolgus, are common non-human primate models for a variety of viral infections. The alpha interferon subtypes from these species are mostly unavailable and hence either mouse or human IFN subtypes will frequently be used in these model systems. In order to understand which mouse or human alpha IFN subtypes could be used in cross-species experiments, we have established antiviral cytopathic effect inhibition assays on rhesus macaque (LLC-MK2/VSV), cynomolgus macaque (JTC-12/EMCV), Syrian golden hamster (BHK-21/VSV) and Norwegian rat (C6/VSV) cell lines. All 14 mouse alpha IFNs were tested on the hamster and rat cells and all 12 of the human subtypes were tested on the rhesus and cynomolgus cell lines. In general, most of the mouse IFN-alpha subtypes exhibited activity on the other rodent cells and most of the human IFN-alpha subtypes displayed activity on the macaque cells. However, there are several notable exceptions with certain subtypes having little or no activity in the cross-species assay. The results of this study may be used to identify and select which human and mouse IFN-alpha subtypes which can be best used in the relevant animal model system.

INTRODUCTION

The Type I interferon system in mammals appears highly redundant. This system includes beta, epsilon, kappa, zeta and tau in a variety of species. The IFN-α family is particularly large in most mammals with 12 members in the human, 14 in the mouse and 18 in the rat. The reasons for this redundancy are unclear and the subject of a number of studies.

Hamsters, rats, rhesus and cynomolgus monkeys are often used as models of various diseases which might be treated with IFN. None of the hamster IFN-α subtypes are readily available and only one each of the rat, rhesus and cynomolgus IFN-α subtypes are available.

All of the human subtypes and several of the mouse subtypes are commercially available, but little data exists about which ones might work well in a cross species manner.

We have examined the antiviral effect of multiple human and mouse IFN-α subtypes in cell lines derived from these species in an effort to understand which subtypes might be suitable for particular experiments.

METHODS

Cell lines and viruses. Mouse fibroblast L929 (CCL-1), Hamster fibroblast BHK-21 (CCL-10), Rat fibroblast C6 (CCL-107), Human epithelial A549 and Rhesus epithelial LLC-MK2 (CCL-7 cell lines were obtained from ATCC (Manassa, Va.). Cynomolgus epithelial JTC-12 (JCRB0607) cell line was obtained from HSRRB (Tokyo, Japan). DMEM with 10% FBS (Invitrogen) was used to maintain A549 and JTC-12 cells. Eagles MEM with 10% FBS (Invitrogen) was used to maintain L929, BHK-21 and LLC-MK2 cells and F12K with 10% FBS (Invitrogen) was used to maintain the C6 cells. Encephalomyocarditis virus (VR-129B) and vesicular stomatitis virus (VR-1238) were also from ATCC and were grown in Vero (CCL-81).

Mouse IFN-α subtypes cloning and purification. All Mu IFN-α genes (Table 1) were synthesized in codon and mRNA optimized forms for expression in *E. coli*. They were expressed using a heat shock promoter system. The individual IFN-α mature proteins found in inclusion bodies were denatured with guanidine chloride, refolded by dilution and purified to >95% purity (Figure 1) using chromatographic methods (combination of ion exchange, sizing and hydrophobic interaction chromatography).

Human IFN-α subtypes. Were obtained from PBL InteferonSource stocks.

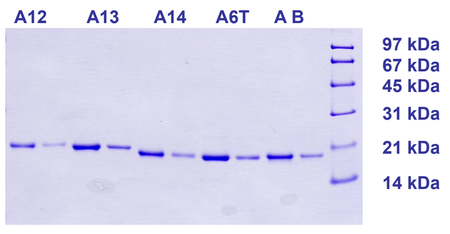
Cytopathic Effect Assay (CPE). All assays were established by plating cells in microtiter plates, allowing them to adhere for 2-4 hours followed by addition of test IFN. After an overnight incubation dilutions of either EMCV or VSV were added. After 24-48 hours cytopathic effect in wells containing no IFN was determined. The dilution of the viral stock to be used in the assay was emperically determined as 2-4 fold more concentrated than the dilution which killed 90 to 100% of the cells in the time frame. Viable cells were stained with crystal violet and excess dye was washed off with tap water. After drying the plates for at least 2 hr, the dye was solubilized with 70% methanol and absorbance was determined at 562 nm in a Molecular Devices UVMax plate reader. Inhibition CPE was calculated by setting 100% inhibition as cells incubate with virus but no IFN (virus control) and 0% inhibition as cells incubate without virus (cell control). Six wells were used for both controls. Generally the cell control was 6-10 times the value of the virus control. All samples data points were run in duplicate wells in at least two independent assays.

