

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogado
JUAN PABLO CADENA SARMIENTO

ASUNTO	Radicación	09-9959
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	1 2 7 6 8
	Folios	04

Patente de Invención	☐	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☐	Cap. I	☐ Cap. II ☐

No. Solicitud Internacional PCT/JP2007/063545
Fecha de Presentación Internacional 25 junio 2005

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 36-48	03 de febrero de 2009
Reivindicaciones:	Folios 49-50	03 de febrero de 2009
Dibujos:	Folios 51-52	03 de febrero de 2009
Prioridad:	JP 2006187943	7 de julio de 2006

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Agente profiláctico o terapéutico para enfermedad viral"

El problema planteado en esta solicitud consiste en la necesidad combinar agentes terapéuticos para tratar la Hepatitis C

Para resolver este problema técnico la solicitud en estudio proporciona una combinación de 22β -metoxioalean-12-eno-3β -24-(4β)-diol, con un interferón como ingredientes activos.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 38/21; A61K 31/56; A61P 35/00; A61P 31/14

4. Excepción a la Patentabilidad (Art 20 D 486 literales (d))

El objeto de las reivindicaciones 6-10 no es patentable de acuerdo con el Art citado.

5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 11-15 no es patentable, de acuerdo con el Art 14.

6. De la Solicitud de Patente

Título

El título no define adecuadamente alcance de la solicitud porque de él no se puede derivar cual es la intención del solicitante, si es una composición que contiene 22β -metoxioalean-12-eno-3β -24-(4β)-diol y un interferón como ingredientes activos, o corresponde a un método de tratamiento. Se sugiere hacer las modificaciones necesarias.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio no es claro cuando utiliza la expresión “Agente profiláctico o terapéutico” porque de ella no se puede derivar el alcance de la presente solicitud. No se comprende si hace referencia a una composición que comprende 22β -metoxioalean-12-eno-3β -24-(4β)-diol y un interferón como ingredientes activos. O a la utilización/ administración de ambos por separado. Se sugiere hacer las aclaraciones necesarias.

En la reivindicación 1 no se define la estructura del interferón (secuencia completa, estructura, etc.), que ha de utilizarse como principio activo. Se sugiere hacer las aclaraciones necesarias de acuerdo con lo soportado en el capítulo descriptivo.

Las reivindicaciones 2 a 5 se encuentran definidas por resultados a alcanzar y no por sus características esenciales puesto que hacer referencia al tipo de enfermedad , virus que es atacado por los agentes terapéuticos. Se sugiere al solicitante definir de manera adecuada la característica esencial que desea proteger, de lo contrario debe retirarlas del pliego reivindicatorio.

7. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de Presentación de la Solicitud de Prioridad: 7 de julio de 2006 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación*
D1	US6306862	Triterpene derivatives and pharmaceuticals for treating hepatic disorders	2001-10-23
D2	Journal Of virology, Vol 76 No. 21 11148-11154	Comparative Analysis of Anti-Hepatitis C Virus Activity and Gene Expression Mediated by Alpha, Beta, and Gamma Interferons, Wayne Chaney Et Al. DOI: 10.1128/JVI.76.21.11148-11154.2002	Noviembre de 2002

D1:<http://www.google.com/patents?id=5g4IAAAAEBAJ&printsec=frontcover&dq=US6306862&hl=en&sa=X&ei=U6rsT4arBKfcmAWX3JHWAq&ved=0CDMQ6AEwAA>
Divulga la administración de 22β -metoxioalean-12-eno-3β -24-(4β)-diol o una sal farmacológicamente aceptable (ejemplo 22. Compuesto 27 y la reivindicación 40) para el tratamiento de un trastorno hepático (columna 4, líneas 25-32 y la reivindicación 28). Divulga que la composición se puede aplicar a la hepatitis viral aguda y crónica, (columna 4. líneas 25-30). D1 divulga la administración de 22β -
Primer Artículo 45- Expediente 09-9959

metoxioalean-12-eno-3β -24-(4β)-diol en la dosificación de aproximadamente 0,1 a 1000 mg, preferiblemente de 1 a 100 mg por día para un adulto humano, una o dos veces al día y puede ser oral o parenteral (columna 7, líneas 1-3).

D2 Journal Of virology, Comparative Analysis of Anti-Hepatitis C Virus Activity and Gene Expression Mediated by Alpha, Beta, and Gamma Interferons, Wayne Chaney Et Al. DOI: 10.1128/JVI.76.21.11148-11154.2002. Divulga la administración de interferones para el tratamiento de infecciones de virales de hepatitis C (todo el documento, folios- 84-89), enseña los efectos antivirales de los interferones IFN-α/β e IFN- γ son efectivos (página 11153. Columna de la derecha, tercer párrafo, folio 89).

