Differential Transcriptional Responses to Interferon- α and Interferon- γ in Primary Human Hepatocytes

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Interferon (IFN) plays a central role in the innate and adaptive antiviral immune responses. While IFN- α is currently approved for treating chronic hepatitis B and hepatitis C, in limited studies, IFN- γ has not been shown to be effective for chronic hepatitis B or C. To identify the potential mechanism underlying the differential antiviral effects of IFN- α and IFN- γ , we used cDNA microarray to profile the global transcriptional response to IFN- α and IFN- γ in primary human hepatocytes, the target cell population of hepatitis viruses. Our results reveal distinct patterns of gene expression induced by these 2 cytokines. Overall, IFN- α induces more genes than IFN- γ at the transcriptional level. Distinct sets of genes were induced by IFN- α and IFN- γ with limited overlaps. IFN- α induces gene transcription at an early time point (6 h) but not at a later time point (18 h), while the effects of IFN- γ are more prominent at 18 h than at 6 h, suggesting a delayed transcriptional response to IFN- γ in the hepatocytes. These findings indicate differential actions of IFN- α and IFN- γ in the context of therapeutic intervention for chronic viral infections in the liver.

Introduction

TYPE I AND TYPE II interferons (IFNs) play a central role in the innate and adaptive immune responses against both acute and chronic viral infections. Type I IFNs, including IFN- α , IFN- β , and IFN- ω , are produced directly by virus-infected host cells, while type II IFN, or IFN- γ , is induced by viral antigen-stimulated immune cells including NK cells and T cells (Stark and others 1998). Although the mechanisms for the antiviral effects of these IFNs are not entirely clear, it is believed that they exert their antiviral function through both direct and indirect mechanisms. Both IFN- α and IFN- γ have a profound effect on host gene expression. The direct antiviral effects of IFNs are thought to involve induction of IFN-stimulated genes (ISGs), which establishes an antiviral state in the infected cells (Samuel 1991; Stark and others 1998; Samuel 2001). The indirect antiviral effects of IFNs involve their regulation of the innate and adaptive immune systems, which is also likely to be mediated by ISGs.

IFN- α is the major FDA-approved therapeutic for chronic infection with hepatitis C virus (HCV) and remains an effective therapeutic in certain circumstances for hepatitis B

virus (HBV) infection. HCV treatment with recently developed peginterferon α -2b and peginterferon α -2a in combination with ribavirin has a significantly higher response rate than treatment with IFN- α alone, resulting in a sustained virological response rate of ~50% (Manns and others 2001; Fried and others 2002). Subgenomic HCV replicon cells and more recently, infectious HCV cell culture systems have been used to study the anti-HCV effects of IFNs *in vitro*. It has been demonstrated that both IFN- α and IFN- γ potently suppress HCV replication in cell culture (Frese and others 2002; Lanford and others 2003; Kato and others 2007). Despite the similarity between IFN- α and IFN- γ regarding their effects on cellular gene expression and subgenomic HCV replicon, the therapeutic effect of IFN- γ on chronic HCV infection has not been evaluated in clinical trials with treatment-naïve patients. A pilot study with IFN- γ in patients with chronic hepatitis C who had failed to achieve a sustained response to IFN- α -based therapies showed no effect of IFN- γ (100–400 μ g, 3 times per week) on HCV RNA levels (Soza and others 2005). In addition to HCV, IFN- α is also one of the current therapeutic agents for chronic HBV

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infection, although the treatment effect on HBV viral load is smaller compared to HCV and only about 30% of treated individuals respond to treatment (Leung 2002; Lau and others 2005; Lai and others 2006). Clinical trials of IFN- γ for chronic HBV infection failed to show a significant anti-HBV effect (Marcellin and others 1990; Kakumu and others 1991; Lau and others 1991).

Previous studies have shown that IFN- α and IFN- γ share a broad range of signal transduction pathways (Stark and others 1998; Samuel 2001; Sanda and others 2006). However, the overall patterns of transcriptional responses of human hepatocytes to IFN- α and IFN- γ have not been compared systematically. We had previously used microarray expression analyses to investigate ISGs in liver or PBMCs from chronic hepatitis C patients starting IFN- α therapy and demonstrated that the outcome of IFN- α therapy is associated with the magnitude of overall ISG induction (Ji and others 2003; He and others 2006; Feld and others 2007). To explore the potential mechanism underlying the differences in the *in vivo* antiviral effects of IFN- α and IFN- γ , we used cDNA microarray to profile the global transcriptional response to IFN- α and IFN- γ in primary human hepatocytes, the major target of infection by hepatitis viruses. Our results reveal distinct patterns of gene expression induced by these 2 cytokines in terms of the kinetics, breadth, and magnitude of transcriptional response in the hepatocytes. These findings suggest differential actions of these 2 IFNs in the context of therapeutic intervention for chronic viral infections in the liver.

