Alpha Interferon Inhibits Hepatitis C Virus Replication in Primary Human Hepatocytes Infected In Vitro

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Chronic hepatitis C is a common cause of liver disease, the complications of which include cirrhosis and hepatocellular carcinoma. Treatment of chronic hepatitis C is based on the use of alpha interferon (IFN-α). Recently, indirect evidence based on mathematical modeling of hepatitis C virus (HCV) dynamics during human IFN-α therapy suggested that the major initial effect of IFN-α is to block HCV virion production or release. Here, we used primary cultures of healthy, uninfected human hepatocytes to show that: (i) healthy human hepatocytes can be infected in vitro and support HCV genome replication, (ii) hepatocyte treatment with IFN-α results in expression of IFN-α-induced genes, and (iii) IFN-α inhibits HCV replication in infected human hepatocytes. These results show that IFN-α acts primarily through its nonspecific antiviral effects and suggest that primary cultures of human hepatocytes may provide a good model to study intrinsic HCV resistance to IFN-α.

Hepatitis C has emerged in recent years as a common cause of liver disease, and an estimated 170 million people are thought to be infected worldwide. Hepatitis C virus (HCV) infection is characterized by viral persistence and chronic liver disease in approximately 80% of cases. The complications of chronic hepatitis C include cirrhosis in 20% of cases and hepatocellular carcinoma, which has an incidence of up to 4 to 5% per year in patients with cirrhosis. Hepatitis C-related end-stage liver disease is now the principal indication for liver transplantation in industrialized countries (2).

HCV is a single-strand positive-sense RNA virus belonging to the family Flaviviridae. Translation of its only open reading frame leads to the synthesis of a single polyprotein which is secondarily cleaved by both host and viral proteases, giving rise to structural and nonstructural proteins (22). The nonstructural protein 5B (NS5B) is an RNA-dependent RNA polymerase (RdRp). The mechanisms of HCV replication in host cells are poorly understood. It is thought that RdRp, along with other nonstructural proteins, the HCV RNA template, and host cell factors, forms a replication ribonucleoprotein complex associated with perinuclear membranous structures that would be the site of RNA replication (13, 43). By analogy with other members of the family Flaviviridae, the replication strategy within this complex would be the production of a negative-strand copy of the RNA genome, which would in turn serve as a template for the production of progeny positive-strand RNA. Indeed, negative-strand HCV RNA has been detected in various cells and tissues supporting HCV replication (1, 18, 20, 28, 39, 40).

HCV RdRp, like other viral RNA polymerases, has a high error rate, with misincorporation frequencies averaging about $10^{-4}$ to $10^{-5}$ per base site, in the absence of a proofreading mechanism. As a result, mutations accumulate in newly generated HCV genomes. Most mutant viral particles are replication deficient, but some propagate efficiently. The fittest infectious particles are selected continuously on the basis of their replication capacities and environmental selective pressures (mainly the host immune response). This explains why each infected individual harbors a pool of genetically distinct but closely related HCV variants referred to collectively as a quasi-species (24, 45).

Treatment of chronic hepatitis C is aimed at preventing complications, especially cirrhosis and hepatocellular carcinoma. It is currently based on subcutaneous injection of recombinant alpha interferon (IFN-α) three times a week or of its pegylated form (i.e., IFN-α combined with polyethylene glycol) once a week. The antiviral efficacy of IFN-α is potentiated by ribavirin, a nucleoside analog with an unknown mechanism of action (2, 10, 21, 25, 36, 47). Combination therapy with pegylated IFN-α plus ribavirin for 24 to 48 weeks leads to permanent viral clearance in 42 to 82% of patients according to HCV genotype; other patients have ongoing viral replication and remain at risk of disease progression [23; M. W. Fried, M. L. Shiffman, R. K. Reddy, C. Smith, G. Marinos, F. L. Goncales, Jr., et al., Gastroenterology 120(Suppl. A):55, 2001].

After subcutaneous administration, IFN-α specifically binds to high-affinity receptors at the surface of target cells. IFN-α binding to its receptor triggers a cascade of intracellular reactions, leading to activation of numerous IFN-induced genes (11, 31, 38, 41, 44). The products of these genes mediate the cellular actions of IFN-α. As IFN-α binds to surface receptors of immune cells, it has immunomodulatory effects (34, 42). IFN-α binding to various cells also induces numerous proteins...
and enzymatic pathways involved in establishing a non-virus-specific antiviral state through distinct but complementary mechanisms (3, 12, 27, 37, 49). Specific IFN-α binding to human hepatocytes and subsequent activation of IFN-α-induced genes leading to the establishment of an antiviral state have not yet been documented.

Recently, indirect evidence based on mathematical modeling of HCV dynamics during human IFN-α therapy suggested that the major initial effect of IFN-α is to block HCV virion production or release (30). Inhibition of HCV replication by both IFN-α and IFN-β has been observed in a human lymphocytic cell line supporting HCV genome replication (40). More recently, IFN-α was shown to inhibit subgenomic HCV RNA replication in HuH-7 human hepatoma cell lines (8, 9) and full-length HCV RNA replication in a binary expression system in CV-1 monkey kidney cell lines (4). Nevertheless, IFN-α blockade of HCV replication has never been demonstrated in healthy, uninfected hepatocytes.