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en un agente profiláctico o terapéutico para una enfermedad viral caracterizado porque combina 22β -metoxioalean-12-eno-3β -24-(4β)-diol con un interferón como ingredientes activos.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Una combinación de:	Una composición	
22β -metoxioalean-12-eno-3β -24-(4β)-diol	22β -metoxioalean-12-eno-3β -24-(4β)-diol	No se menciona
Un interferón	Otros agentes antihepatitis	Un interferón

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque

D1 divulga composiciones para el tratamiento de enfermedades virales asociadas a la Hepatitis C con 22β -metoxioalean-12-eno-3β -24-(4β)-diol (D1-ejemplo 22, compuesto 27 y reivindicación 40) y la administración de 22β -metoxioalean-12-eno-3β -24-(4β)-diol o una sal farmacológicamente aceptable (ejemplo 22. Compuesto 27 y la reivindicación 40) para el tratamiento de un trastorno hepático (columna 4, líneas 25-32 y la reivindicación 28) asimismo que la administración de 22β -metoxioalean-12-eno-3β -24-(4β)-diol en la dosificación de aproximadamente 0,1 a 1000 mg, preferiblemente de 1 a 100 mg por día para un adulto humano, una o dos veces al día y puede ser oral o parenteral (columna 7, líneas 1-3).por ultimo divulga el tratamiento en combinación con otros agentes terapéuticos.

La diferencia entre la invención definida en la reivindicación 1 y la composición de D1 consiste en que la invención combina el 22β -metoxioalean-12-eno-3β -24-(4β)-diol con el interferón.

Por su lado el documento D2 hace referencia a el uso de composiciones que continenen con interferones de tipo β y α para el tratamiento de infecciones de virales de hepatitis C (todo el documento, folios 84-89) también que enseña los efectos antivirales de los interferones IFN-α/β e IFN-γ son efectivos (página 11153. Columna de la derecha, tercer párrafo, folio 89).

La diferencia entre la invención, definida en la reivindicación 1 y D2 consiste en que la invención combina el interferón con el 22β -metoxioalean-12-eno-3β -24-(4β)-diol.

No se evidencia un efecto técnico sinérgico ó una mejora en la inhibición de la replicación del virus de la Hepatitis C, por la combinación del 22β -metoxioalean-12-eno-3β -24-(4β)-diol y el interferón en comparación con los efectos individuales de ambos compuestos.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "¿cómo obtener composiciones alternativas a las conocidas en D1 que contienen 22β -metoxiolean-12-eno-3β -24-(4β)-diol en donde se combine con otro tipo de agente terapéutico como un interferón para mejorar la inhibición de la replicación del virus de la Hepatitis C?"

Sin embargo, el uso de un interferón para presentar una eficiente inhibición de la replicación del virus de la Hepatitis C, ya está divulgado en el documento D2, en donde se evidencia claramente una mejora en la actividad inhibitoria de la replicación por la administración de interferones IFN-α/β e IFN-γ (página 11153. Columna de la derecha, tercer párrafo, folio 89).

En consecuencia, la persona del oficio normalmente versada en la materia que pretenda lograr una mejora en la inhibición de la replicación del virus de hepatitis C, estaría motivado a realizar una combinación de agentes inhibidores ya conocidos en el estado de la técnica como son 22β -metoxiolean-12-eno-3β -24-(4β)-diol e interferones IFN-α/β e IFN-γ, y esperaría lograr un efecto al menos aditivo de las actividades llegando de esta manera al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

El objeto de las reivindicaciones 2-5 no hacen nueva o inventiva la reivindicación 1 por lo cual, también se consideran obvias.

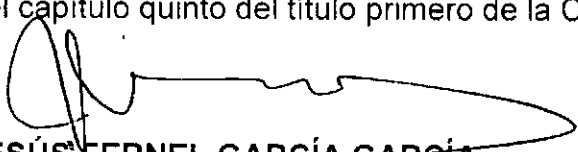
8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US6306862	1-5	Nivel Inventivo
D2	Journal Of virology, Vol 76 No. 21 11148-11154	1-5	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

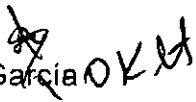

JESÚS FERNEL GARCÍA GARCÍA
 Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha

25 JUL. 2012

Elaborado por: Harvey Jaramillo

Revisado Por: Ana Delia Pinzón García



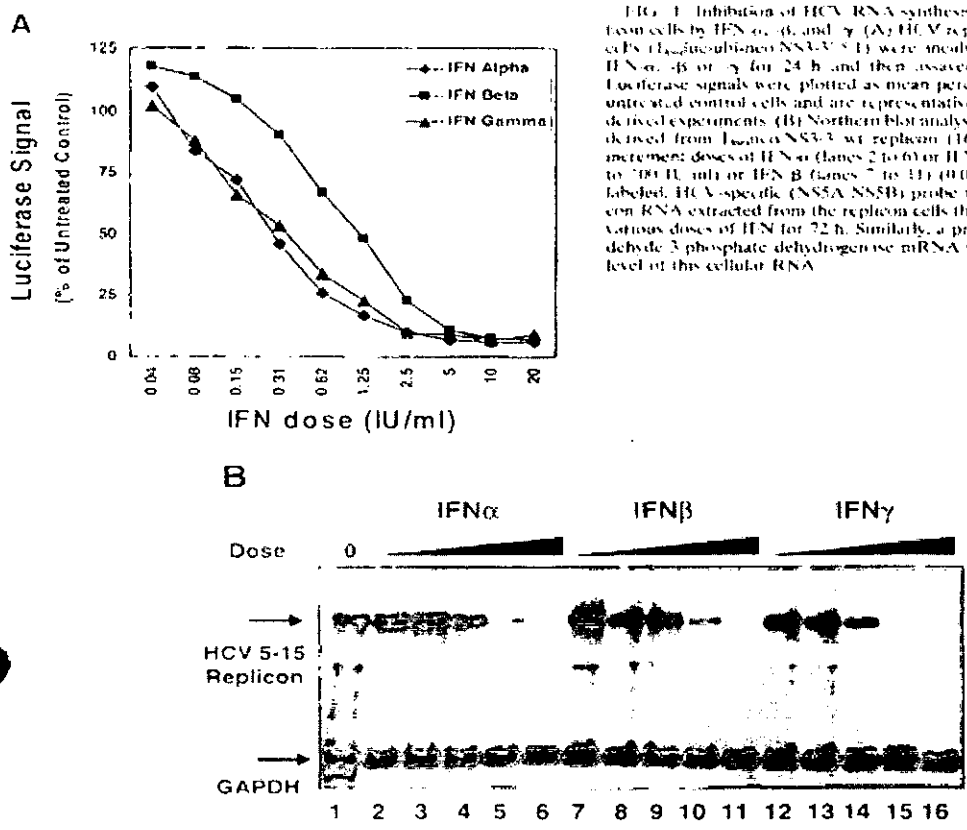


FIG. 1. Inhibition of HCV RNA synthesis in the subgenomic replicon cells by IFN α , β , and γ . (A) HCV replicon luciferase reporter cells (Luciferase-NeoNS3-3'UTR) were incubated in the presence of IFN α , β , or γ for 24 h and then assayed for luciferase activity. Luciferase signals were plotted as mean percentages of those for the untreated control cells and are representative of three independently derived experiments. (B) Northern blot analysis of HCV replicon RNA derived from LucNeoNS3-3'UTR replicon (16) cells treated with log increment doses of IFN α (lanes 2 to 6) or IFN γ (lanes 12 to 16) (0.04 to 100 IU/ml) or IFN β (lanes 7 to 11) (0.003 to 33 IU/ml). A ^{32} P-labeled, HCV-specific (NS5A-NS5B) probe was used to detect replicon RNA extracted from the replicon cells that had been treated with various doses of IFN for 72 h. Similarly, a probe specific for glyceraldehyde 3-phosphate dehydrogenase mRNA was used to monitor the level of this cellular RNA.

Nevertheless, the recent establishment of a selectable HCV subgenomic replicon system (genotype 1b) in Huh-7 hepatoma cells has provided a useful system for studying HCV RNA replication [3, 10, 17, 20]. IFN α/β has antiviral activity against the HCV replicon system [3, 10, 13], however, a comparative analysis of the *in vitro* antiviral activity of IFN α/β and IFN γ has not been demonstrated in this model. The HCV replicon system provides us with a unique opportunity to perform this comparison. First, some of the classical IFN α/β -induced antiviral effector pathways do not appear to be of importance in this *in vitro* model [10], and second, the absence of infiltrating IFN γ -responsive immune cells, such as lymphocytes, in this model allows us to focus on the IFN γ -induced antiviral effector molecules present in liver cells. We therefore monitored the effects of recombinant IFN α (Intron A; Schering-Plough, Kenilworth, NJ), IFN β (Biosource, Camarillo, CA), and IFN γ (Actimmune; InterMune Pharmaceuticals, Palo Alto, Calif.) on viral RNA synthesis in 5-2 cells (a non-hepatoma cell line, Huh-7, carrying the firefly luciferase gene) and HCV subgenomic replicon based on the LucNeo-

NS3-3' 5.1 replicon backbone [15]. Cells were maintained in growth medium (Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 0.5 to 1 mg of G418/ml). To assess the antiviral activity or potential cytokine-induced toxicity from the IFN treatments, luciferase activity (a direct measurement of reporter replicon RNA synthesis) and cell viability assays were performed. In these assays, replicon cells were incubated for 2 days in the presence of G418 and then treated with 0.04 to 20 IU of IFNs/ml in growth media without phenol red or G418. Luciferase and cellular viability [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS); Promega, Madison, Wis.] assays were performed at 24 h posttreatment. All three IFNs were capable of suppressing HCV RNA replication as demonstrated by decreased luciferase activity (Fig. 1A) without affecting viability. The 50% effective concentrations (EC_{50} s) for the various IFNs tested were approximately 0.3, 1.2, and 0.3 IU/ml for IFN α , IFN β , and IFN γ , respectively. Interestingly, complete suppression of the replicon-directed luciferase signal was not achieved at IFN concentrations

30- to 90-fold higher than the EC_{50} 's. Similar data for IFN- γ has been generated recently with the HCV replicon cell culture model using concentrations ranging from 5 to 5,000 IU/ml (17).