Materials and Methods

Human hepatocyte culture and interferon treatment

Primary human hepatocytes were obtained from Stephen C. Strom of University of Pittsburgh, through Liver Tissue Procurement and Distribution System (Pittsburgh, Pennsylvania). Five donor livers were used for this study. These hepatocytes maintain liver-specific functions for many days under a defined culture condition (Kostrubsky and others 1999). In brief, human hepatocytes were prepared from livers not used for whole organ transplant within 24 h of procurement. Hepatocytes were isolated by a 3-step collagenase perfusion technique, as described (Strom and others 1996). Hepatocytes with a viability of >80% (assessed by a Trypan Blue exclusion method) were plated at a cell density of 2×10^6 cells per well on 6-well culture plates previously coated with rat-tail collagen in human hepatocyte maintenance medium (HMM) supplemented with 0.1 μ M insulin, 0.1 μ M dexamethasone, 0.05% streptomycin, 0.05% penicillin, 0.05% amphotericin B, and 10% bovine calf serum (HMM⁺) (Runge and others 2000). After 4 h of culturing for cell attachment, the medium was replaced with serum-free HMM⁺. After another 24 h in culture, unattached cells were removed by gentle agitation and washing with PBS. The cells were maintained in culture at 37°C in serum-free HMM containing 5% CO₂ for 6 and 18 h, with or without IFN- α -2b (Schering, Kenilworth, NJ) at a concentration of 200 U/mL as previously described (Ji and others 2003; He and others 2006), or with IFN- γ -1b (Actimmune®, kindly provided by Larry Blatt, InterMune, Inc., Brisbane, CA) at a concentration of 20 ng/mL. This concentration of IFN- γ was chosen based on a similar ratio of concentrations of IFN- α versus IFN- γ

in the hepatocyte cultures compared to the standard clinical dosage of IFN- α -2b (3 million U) versus the dosage of IFN- γ -1b (100–400 μ g) in a previous clinical trial of IFN- γ for treating chronic HCV infection (Soza and others 2005).

Microarray assay

Hepatocyte total RNA isolation, cDNA synthesis, as well as preparation of fluorescence labeled targets, and cDNA microarray hybridization were carried out as previously reported (He and others 2006). Microarrays were obtained from Stanford Functional Genomics Facility (Stanford, CA). Each microarray contained ~43,000 spots, including 85.8% I.M.A.G.E. consortium clones from the Research Genetics Sequence Verified clone set (http://www.invitrogen.com/content/sfs/manuals/sequenceverifiedclones_man.pdf), 9.8% CGAP clone set (<http://cgap.nci.nih.gov/Genes/PurchaseReagents>), and 4.4% control spots or customer spots, corresponding to ~38,500 human sequence-verified genes. Detection and processing of the fluorescence signals on microarrays were carried out as described (He and others 2006). For all subsequent analyses, we included 7,379 array elements whose expression was well-measured. We defined well-measured genes by having (1) a ratio of normalized signal intensity to background noise of >2 for either the Cy5 signal derived from IFN-stimulated hepatocytes or the Cy3 signal derived from unstimulated hepatocytes and (2) a pixel regression correlation of $R < 0.6$ in at least 70% of all arrays (He and others 2006).

Microarray data analysis

One-class SAM analysis (Tusher and others 2001) was performed to identify IFN-regulated genes under each experimental condition at a False Discovery Rate (FDR) of <10%. Ingenuity Pathways Analysis (IPA; Ingenuity, Redwood City, CA) was used to identify pathways significantly ($P < 0.002$) activated by IFN- α at 6 h and by IFN- γ at 18 h.

Quantitative real-time PCR analysis

To validate the results of microarray analysis, several ISGs—*MxA*, *IFIT1*, *IP10*, and *interferon responsive factor 1* (*IRF1*)—were selected for TaqMan real-time reverse transcription (RT)-PCR analysis using the iCycler (Bio-Rad, Hercules, CA). In brief, cDNA was generated using 500 ng of total RNA in a 20- μ L RT reaction with the First-Strand cDNA Synthesis System for Quantitative RT-PCR Kit (Marligen Biosciences, Ljamsville, MD) as templates. TaqMan probes and primers for the genes of interest were purchased from PE Applied Biosystems (Foster City, CA) and the reactions were performed according to the manufacturer's guidelines. Standard curve for each individual target amplicon was constructed using cDNA from Huh7. All PCR assays were performed in duplicates, and results are represented by the mean values. The 18s rRNA was used as a housekeeping gene against which all samples were normalized.