Table 1. Murine IFN genes and proteins used in this study.

IFN Gene	PBL Cat. #	GenBank Accession # mRNA	GenBank Accession # Protein	Mouse Strain
IFNA1	12105-1	AY225950	AAD63592	C57BL/6
IFNA2	NYA	X01969	CAA26002	BALB/c
IFNA4	12110-1	AY220463	AAD64456	129/Sv
IFNA5	NYA	AY220464	AAD64457	129/Sv
IFNA6T	NYA	AY220465	AAD64458	129/Sv
IFNA7/10	NYA	M1310	AAA37888	Swiss
IFNA8/6	NYA	AY225953	AAD63595	C57BL/6
IFNA9	NYA	M13660	AAA37886	BALB/c
IFNA11	12125-1	AY225954	AAD63596	C57BL/6
IFNA12	NYA	AY225951	AAD63593	C57BL/6
IFNA13	12130-1	AY220461	AAD64454	129/Sv
IFNA14	NYA	AY220462	AAD64455	129/Sv

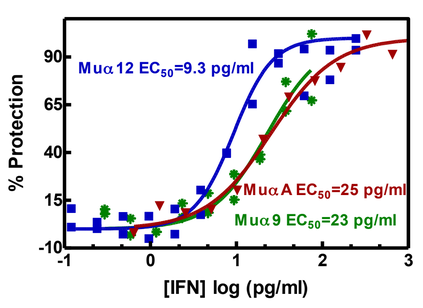
NYA is not yet available.

Figure 1. Purified Mu IFN-α proteins show >95% purity.



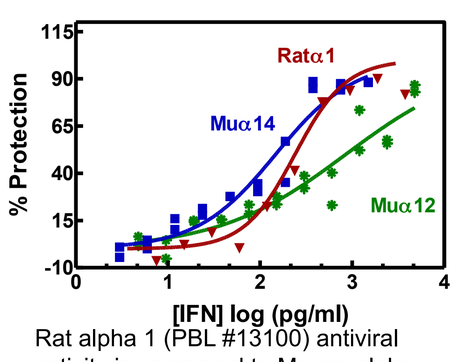
Variable expression level and yield among the purified mouse IFN-α subtypes (0.3 to 10 mg/L of bacterial culture) were obtained. Mouse IFN-α12, 13, 14, 6T and B are loaded on 12.5 % SDS-PADE at 3 and 1 µg per lane, respectively.

Figure 2. Standard CPE assay on L929 cells challenged with EMCV.



PBL laboratory standard (Mu IFN-αA) has been calibrated to the NIH Mouse IFN-α standard (Ga02-901-511).

Figure 3. Murine IFN-α12, murine IFN-α14 and Rat IFN-α1 protection of rat C6 cells from VSV challenge.



Rat alpha 1 (PBL #13100) antiviral activity is compared to Mouse alpha 12 and 14 on the rat C6 cells challenged with VSV.

Figure 4. Murine IFN-α6T, murine IFN-α13 and human IFN-α2a protection of Syrian golden hamster BHK-21 cells from VSV challenge.

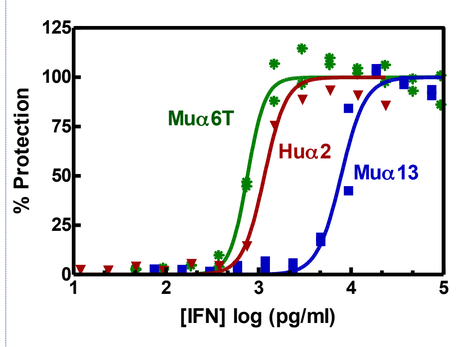


Figure 5. Comparison of Activity of Murine IFN-α Subtypes Across Assays.