To confirm that the inhibitory effect on the luciferase signal was due to suppression of viral RNA synthesis, Northern blot analysis was performed to measure the replicon RNA levels in the IFN- γ -treated cells. Replicon cells that had been plated 24 h prior to IFN- γ treatment (24 h) were treated with IFN- γ at 20 IU/ml for another 72 h. All harvests of total RNA were subjected to Northern blotting against an HCV-specific probe. As shown in Fig. 1B, no inhibitory effects on replicon RNA levels were observed by the three IFNs. This inhibition was specific for HCV RNA, as the level of cellular mRNA (glyceraldehyde-3-phosphate dehydrogenase) was unaffected. We were unable to correlate temporally the IFN-mediated decreases in HCV replicon RNA levels with suppression of the luciferase reporter gene, because 24 h post-IFN treatment would be insufficient time to detect RNA differences by Northern blotting. Nonetheless, these results clearly demonstrated that both IFN- α/β and IFN- γ can effectively inhibit HCV RNA replication with similar efficiency in cell culture.

The effect of the IFN exposure time on the antiviral activity of IFN- γ was further assessed by evaluating the luciferase signal in IFN-pulse treatments (20, 100, and 320 IU/ml) in 5-2 cells for 4, 24, or 72 h (Fig. 2A). The IFN-containing medium was replaced with IFN-free medium after each treatment period, and luciferase signal was measured 24 h from the start of viral RNA cultivation. Although IFN pulse times varied, luciferase measurements were not made sooner than 24 h after treatment due to the IFN pulse treatment due to the preexisting luciferase in pool of replicon RNA and the slow degradation of replicon RNA. The response rate of HCV replication inhibition to each IFN was dose dependent, although IFN- γ was able to achieve maximal suppression of replication with only a 4-h pulse at the lowest IFN dose of 20 IU/ml (Fig. 2A). This result indicated that IFN- γ induced a more robust response than IFN- α and - β in the HCV replicon cell culture. More strongly, a recent report (22) on HBV revealed that IFN- γ induced a stronger antiviral effect than IFN- α , suggesting that a similar temporal mechanism may be employed by IFN- γ to

increase in the luciferase signal (Fig. 2B). The dose of IFN was not the determining factor for recovery of HCV replication. The durability of response by an 8- or 16-fold-higher dose of IFN- α and - β , respectively, paralleled the trends seen when comparing the durability of responses at the 20 IU/ml treatment level (Fig. 2B). The maximal inhibition of HCV replicon replication induced by IFN- γ at all doses tested after 24 h was not reversible over a 6-day period. In contrast, the maximal inhibition of HCV replicon replication after 24 h induced by IFN- α and - β was reversible even when the dose of IFN- α and - β was 8 to 16-fold higher than that of IFN- γ . Cell proliferation assays (MTS) performed on parallel and identically treated cell cultures suggested that the replicon cells were viable and continuously growing over the 168-h test period. IFN-mediated cytotoxicity was not a contributing factor for inhibition of HCV replication in all experiments, since the viability of the replicon cells following various doses and exposure times to IFN- α , IFN- β , and IFN- γ was $100\% \pm 2.4\%$, $99.6\% \pm 4.3\%$, and $98.3\% \pm 2.4\%$, respectively. In addition, an experiment addressing the long-term survival of IFN-treated replicon cells in the presence of G418 showed that IFN- γ -pulsed cells (20 and 320 IU/ml) were unable to replicate and survive under G418 selection whereas a significant proportion of IFN- α/β -treated cells grew steadily (data not shown). Collectively, these results showed greater durability of the antiviral response by IFN- γ than of that by IFN- α and - β and suggest a possible alternate mechanism of anti-HCV replication effect by IFN- γ .

Correlation of the differential IFN-induced gene expression profiles between IFN- α/β and IFN- γ treatments and the strength and durability of the IFN-induced inhibition of HCV replicon replication could provide insight into previously unrecognized IFN-induced anti-HCV pathways and antiviral effector molecules present in the liver. To address this, DNA microarray analyses were performed on RNA samples isolated from untreated or IFN-treated HCV replicon cells that contained the replicon construct I_{cgneo} NS3-3' wt (16). The cells (2×10^6 to 4×10^6) were plated in 10-cm tissue culture plates, incubated for 2 days in the presence of G418, and then treated for 4 or 24 h with 20 IU of IFN- α 2b, IFN- β , or IFN- γ ml (in growth media without G418) prior to isolation of total cellular RNA. The synthesis of double-stranded cDNA from total cellular RNA (10 μ g) and the generation of biotin-labeled cRNA from the double-stranded cDNA template were performed