Results

To investigate the transcriptional responses of hepatic genes induced by IFN- α or IFN- γ , we prepared primary hepatocytes from 5 healthy human donor livers. Each

hepatocyte culture was incubated with or without IFN- α or IFN- γ for 6 and 18 h, followed by microarray analysis to identify genes regulated by each cytokines at the 2 time points. Across these 20 microarrays, a total of 7,379 array elements were identified as well-measured genes based on the pre-set criteria (He and others 2006).

First, we examined the kinetics of transcriptional responses to IFN- α and IFN- γ . For each experimental condition (IFN- α /6 h, IFN- α /18 h, IFN- γ /6 h, and IFN- γ /18 h), average fold change (aFC) was calculated for each gene across all 5 samples. The aFC values of the 7,379 genes were sorted by descending order and plotted against their ranking (Fig. 1). While the vast majority of these genes had an aFC close to 1, both up-regulated genes, or by definition, ISGs, and down-regulated genes were identified under each experimental condition. In hepatocytes incubated with IFN- α , the numbers of ISGs with aFC > 1.4 or aFC > 2 are greater at the 6-h time point than at the 18-h time point (Fig. 1, insets A and B). Similarly, the numbers of down-regulated genes with aFC < 1/1.4 or aFC < 1/2 are both greater at 6 h than at 18 h (Fig. 1, inset C). In contrast to IFN- α , when hepatocytes were incubated with IFN- γ , the numbers of ISGs with aFC > 1.4 or aFC > 2 are both smaller at the 6-h time point than at the 18-h time point (Fig. 1, insets A and B), while the numbers of down-regulated genes with aFC < 1/1.4 or aFC < 1/2 are both smaller at 6 h than at 18 h (Fig. 1, inset C).

We further compared the aFC of individual ISG between the 2 time points for each IFN treatment. In hepatocytes incubated with IFN- α (Fig. 2A), the majority (274 of 331, 83%) of genes with an overall aFC > 1.4 had a greater aFC at 6 h than at 18 h. In contrast, in hepatocytes incubated with IFN- γ (Fig. 2B), the majority (188 of 308, 61%) of genes with an overall aFC > 1.4 had a greater aFC at 18 h than at 6 h. Taken together, these results indicated that the effects of IFN- α on hepatocyte gene expression decrease from 6 h to 18 h, while the effects of IFN- γ increase during the same time frame, suggesting that compared to IFN- α , the hepatocyte transcriptional responses to IFN- γ were delayed.

To compare the pattern of ISG induction by IFN- α versus IFN- γ in hepatocytes, we first compared the aFC of individual ISGs in hepatocytes incubated with these 2 IFNs at each time point. At the 6-h time point, the majority (314 of 414, 76%) of genes with an overall aFC > 1.4 were induced to a greater FC by IFN- α than by IFN- γ (Fig. 2C). At 18 h, however, the majority (154 of 214, 72%) of the ISGs with an overall aFC > 1.4 were induced to a greater aFC by IFN- γ than by IFN- α (Fig. 2D).

Next we used SAM to identify genes significantly induced by IFN- α or IFN- γ at the 2 time points, with a false discovery rate of <10%. Table 1 lists all the identified genes and their aFC values. For IFN- α , 107 genes were identified as significantly up-regulated at 6 h. At 18 h, not a single gene was

FIG. 1. Distribution of average fold change (aFC) values for mRNA levels of hepatocyte genes induced by interferon (IFN)- α or IFN- γ . Primary human hepatocyte cultures from 5 donors were incubated with and without IFN- α or IFN- γ for 6 and 18 h. Microarray assay was used to measure FC of mRNA levels for individual genes in response to IFN- α or IFN- γ , respectively, at each time point. For each experimental condition, the aFC values for individual genes were calculated across all 5 donors, sorted by descending order, and normalized to make the median aFC equal to 1. The normalized aFC values were plotted against the rank number of genes. Inset A shows the number of genes with aFC > 2 and aFC > 1.4 under each experimental condition. Inset B shows a close-up look of the first 400 genes with the greatest aFC values (up-regulated genes) from the main graph. Inset C shows a close-up look of the last 400 genes with the smallest aFC values (down-regulated genes) from the main graph.