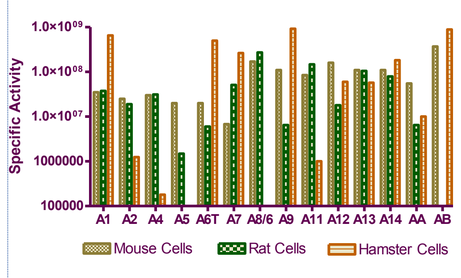


Table 2. Comparison of the antiviral activities of Murine IFN-α proteins on L929, C6 and BHK-21 cells.

IFN Subtype	L929/EMCV	RatC6/VSV	Hamster BHK-21
IFN-α1	3.5E+07	3.8E+07	1.1E+08
IFN-α2	2.5E+07	1.9E+07	1.2E+06
IFN-α4	3.0E+07	3.1E+07	1.8E+05
IFN-α5	2.1E+07	1.5E+06	NT
IFN-α6T	2.0E+07	6.0E+06	5.0E+08
IFN-α7	6.0E+06	5.1E+7	2.6E+8
IFN-α8	1.7E+08	2.7E+8	NT
IFN-α9	2.00E+08	6.5E+6	9.2E+8
IFN-α11	8.5E+07	1.5E+08	<1.1e+6
IFN-α12	1.6E+08	1.8E+07	6.0E+07
IFN-α13	3.4E+07	1.1E+08	5.7E+07
IFN-α14	1.1E+08	7.9E+07	1.8E+08
IFN-αA	4.6E+07	6.5E+06	<1e7
IFN-αB	3.7E+08	NT	8.9e8

On the mouse L929 cells, murine IFN-α 8,9,12,14 and B exhibit the highest activities. On the Rat C6 cells, IFN-α 8, 11, 13 exhibit the highest activities while on the hamster BHK-21 cells, IFN-α 6T, 7, 9, 14 and B exhibit the highest activities.

In general it appears that Murine IFN-α1, 13, 14 and perhaps B exhibit the best cross species reactivity. Thus these would be the choices for cross species studies.

Table 3. Human IFN genes and proteins used in this study.

IFN Gene	PBL Cat #	GenBank Accession # DNA	GenBank Accession # Protein
IFN-α1b	11125	V00538	CAA23799
IFN-α2a	11100	V00549	CAA23810
IFN-α4a	11177	NM002168	NP 066546
IFN-α4b	11180	X02955	CAA26701
IFN-α5	11135	X02956	CAA26702
IFN-α6	11165	X02958	CAA26704
IFN-α7a	11160	X02960	CAA26706
IFN-α8b	11115	X03125	CAA26903
IFN-α10a	11120	NM002171	NP 002162
IFN-α14c	11145	V00533	CAA23794
IFN-α16	11190	X02957	CAA26703
IFN-α17a	11150	J00216	AAA52723
IFN-α21a	11130	V00540	CAA23801

Figure 6. Activity of selected Human IFN-α subtypes on A549 cells

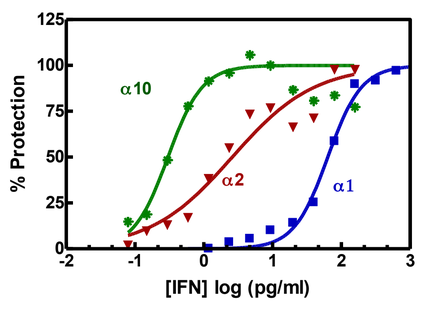


Figure 7. Activity of selected Human IFN-α subtypes on Rhesus LLC-MK2 cells

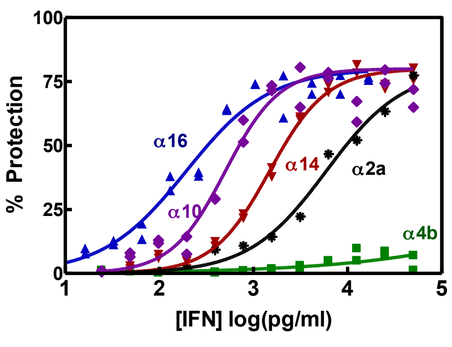


Figure 8. Sequence Relationships between Human, Rhesus and Cynomolgus Interferon Alphas.