induced gene expression changes were determined following comparison with the untreated cells and represented as increased or decreased fold changes. Only genes that had a fold change in expression of ≥ 3 were considered significant. The comparisons of the genes upregulated or downregulated by IFN- α , IFN- β , or IFN- γ in the HCV replicon cell culture model are detailed in Table 1. Distinct sets of genes were upregulated at 4 and 24 h by both IFN- α/β and IFN- γ . However, the magnitude of induced gene expression could be clearly categorized into three classes: (i) 18 genes that were preferentially upregulated by IFN- γ , (ii) 19 genes that were preferentially upregulated by IFN- α and IFN- β , and (iii) 8 genes that were upregulated by all three IFNs. The downregulated genes, however, showed no similar trend (Table 1). The differential effect on gene expression by IFN- α/β versus IFN- γ , coupled with similarity in magnitude but differences in dura-

ability of the antiviral response to each of the three IFNs, was addressed by following the temporal effect (24 to 168 h) on the IFN-pulse treatment with IFNs at 20, 100, or 320 IU/ml. IFN-pulsed replicon cells were cultured and assayed for luciferase signal as described previously, 24, 48, or 72 h post-IFN treatment. In addition, a parallel culture was split 1:3 to maintain subconfluent growth and then replated onto new microtiter plates for further culturing out to 168 h and 168 h post-IFN treatment. IFN- γ produced long-term HCV inhibitory effects than similar pulses with IFN- α and - β (Fig. 2B). IFN- γ -treated cells were unable to recover HCV replication 72 h following the IFN pulse treatment or even after continuation of the culture (with one culture split) out to 168 h after treatment. In contrast, replicon cells treated with IFN- α and - β appeared to recover slowly following the IFN pulse treatments, as evidenced by a temporal