FIG. 2. Group-wise comparison of average FC (aFC) of mRNA levels of individual genes in human hepatocytes incubated with interferon (IFN)- α or IFN- γ at 6 and 18 h. Included are all genes with aFC > 1.4. The genes are ranked by descending order of the overall average FC across all the individual samples in the compared groups. (A) Comparison of IFN- α -induced changes for 331 genes, 6 h versus 18 h. (B) Comparison of IFN- γ -induced changes for 308 genes, 6 h versus 18 h. (C) Comparison of 412 genes, IFN- α versus IFN- γ at 6 h. (D) Comparison of 214 genes, IFN- α versus IFN- γ at 18 h.

significantly up-regulated. In contrast, the number of genes significantly up-regulated by IFN- γ increased from 56 at 6 h to 77 at 18 h. We then examined the overall pattern of ISG induction in response to IFN- α and IFN- γ by comparing the list of significantly up-regulated genes identified with SAM. As shown in Figure 3, the majority (64%), or 69 of 107 genes significantly up-regulated by IFN- α at 6 h were not up-regulated by IFN- γ , while the majority of IFN- γ up-regulated genes at 6 h (but not at 18 h) were also up-regulated by IFN- α at the same time point. Of note, even when the genes significantly up-regulated by IFN- γ at 18 h were compared to those up-regulated by IFN- α at 6 h, <50% of the 2 gene lists overlapped with each other. These results suggest that IFN- α and IFN- γ induced distinct sets of ISGs in hepatocytes with limited overlap.

To validate the results of microarray analysis, we performed real-time quantitative PCR. The ISGs, *Mx1* and *IFIT1*, were chosen as representative genes more specific for IFN- α , while *IRF1* and *IP10* (*CXCL10*) were chosen for IFN- γ . Both *Mx1* and *IFIT1* were highly induced in comparison to their respective controls in the IFN- α -treated hepatocytes at the 6-h time point (Fig. 4). Their strength of induction diminished at 18 h of IFN- α treatment. In contrast, the more IFN- γ -specific genes *IRF1* and *IP10* followed the opposite trend and were induced to higher levels at the 18-h time point (Fig. 4). Quantitative PCR of these selected ISGs supported the overall trend as defined by microarray analysis.

To examine the functional pathways activated by IFN- α and IFN- γ , we used Ingenuity Pathways Analysis (IPA) to identify functional pathways that were significantly activated by IFN- α (at 6 h) or by IFN- γ (at 18 h). At the significance level of $P < 0.002$, 5 functional pathways were commonly activated by IFN- α and IFN- γ , 2 functional pathways were significantly activated by IFN- α only, while 4 functional pathways were significantly activated by IFN- γ only (Table 2).

Discussion

We used microarrays to investigate the transcriptional responses in primary hepatocytes induced by IFN- α and IFN- γ , 2 cytokines that have been approved or tested in clinical trials for treating chronic hepatitis B or C. The antiviral effects of IFNs are believed to be mediated by the induction of ISGs, which have been examined extensively in previous studies. However, most of the previously documented human ISGs were identified either in the cell lines (Der and others 1998), or in PBMCs (Ji and others 2003). In this study, we used primary human hepatocytes derived from donor livers to examine the transcriptional responses to IFN- α and IFN- γ , which should generate more relevant information for the therapeutic effects of these 2 cytokines against the hepatitis viruses in their target organ.

Our results indicated that in the primary hepatocytes, while both IFN- α and IFN- γ induced large numbers of gene transcripts, a striking difference between the cellular transcriptional responses to these 2 IFNs is the kinetics of gene induction. A vigorous response to IFN- α was seen at 6 h that disappeared completely at 18 h. In contrast, a slower transcriptional response to IFN- γ was observed that was greatly enhanced at 18 h compared to 6 h. Although the implication of this kinetic difference to the antiviral effects of IFN- α versus IFN- γ is not clear at this time, it is likely that the vigorous early transcriptional response to IFN- α in hepatocytes is directly related to the rapid decline of serum HCV viral load observed within hours of IFN- α injection (Neumann and others 1998).

The best understood signal transduction pathway triggered by IFNs is the JAK-STAT pathway (Stark and others 1998; Sen 2001; Plataniias 2005). Binding of IFN to its cognate transmembrane receptors on the cell surface triggers dimerization of the receptor subunits and activation of the receptor-associated Janus kinases, JAK1 and TYK2 for the