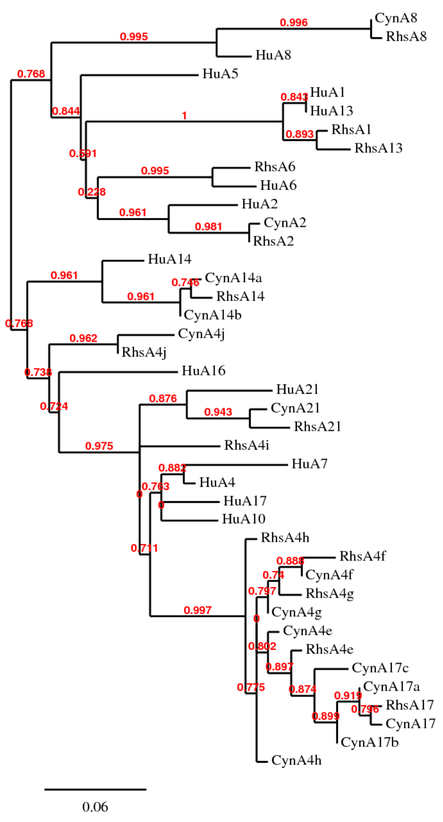


Figure 9. Comparison of Activity of Human IFN-α subtypes on human and monkey cells.

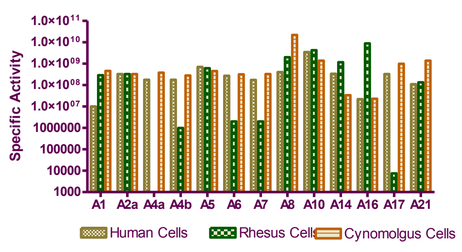


Table 4. Antiviral Activity comparison of Human IFN-α proteins on Human, Rhesus and Cynomolgus Cells.

IFN Subtype	A549/EMCV	LLC-MK2/VSV	JTC-12/VSV
Gene	Cat #	DNA	Protein
IFN-α1b	1.1E+7	2.9E+8	4.6E+8
IFN-α2a	3.3E+8	3.3E+8	3.3E+8
IFN-α4a	1.8E+8	<1e6	3.8E+8
IFN-α4b	1.7E+8	<2e6	2.8E+8
IFN-α5	7.1E+8	6.2E+8	4.6E+8
IFN-α6	2.7E+8	<2e6	3.2E+8
IFN-α7a	1.8E+8	<2e6	3.3E+8
IFN-α8b	4.1E+8	2.0E+9	2.2E+10
IFN-α10a	3.5E+9	4.2E+9	1.4E+9
IFN-α14c	3.4E+8	1.2E+9	3.4E+7
IFN-α16	2.2E+7	8.9E+9	2.3E+7
IFN-α17a	3.3E+8	5.3E+8	1.0E+9
IFN-α21a	1.1E+8	1.3E+8	1.4E+9

Human IFN-α2, 5 and 10 seem to have consistent activity across the cell lines. IFN-α1 has higher activity on the monkey cells.

CONCLUSIONS

We have examined the ability of the panel of mouse and human IFN-α subtypes to protect heterologous cells from viral challenge.

Some of the subtypes exhibit very significant lack of cross reactivity between the species.

The most readily available mouse IFN-α subtype, IFN-αA shows relatively low activity on rat and Syrian golden hamster cells.

Murine IFN-α 5, 9 and 12 have weak activity on rat cells while at least 9 and 12 have good activity on hamster cells.

Human IFN-α4a, 4b, 6 and 7 have very weak activity on rhesus cells but good activity on cynomolgus cells.

Some of the IFN-α subtypes do appear appropriate for experimental work involving multiple species.

Mouse IFN-α1 and 11 have good activity across the three species.

Human IFN-α2, 5 and 10 have good reactivity across the species tested and IFN-α10 in particular has very potent activity on all the tested cells.

This result is somewhat intriguing since neither the rhesus or cynomolgus genome contains a clear homologue of the human IFN-α10 sequence.