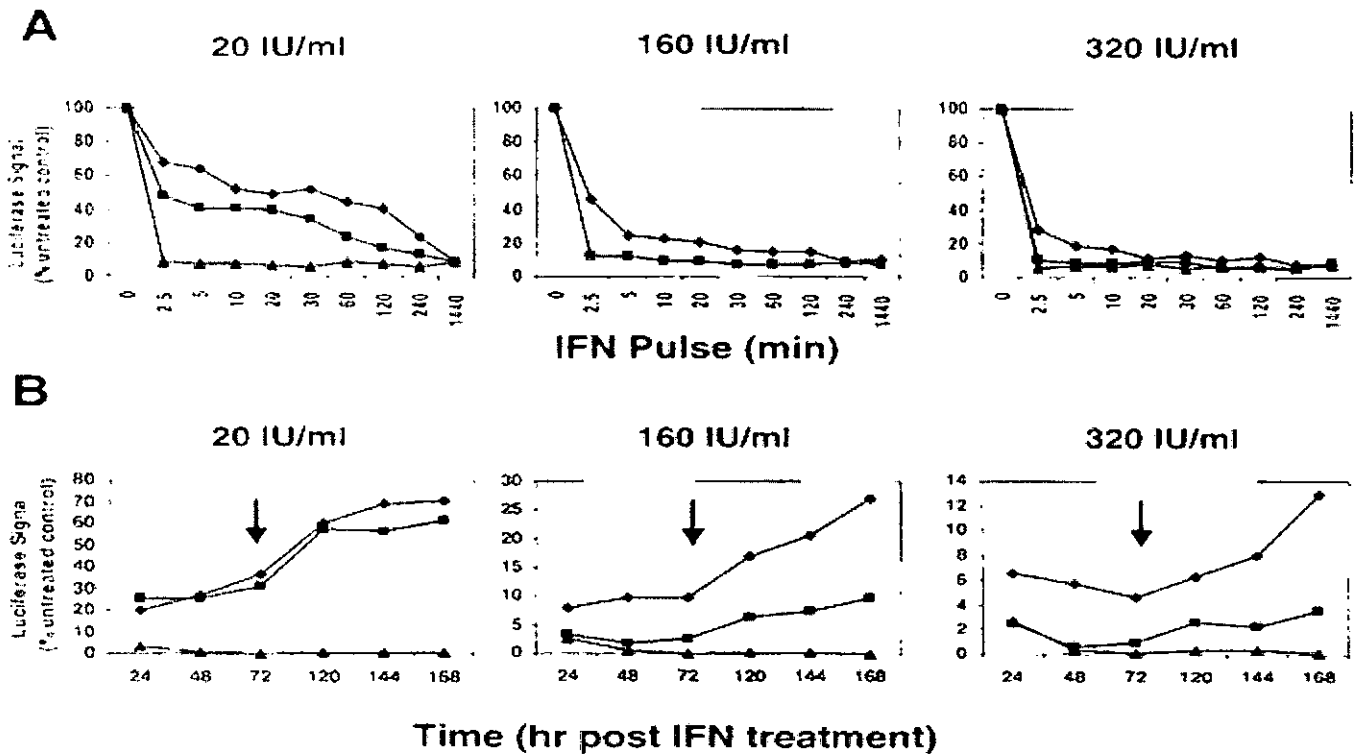


FIG. 2. Differential response rates of HCV replicon reporter cells to IFN treatment and durability of IFN inhibitory effect. (A) HCV replicon cells in 293T cells (HCV-G subgenotype NS5B-5.3) were treated with 20, 160, or 320 IU of IFN- α (diamonds) and IFN- β (squares) ml or with 20 and 320 IU of IFN- γ for 2.5 min to 24 h and assayed for luciferase activity at the 24-h time point from the start of IFN treatment. Luciferase signals were plotted as mean percentages of the untreated control cells and are representative of three independently repeated experiments. (B) Reporter cells were treated with IFN doses as described above for 4 h and then assayed for the luciferase signal 24, 48, 72, 120, 144, or 168 h post-treatment. In addition, a parallel and equally treated cell culture was trypsinized at the 72-h mark (see arrow), diluted 1:4 into new cells, and then assayed at 24, 48, 72, or 96 h from this point (120, 144, and 168 total hours post-IFN treatment). Durability of IFN-HCV-G₂ inhibitory effect was plotted over time as a percentage of the luciferase signal derived from mock-treated cells. Data are mean $\pm$ SD of duplicate assays from a typical assay.

Our data on the HCV response by IFN- α/β and IFN- γ in the HCV replicon system, suggests that distinct, IFN-type-specific antiviral HCV pathways exist in liver cells. One possible explanation for the difference in durability of the IFN- γ antiviral response, from that of IFN- α/β was provided by examining the kinetics of the gene expression induced by IFN- α/β and IFN- γ . The magnitude of antiviral activity (decrease in luciferase signal) of each IFN was similar after either a 4-h pulse or continuous exposure for 24 h. Nevertheless, all of the IFN- γ -induced genes continued to increase expression between 4 and 72 h whereas expression of IFN- α/β -induced genes was maximally induced at 4 h in 10 of the 19 genes. Therefore, we can assume that the differential magnitude and kinetics of gene expression have little impact on the induction of an antiviral effect by the IFN-stimulated genes but more likely influence the strength and durability of the antiviral response in the HCV replicon system.

Our gene expression data generated in the HCV replicon system, replicon-containing Huh7, showed some striking similarities to data generated in previous comparative gene pro-

filings experiments with IFNs. IFN- α/β or IFN- γ (1,000 IU/ml, 6-h exposure)-stimulated gene expression profiles were demonstrated in a human fibrosarcoma cell line, HT1080, in the absence of viral replication, by DNA microarray analysis (9). Of the 18 genes preferentially upregulated by IFN- γ in the HCV replicon-containing cell line shown in Table 1, 10 of those genes were upregulated in the IFN-stimulated HT1080 cells, and 7 of those 10 genes (encoding IRT-1, TAP-1, LMP7, LMP2, MECL-1, C11, and HLA-E) showed preferential upregulation by IFN- γ . In addition, of the 19 genes preferentially upregulated by IFN- α and - β in the HCV replicon-containing cell line (Table 1), 16 were present in the IFN-stimulated HT1080 cells and 12 of 16 showed preferential upregulation by IFN- α and IFN- β . These data show that in the absence of viral replication, the differential gene profiles induced by IFN- α/β and IFN- γ are strikingly similar to our observations with the HCV replicon-containing Huh7 cells.

Our data correlating in vitro antiviral activity by the three IFNs and gene expression profiles identified by microarray analysis suggest that some of these genes may initiate or my-

TABLE 1. Genes upregulated and downregulated by 4 h and 24 h treatment with IFN- α , IFN- β , and IFN- γ