TABLE 1. SIGNIFICANTLY UP-REGULATED HUMAN HEPATOCYTE GENES BY IFN- α AND IFN- γ AT 6 AND 18 h

ID	Name	IFN- α /6 h	IFN- γ /6 h	IFN- γ /18 h
ARL5B	ADP-ribosylation factor-like 5B	2.6		
BAZ1A	Bromodomain adjacent to zinc finger domain, 1A	2.7		
BLZF1	Basic leucine zipper nuclear factor 1 (<i>JEM-1</i>)	2.6		
BST2	Bone marrow stromal cell antigen 2	4.3	3.6	5.9
C1orf38	Chromosome 1 open reading frame 38	3.8		
C3AR1	Complement component 3a receptor 1	2.8		
CA7	Carbonic anhydrase VII			6.2
CARHSP1	Calcium-regulated heat stable protein 1, 24 kDa			2.6
CASP1	Caspase 1, apoptosis-related cysteine peptidase	3.0		
CCRL1	Chemokine (C-C motif) receptor-like 1			3.9
CD38	CD38 molecule			2.7
CD40	CD40 molecule, TNF receptor superfamily member 5			5.0
CD53	CD53 molecule	2.5		
CDC25B	Cell division cycle 25 homolog B (<i>Schizosaccharomyces pombe</i>)			2.5
CMPK2	Cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	3.7		
CSF2RB	Colony-stimulating factor 2 receptor, β , low affinity			3.5
CTSO	Cathepsin O			2.8
CTSS	Cathepsin S		3.1	2.7
CX3CL1	Chemokine (C-X3-C motif) ligand 1	5.2	7.5	7.3
CXCL10	Chemokine (C-X-C motif) ligand 10	8.2	7.0	3.9
CXCL9	Chemokine (C-X-C motif) ligand 9		3.5	3.8
CYorf15A	Chromosome Y open reading frame 15A	6.8	3.2	3.0
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1			2.8
DDO	D-Aspartate oxidase	3.2		
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	8.9	3.0	
DDX60L	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60-like	4.5		
DUOXA1	Dual oxidase maturation factor 1			2.9
DUOXA2	Dual oxidase maturation factor 2			2.9
DUSP6	Dual specificity phosphatase 6		2.6	
EIF2AK2	Eukaryotic translation initiation factor 2- α kinase 2	3.0		
EPSTI1	Epithelial stromal interaction 1 (breast)	8.8	7.2	9.2
ETV6	Ets variant gene 6 (TEL oncogene)	2.5		
EXT1	Exostoses (multiple) 1	2.9		
FAM111A	Family with sequence similarity 111, member A	2.9		
FAM26F	Family with sequence similarity 26, member F	6.2		5.0
FAM72B	Family with sequence similarity 72, member B	3.6		
FLJ11171	Hypothetical protein FLJ11171	3.8	2.7	
FLJ39639	Hypothetical protein FLJ39639			2.5
FST	Follistatin			2.4
GBP1	Guanylate-binding protein 3		3.3	
GBP2	Guanylate-binding protein 2, interferon-inducible		3.6	4.5
GCNT1	Glucosaminyl (N-acetyl) transferase 1, core 2	3.5		
GLB1	Galactosidase, β 1		2.8	
GMPR	Guanosine monophosphate reductase	7.9		
GNB4	Guanine nucleotide-binding protein (G protein), β -polypeptide 4	2.9		
HIST2H2AA3	Histone cluster 2, H2aa3	2.6		
HK2	Hexokinase 2	2.8		3.2
HLA-DPA1	Major histocompatibility complex, class II, DP- α 1			3.3
HLA-DQB1	Major histocompatibility complex, class II, DQ- β 1			3.2
HLA-DRB1	Major histocompatibility complex, class II, DR- β 3	3.2		3.6
HLA-DRB4	Major histocompatibility complex, class II, DR- β 4	3.8		5.1
HLA-E	Major histocompatibility complex, class I, E	2.5	2.8	4.8
HMCN2	Hemicentin 2	5.8		
IFI16	Interferon, γ -inducible protein 16	2.5		
IFI30	Interferon, γ -inducible protein 30			2.9
IFI44L	Interferon-induced protein 44-like	6.3	3.8	5.4
IFI6	Interferon, α -inducible protein 6	3.1		
IFIH1	Interferon induced with helicase C domain 1	9.0	3.0	3.0

(Continued)

TABLE 1. (CONTINUED)