ORF	Gene	IFN α		IFN β		IFN γ	
		4h	24h	4h	24h	4h	24h
MT004	ADP-5	6.5	3.2	11	2.7	9.6	
MT005	PKP	17.5	4.5	10.8	15.6		
MT006	PKP	1.6	1.1	1.3	1.2		
MT007	PKP	9	13.5	11.9	23.4	12.9	
MT008	PKP	4.7	5.4	9.4	10.8	9.8	
MT009	PKP	1.2	1.7	1.2	2.5	1.8	
MT010	PKP	22.5	9.7	23.5	19.6		
MT011	PKP	5.3	4.9	5.4	9.1		
MT012	PKP	3.4	2.7	4.2	3.8	3.5	
MT013	PKP	5.4	1.9	5.3	1.6		
MT014	PKP	6	7.7	8.5	10.7	4	
MT015	Adenovirus hexonally transmembrane	4.3	4.5	5.1	8.1		
MT016	Phosphatidylserine synthase	2.6	1.8	2.8	2.3		
MT017	Glucocorticoid-induced leucine-rich repeat	2.1	2.4	2.2	3.2		
MT018	Thyroid receptor-interactor	5.8	21.9	9.5	59.9	7.3	
MT019	CRABP	5.3	1	5.9	1.3	1	
MT020	HLA-B	2.5	2.5	4.3	4.9	6.6	
MT021	HLA-B	4.1	2.8	6.2	4.2	5.1	
MT022	MxA	38.1	62.7	48.6	111	2.5	23.5
MT023	2',5'-oligoadenylate synthetase	11.7	19.5	19.3	38.8	1.5	6.2
MT024	PKP	4.7	5.5	4.7	5.8	1.4	2.9
MT025	IFN- α	18.8	71.8	23.3	96.7	2.7	30
MT026	ISGF-3	14.3	23.8	12.7	34	1.5	8.8
MT027	p56	86.2	48.5	108	47.8	2.1	4
MT028	Phospholipid scramblase	22.8	9.2	20.7	8.7	9.2	4.3
MT029	ISGF-3	15.8	16.2	16.8	16.8	9.3	12.3
MT030	ISGF-3	4.6	1.4	6.9	1.3	1.8	1.1
MT031	RIG-1	8.9	2.2	12.6	2.1	6.2	5.2
MT032	p56	3.6	2.3	3.4	2	1.3	1.2
MT033	p56	2.2	5.6	4.3	19	2.7	2.7
MT034	HLA-B	6.4	24.2	10.3	71.4	1.8	58.4
MT035	Nuclear phosphoprotein	4.6	3.1	4.7	3.6	1.9	3.2
MT036	BST-2	2.7	10.4	3.9	33	1.1	22.2
MT037	GyDab	2.8	1.6	3.2	1.6	1.6	1.8
MT038	Thyroid receptor-interactor (TRIP14)	6.3	1.3	6.9	1.4	1.5	1.7
MT039	PMIL-2	5.8	2.4	5.9	2.4	1.5	2.2
MT040	Estrogen responsive finger protein	7.9	3.3	12.3	3.6	2.8	1.9
MT041	TAT-1	7.2	6.7	5.9	8.6	6.1	10.1
MT042	BHL-1	13.7	4.6	2.5	3.4	1.1	5
MT043	2',5'-oligoadenylate synthetase E1 (ADE1L)	1.3	4.3	2.5	3.7	1.2	2.6
MT044	2',5'-oligoadenylate synthetase	6	1.9	8.6	2.3	6.9	4.7
MT045	2',5'-oligoadenylate synthetase	6.9	1.4	9	1.6	9.8	1.5
MT046	2',5'-oligoadenylate synthetase (GPT)	5.1	2.7	10.3	1.1	8.7	5.8
MT047	2',5'-oligoadenylate synthetase	1.9	2.7	1.8	3.4	1.4	3.1
MT048	2',5'-oligoadenylate synthetase	3.9	2.6	4.2	2.5	3	4.4
MT049		-2.7	-5.1	-1.8	-2	1.8	-5.7
MT050		-2.6	-4.9	-1	-4.3	1.4	-3.9
MT051		-1	-4.6	-2	-7.1	1.3	-4.8
MT052		-3.9	-3.1	-2.7	-4.3	1.1	-2.7
MT053		-3.5	-3.1	1.1	-3.5	2	-4
MT054		-3.4	-2.8	-2.3	-6.3	1.4	-3.1
MT055		-1	-2.5	-2.2	-6.2	1.1	-1.1
MT056		-1.7	-2.5	-1.8	-1.1	1.4	-2.4
MT057		-1.2	-2.5	-1.3	-3.7	1.4	-1.8
MT058		-1.4	-2.4	-1.5	-5.2	1.5	-2.6
MT059		-1.2	-2.3	1.2	-4	1.1	-1.5
MT060		-1.8	-2.3	-1.9	-4.4	-1.1	-2.3
MT061		-1.4	-2.3	-1.6	-3.3	1.1	-3.4
MT062		-1.6	-2.1	-1.6	-4.2	1.1	-2.3
MT063		-1.8	-2.1	-1.2	-3.3	1.4	-1.9
MT064		-1.2	-1.9	-1.1	-3.7	1.2	-3.1
MT065		-1.6	-2.2	1.5	-3.5	1.1	-3

during the antiviral action needed to eliminate HCV replication. Which of these genes are functionally relevant can be determined only with an *in vivo* antiviral response to HCV. Interestingly, DNA microarray analyses have been performed to determine changes in liver gene expression during the course of an acute-resolving HCV infection in a chimpanzee (2). Changes in liver gene expression occurred between infection and viral clearance, and some of the changes in gene expression corresponded to the 15 IFN- α -responsive genes which were grouped into three different expression patterns: (i) genes which peaked early (day 0) and then declined, (ii) genes which peaked at week 6, and (iii) genes which peaked early and then declined but were sustained until clearance of viremia. Interestingly, 10 of the 15 genes preferentially upregulated by IFN- γ in the HCV replicon-containing cells, expression of 8 of these genes was found in the liver biopsies from the HCV-infected chimpanzee. More importantly, all eight of these genes (genes for p21, p27, p28, p30, p36, p40, p44, p54, p58, p60, RIG-I, and p27) have also been found to be upregulated in the HCV-infected liver biopsies. In contrast to the eight IFN- γ -induced genes, only one IFN- α -induced gene, that for MxA, had a peak expression at week 6. The other seven genes peaked early, remained high at week 6, and maintained high expression levels at week 8, genes for 2',5'-OAS, ISG15, p56, RIG-I, and p27. These data imply that the IFN- γ -stimulated genes have more relevance since they are IFN- α -induced genes in initiating the antiviral response to HCV infection. Interestingly, expression of IFN- γ -stimulated genes might be a marker for viral clearance in HCV infections. Evidence supporting these hypotheses comes from a recent study with 25 patients with chronic HCV infection. Patients were treated with the combination IFN- α ribavirin or IFN- α alone (6), and the HCV-specific T-cell reactivity was monitored during treatment, at the end of treatment, and at 24-week follow-up. Similar to the chimpanzee data, this study showed that T-cell reactivity increased at around treatment weeks 4 to 8. Resolution of HCV viremia (sustained antiviral response) in patients treated with the combination IFN- α ribavirin or IFN- α alone was more likely for patients who developed HCV-specific T-cell proliferation with increased IFN- γ production.

Using DNA microarray analyses, Robek and colleagues compared changes in cellular gene expression that accompany the IFN- α -induced inhibition of replication in the livers of the chimpanzees compared to the HCV-Met hepatocyte cell lines (12). A number of genes were common to all three cell types, but only a few were common also to our list

of genes preferentially upregulated by IFN- γ , namely, the MxG, IP10, LMP2, LMP7, MECL-1, and GBP-1 genes. Collectively these data suggest that a common IFN-induced antiviral mechanism exists in HBV and HCV that can occur in host nonlymphocyte cells. This antiviral activity is presumably part of the host adaptive immune response to virus infection and is independent of the classical IFN- α -induced innate host antiviral mechanisms (MxA, PKR, and 2',5'-OAS).

The remarkable consistency of the profile of IFN- γ -induced gene expression, irrespective of cell type and presence of replicating virus, coupled with their peak expression at the initiation of viral clearance (HCV) *in vivo* provides strong evidence that these IFN-induced genes may have direct antiviral activity. The question remaining is which of the key IFN- γ -regulated genes mediate these direct antiviral effects. Our preliminary data suggest that the IFN-induced chemokines IP10 and MxG and proteasome subunits LMP2, LMP7, and MECL-1 do not have direct antiviral activity using the HCV subgenomic replicon system (data not shown) or may require more than one component to trigger the antiviral state.

In summary, we have shown that IFN- α and IFN- γ mediate antiviral effects of similar magnitude in HCV replicon-containing Huh 7 cells. However, kinetic analyses showed that the antiviral effect of IFN- γ was strong and durable, whereas IFN- α and IFN- β induced a less robust and more transient antiviral response against HCV. Indeed, IFN- γ -treated HCV replicon cells under drug selection failed to recover and form colonies after long-term culture, suggesting either extended suppression or potential eradication of HCV replicons. Studies are ongoing to distinguish between these two hypotheses. Furthermore, two classes of IFN-regulated genes were also identified. The class of genes that were preferentially upregulated by IFN- γ correlated with some of the genes shown by others to be (i) expressed highly at the peak of an acute-resolving host response to HCV in a chimpanzee and (ii) important in the noncytolytic cytokine-mediated antiviral effects of the host adaptive immune response. This study along with other previous observations suggests that IFN- α and IFN- γ induce distinct direct antiviral effector molecules and alternate antiviral pathways in the IFN-mediated suppression of HCV replication. The contribution of these distinct IFN-induced pathways to host innate and adaptive direct antiviral responses warrants further investigation.

We thank Ralf Bartenschlager and colleagues for supplying the HCV subgenomic replicon system.

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Manuscript analysis of gene expression changes induced by 20 IU/ml of IFN- α and IFN- β or IFN- γ in HCV replicon cells (HepG2-NS3). Total RNA (1 μ g) was used to synthesize double-stranded cDNA, which was subsequently used to generate biotin-labeled cRNA. The cRNA (10 μ g) target was hybridized to a Haffsall full-length GeneChip array (Affymetrix) at 45°C overnight. The chips were washed and stained with phycoerythrin-labeled streptavidin (Molecular Probes, Eugene, Oreg.) and then scanned on a GeneChip Array Scanner. Data was analyzed using the Affymetrix Data Mining Tool (version 3.0). The manufacturer recommended analyses to assess variability in array hybridization (efficiency and normalization), and cDNA synthesis efficiency were performed to process the data. Genes are listed by open reading frame (ORF) GenBank accession numbers or TrEMBL database ID number. Genes preferentially upregulated by IFN- γ are listed in red with fold changes highlighted in red, whereas genes preferentially upregulated by IFN- α and IFN- β are listed in blue with fold changes highlighted in blue. Genes not preferentially upregulated by all three IFNs are listed in black. Down-regulated genes are listed in green. Bold numbers represent fold changes of ≥ 3 .