ID	Name	IFN- α /6 h	IFN- γ /6 h	IFN- γ /18 h
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1	17.6	4.8	2.9
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	19.7	5.6	6.0
IFITM1	Interferon-induced transmembrane protein 1 (9–27)	5.6	3.6	5.8
IL18BP	Interleukin 18-binding protein		4.0	7.1
IL1RN	Interleukin 1 receptor antagonist	5.0		
INDO	Indoleamine-pyrrole 2,3 dioxygenase			3.1
IRF2	Interferon regulatory factor 2	10.7	3.6	
IRF7	Interferon regulatory factor 7	2.9		
IRF8	Interferon regulatory factor 8		2.9	
ISG15	ISG15 ubiquitin-like modifier	7.2	4.1	5.3
ISG20	Interferon-stimulated exonuclease gene 20 kDa	3.0	2.7	4.1
JAK2	Janus kinase 2 (a protein tyrosine kinase)			3.4
KLHL17	Kelch-like 17 (<i>Drosophila</i>)	3.5		
LAMP3	Lysosomal-associated membrane protein 3	6.0		3.8
LAP3	Leucine aminopeptidase 3	2.7		3.1
LMO2	LIM domain only 2 (rhombotin-like 1)	4.2		
LOC100129349	Hypothetical protein LOC100129349	11.8	4.5	5.5
LOC285194	Hypothetical LOC285194	3.6		
LOC644151	Similar to calpain 8			2.6
LOC727820	Hypothetical protein LOC727820	3.0		
LOC730054	Similar to hCG2040642			2.8
LRRC44	Leucine-rich repeats and IQ motif-containing 3	2.2		
MAP2K5	Mitogen-activated protein kinase kinase 5	2.7		
MASTL	Microtubule-associated serine/threonine kinase-like	3.9		
MEG3	Maternally expressed 3			4.0
MS4A4A	Membrane-spanning 4-domains, subfamily A, member 4	3.1		
MS4A6A	Membrane-spanning 4-domains, subfamily A, member 6A	3.6		
MX1	Myxovirus (influenza virus) resistance 1	4.3	2.7	
MX2	Myxovirus (influenza virus) resistance 2	6.5		
MXD1	MAX dimerization protein 1		2.5	2.7
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	2.8		
NLRC5	NLR family, CARD domain containing 5	3.0	3.2	
NMI	N-myc (and STAT) interactor	2.9		
NT5C3	5'-Nucleotidase, cytosolic III	5.2		
OAS1	2',5'-Oligoadenylate synthetase 1, 40/46 kDa	6.4	3.5	3.0
OAS2	2'-5'-Oligoadenylate synthetase 2, 69/71 kDa	7.0	4.3	4.9
OAS3	2'-5'-Oligoadenylate synthetase 3, 100 kDa	5.7	3.8	4.2
OTUD4	OTU domain containing 4	3.1		
PARP14	Poly (ADP-ribose) polymerase family, member 14	3.7	3.7	
PLA1A	Phospholipase A1 member A		2.7	3.6
PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)			4.3
PLAUR	Plasminogen activator, urokinase receptor			3.1
PLEK	Pleckstrin			2.6
PLEKHA4	Pleckstrin homology domain containing, family A	3.2		
PLSCR1	Phospholipid scramblase 1	4.6	3.4	
PML	Promyelocytic leukemia	3.2		
PPM1K	Protein phosphatase 1K (PP2C domain containing)	2.6		
PRIC285	Peroxisomal proliferator-activated receptor A interacting complex 285	8.4	3.8	4.2
PSMB10	Proteasome (prosome, macropain) subunit, β -type, 10			2.9
PSMB8	Proteasome (prosome, macropain) subunit, β -type, 8		2.8	3.1
RAB4B	Member RAS oncogene family	3.8		
RAB8B	Member RAS oncogene family	2.4		
RABGAP1L	RAB GTPase-activating protein 1-like	7.6	2.6	
RARRES3	Retinoic acid receptor responder (tazarotene-induced) 3		5.4	6.8
RASGRP3	RAS guanyl-releasing protein 3 (calcium and DAG-regulated)	3.1		
RASSF5	Ras association (RalGDS/AF-6) domain family member 5		2.6	
RNF213	Ring finger protein 213	3.6	3.7	

(Continued)

TABLE 1. (CONTINUED)

ID	Name	IFN- α /6 h	IFN- γ /6 h	IFN- γ /18 h
<i>RNF34</i>	Ring finger protein 34			2.5
<i>RSAD2</i>	Radical S-adenosyl methionine domain containing 2	11.6	3.0	2.9
<i>RTP4</i>	Receptor (chemosensory) transporter protein 4	10.2	3.7	
<i>SAMD9L</i>	Sterile α motif domain containing 9-like	4.2	3.1	3.2
<i>SAMHD1</i>	SAM domain and HD domain 1	2.5	2.6	2.5
<i>SDS</i>	Serine dehydratase	3.4		
<i>SELL</i>	Selectin L (lymphocyte adhesion molecule 1)	2.6		
<i>SLC15A3</i>	Solute carrier family 15, member 3	3.5		
<i>SLC22A23</i>	Solute carrier family 22, member 23	6.0		4.6
<i>SOCS1</i>	Suppressor of cytokine signaling 1			3.3
<i>SP110</i>	SP110 nuclear body protein	3.9	2.8	
<i>SRGN</i>	Serglycin	3.1		2.9
<i>STAT1</i>	Signal transducer and activator of transcription 1, 91 kDa	4.1	4.0	4.7
<i>STAT2</i>	Signal transducer and activator of transcription 2, 113 kDa	3.3		
<i>TAP1</i>	Transporter 1, ATP-binding cassette, subfamily B	2.5	2.9	
<i>TAP2</i>	Transporter 2, ATP-binding cassette, subfamily B			2.8
<i>TLR2</i>	Toll-like receptor 2	2.7		
<i>TMEM110</i>	Transmembrane protein 110	3.3		
<i>TMEM192</i>	Transmembrane protein 192	3.2		
<i>TMEM50A</i>	Transmembrane protein 50A	2.5		
<i>TMEM86A</i>	Transmembrane protein 86A			2.7
<i>TNFSF10</i>	Tumor necrosis factor (ligand) superfamily, member 10	4.2	3.3	2.8
<i>TNFSF13B</i>	Tumor necrosis factor (ligand) superfamily, member 13b	7.3	3.7	
<i>TRAFD1</i>	TRAF-type zinc finger domain containing 1	2.9	2.9	2.9
<i>TRIM22</i>	Tripartite motif-containing 22	2.3		
<i>TRIM31</i>	Tripartite motif-containing 31			3.8
<i>TRIM46</i>	Tripartite motif-containing 46	2.5		
<i>TXNIP</i>	Thioredoxin-interacting protein	3.9		2.4
<i>UBD</i>	Ubiquitin D		3.4	3.8
<i>UBE2D4</i>	Ubiquitin-conjugating enzyme E2D 4 (putative)		2.8	3.0
<i>UBE2L6</i>	Ubiquitin-conjugating enzyme E2L 6	3.6	4.5	4.9
<i>USP18</i>	Ubiquitin-specific peptidase 18	5.6		
<i>VAMP5</i>	Vesicle-associated membrane protein 5 (myobrevin)			3.0
<i>VCAM1</i>	Vascular cell adhesion molecule 1	3.4		
<i>WARS</i>	Tryptophanyl-tRNA synthetase		3.0	5.4
<i>WNT10B</i>	Wingless-type MMTV integration site family, member 10B	4.1		
<i>XRN1</i>	5'-3' Exoribonuclease 1	2.6		
<i>ZCCHC2</i>	Zinc finger, CCHC domain containing 2			2.4
<i>ZNF313</i>	Zinc finger protein 313	3.4	2.7	
<i>ZNFX1</i>	Zinc finger, NFX1-type containing 1	3.9	3.6	

Note: At 18 h, significantly up-regulated genes by Interferon (IFN)- α was not detected.

IFN- α receptor, while JAK1 and JAK2 for the IFN- γ receptor (Müller and others 1993; Watling and others 1993). IFN- α receptor-associated tyrosine kinases JAK1 and TYK2 phosphorylate and activate STAT1 and STAT2, 2 members of the signal transducer and activator family of proteins. The phosphorylated STAT1 and STAT2 proteins assemble in a complex with another protein, IRF9, to form a heteromeric transcription factor ISGF3, which translocates into the nucleus and binds to IFN-stimulated response elements (ISREs) in the promoter area of ISGs to initiate transcription. In contrast, IFN- γ receptor-associated JAK1 and JAK2 phosphorylate and activate STAT1 only, which forms homodimers, and translocate to the nucleus to bind another element GAS (IFN- γ -activated site) in the promoter of certain ISGs, thereby initiating the transcription of these genes. The

phosphorylated STAT1 homodimers can also be induced by IFN- α (Platanias 2005). Hence the type I and type II IFNs share the same JAK-STAT pathway, while some of its component signal molecules also utilize unique receptors and other elements. Our results indicate that both IFN- α and IFN- γ specifically induced up-regulation of genes encoding the signal proteins in the JAK-STAT pathway. *STAT1*, which is involved in IFN- α and IFN- γ pathways, was up-regulated by both IFN- α and IFN- γ to similar degrees. In contrast, *JAK2*, which is associated with the IFN- γ receptor but not IFN- α receptor, was up-regulated by IFN- γ but not IFN- α . Differences in the levels and functional status of these and other signal elements in hepatocytes are likely to form the basis for the observed differential transcriptional responses to these 2 IFNs.

FIG. 3. Numbers of significantly up-regulated genes, including commonly and uniquely up-regulated genes, induced by interferon (IFN)- α or IFN- γ in primary hepatocytes. Genes were identified by one-class SAM analysis at false discovery rate (FDR) < 10%.

Analyses of the overall induction of hepatocyte ISGs suggested that while some genes were commonly induced by IFN- α and IFN- γ , these 2 cytokines induced very distinct

patterns of genes expression (Fig. 3). This conclusion is also supported by the pathway analysis (Table 2), which indicated that at the same significance level of $P < 0.002$, functional pathways commonly activated by both IFN- α and IFN- γ , as well as those activated by either IFN- α or IFN- γ , were identified. Previous studies in a human fibrosarcoma cell line suggested some ISGs are preferentially induced by IFN- α but not by IFN- γ , including the 2',5'-oligoadenylate synthetase family of genes (*OAS*), *MxA* (*MX1*) and *MxB* (*MX2*) genes, and the gene family comprising *ISG54*, *ISG56*, and *ISG60* collectively known as IFN-induced proteins with tetratricopeptide repeats (*IFIT1*, *IFIT2*, and *IFIT3*; Der and others 1998). In the primary hepatocytes, we found that *OAS1*, *OAS2*, and *OAS3* were induced by both IFN- α and IFN- γ , with differences in aFC between the 2 IFNs smaller than 2-fold (Table 1). These results are largely in agreement with previous findings derived from limited number of human ($n = 2$) and chimpanzee ($n = 1$) hepatocyte samples (Lanford and others 2006).

IFIT1 and *IFIT2* were induced by both IFN- α and IFN- γ , although the aFC values of IFN- α were substantially greater than those by IFN- γ . Similarly, *RSAD2* (viperin) was also induced to a greater degree by IFN- α than IFN- γ . In addition,

FIG. 4. Induction of interferon (IFN)-stimulated genes (ISGs) by interferon (IFN)- α and IFN- γ . Primary hepatocytes were treated with IFN- α or IFN- γ for 6 and 18 h as described. Total RNA was extracted and subjected to TaqMan quantification of *Mx1*, *IFIT1*, *IRF1*, and *IP10* mRNA levels. Data from 2 sets of hepatocytes (PH 1 and PH 2) were analyzed and the fold induction of the ISG for each condition is shown (numerical value on top of the bar).

TABLE 2. FUNCTIONAL PATHWAYS SIGNIFICANTLY ACTIVATED BY IFN- α AND IFN- γ ($P < 0.002$)

Pathway	IFN- α /6 h	IFN- γ /18 h
Cellular growth and proliferation	yes	yes
Cellular development	yes	yes
Cell-to-cell signaling and interaction	yes	yes
Hematological system development and function	yes	yes
Immune response	yes	yes
Cardiovascular system development and function	yes	no
Cell signaling	yes	no
Cancer	no	yes
Immune and lymphatic system function and development	no	yes
Cellular movement	no	yes
Cardiovascular disease	no	yes

Abbreviation: IFN, interferon.

MX2 was induced by IFN- α but not by IFN- γ (Table 1). Of note, *IFIT1* and *RSAD2* have been implicated in the anti-HCV effect of IFN- α in the HCV cell culture systems (Wang and others 2003; Helbig and others 2005; Jiang and others 2008) while *MX2* is an important effector gene in the anti-influenza effect of IFN- α , although its *in vivo* effects on HCV are not known. On the other hand, *ISG20*, *ADAR1*, and *PKR*, all of which have been shown to exhibit anti-HCV effects in cell culture (Taylor and others 2005; Chang and others 2006; Jiang and others 2008), were either induced similarly or not induced substantially by IFN- α and IFN- γ in primary hepatocytes. Interestingly, suppressor of cytokine signaling, specifically *SOC1*, of a family of ISGs that have been associated with non-response of HCV to IFN- α therapy (Walsh and others 2006; Feld and others 2007; Huang and others 2007; Persico and others 2007), was significantly induced by IFN- γ but not by IFN- α in the primary hepatocytes. Further study should be carried out to determine if this difference contributes to the lack of virological response to IFN- γ . Of note, the biological effects of the transcriptional responses cannot be fully understood without a systematic analysis of the IFN-induced proteins, including their expression levels, post-translational modifications, and functional status. In addition, while this study addresses the type I and type II IFN-induced transcriptional responses in hepatocytes, the major target of infection by hepatitis viruses, it should be emphasized that the *in vivo* antiviral effects of IFNs could also involve the IFN-induced autocrine and paracrine signals on non-parenchymal cells in the liver, such as dendritic, stellate, or Kupffer cells (Pan and others 2004; Gautier and others 2005). These issues should also be addressed in future studies to fully understand the different antiviral effects of IFN- α versus IFN- γ .

In summary, our findings in the differential patterns of ISG induction suggest potential mechanisms for an inferior efficacy of IFN- γ against hepatitis viruses as compared to IFN- α . They also provide useful information for future mechanistic studies of the therapeutic effects of IFNs against hepatitis viruses.

Acknowledgments

We would like to thank Stephen Strom and his colleagues at the University of Pittsburgh for providing the primary human hepatocytes through the Liver Tissue Procurement and Distribution System (N01-DK-7-0004/HHSN26700700004C). This work was supported in part by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases, NIH, the NIH grants U19 AI40034 and P30 DK56339, and a REAP Center grant from the Department of Veterans Affairs.

Author Disclosure Statement

No competing financial interests exist.

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Received 17 July 2009/Accepted 29 August 2009