### MATERIALS AND METHODS

**Primary cultures of human hepatocytes.** Healthy human hepatocytes were isolated from surgical liver resection specimens from 15 liver donors (Table 1). The two-step collagenase perfusion method was used (35). Cell viability, assessed by the trypan blue exclusion test, was greater than 85%. Hepatocytes were plated at confluence (14 × 10⁴ cells/cm²) in 60- or 35-mm-diameter culture dishes precoated with type I collagen (Iwaki Glass, Chiba, Japan) in a total volume of 3.0 or 1.5 ml of standard culture medium consisting of a mixture of William's E medium and Ham F12 medium (1:1 volume) supplemented as recommended elsewhere (14) and containing 5% fetal calf serum to favor cell attachment. After 4 h, the standard medium was replaced with 3.0 or 1.5 ml of a previously described serum-free long-term culture medium (6, 17). This medium was renewed after 24 h and then every 48 h. The cultures were maintained in humidified 95% air-5% carbon dioxide at 37°C.

**In vitro infection of primary cultures of human hepatocytes.** Serum samples from nine patients with chronic HCV genotype 1 infection were used for cell infection (the HCV subtypes and viral loads are shown in Table 1). Three days after plating (to permit cell recovery from isolation), the cells were infected in vitro by overnight incubation with 25 µl of HCV-positive serum in 3.0 ml of medium. The cells were then washed three times with 3.0 ml of fresh medium, and the cultures were continued under normal conditions in long-term culture medium. Cells and culture medium were collected at various times during culture and stored at −80°C. All experiments were carried out in duplicate.

**Treatment of primary hepatocyte cultures with IFN-α.** Recombinant IFN-α 2a (Roche) was used at final concentrations of 500 to 10,000 U/ml. IFN-α treatment was started at the time of HCV infection in most experiments and 3 days after infection in some cases. IFN-α was replaced on a daily basis as the culture medium was changed. All experiments were carried out in duplicate.

**Western blot analysis of IRF-1 and PKR induction.** Total protein corresponding to 400,000 cells was extracted in Laemmli buffer, electrophoresed on 12.5% polyacrylamide gels, and then transferred electrophoretically to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, Mass.). Western blotting was performed using the Santa Cruz polyclonal antibodies against interferon-responsive factor 1 (IRF-1) (kindly provided by N. Mechti, INSERM U475, Montpellier, France) and a monoclonal antibody against human RNA-dependent protein kinase (PKR) (kindly provided by E. Meurs, Institut Pasteur, Paris, France) and a polyclonal antibody against interferon-inducible protein kinase (IFN-α-induced) (kindly provided by E. Meurs, Institut Pasteur, Paris, France). The proteins were visualized by using an enhanced chemiluminescence detection method (Amersham Pharmacia, Abingdon, United Kingdom), and the blots were analyzed with a National Institutes of Health image analyzer in order to measure the amounts of induced proteins.

**Cellular RNA extraction and strand-specific RT-PCR.** At the time of cell harvest, the medium was removed, and the cultures were washed three times with cold phosphate-buffered saline. RNA was purified from 4 × 10⁴ or 2 × 10⁴ cultured hepatocytes or from 100 µl samples of HCV-positive sera, using a guanidinium isothiocyanate-acid phenol extraction procedure (RNALIBE; Eurobio, Les Ulis, France). Precipitated RNA was dissolved in 50 µl of diethyl pyrocarbonate-treated water and quantified by UV spectrophotometry. Extracted RNA was analyzed with a modification of the previously described strand-specific

| TABLE 1. Characteristics of the primary cultures of human hepatocytes and the corresponding HCV-positive sera used for inoculation and summary of experiments and results |
|---|---|---|---|---|---|---|---|---|
| Donor | Age | Gender | Serum | Origin | Genotype | Viral load | Accumulation of positive-sense (HCV) RNA strands | Effect of IFN-α on genetic evolution |
| FT144 | 51 | F | Left lobe | S31 | 1b | No | Present | Reduced |
| FT147 | 54 | M | Right lobe | S27 | 1b | Yes | Present | Reduced |
| FT154 | 57 | M | Left lobe | S23 | 1b | Yes | Present | NT |
| FT155 | 59 | M | Right lobe | S20 | 1b | No | Present | NT |
| FT156 | 77 | M | Right lobe | S17 | 1b | Yes | Present | NT |
| FT161 | 54 | M | Right lobe | S42 | 1b | No | Present | NT |
| FT164 | 47 | F | Right lobe | SI | 1b | Yes | Present | NT |
| FT168 | 75 | F | Left lobe | S34 | 1b | Yes | Present | NT |
| FT187 | 66 | M | Left lobe | S155 | 1b | No | Present | NT |
| FT189 | 48 | M | Left lobe | S155 | 1b | Yes | Present | NT |
| FT195 | 17 | M | Left lobe | S155 | 1b | No | Present | NT |

M, male; F, female; NA, not applicable.
rTth reverse transcription-PCR (RT-PCR) assay (7). Primers located in the HCV 5' noncoding region and antisense primer KY80 (5'-GCAGAAAGCGTCTAGCCATGGCGT-3', nt 311 to 288) and sense primer KY80 (5'-GCAGAAAGCGTCTAGCCATGGCGT-3', nt 68 to 91) (46). One microgram of cellular RNA was used for cDNA synthesis in a 20-μl reaction mixture containing 50 ng of primer HCV-I and 3.75 mM MgCl₂ added. PCR was performed on the GeneAmp PCR System 9600 (PerkinElmer, Norwalk, Conn.) in a 50-μl reaction mixture containing 50 ng of primer HCV-II and 3.75 mM MgCl₂ was added. PCR products were analyzed by agarose gel electrophoresis. The negative-strand HCV RNA assay was performed by the same procedure, except that the primers were used in reverse order.

Real-time PCR quantification of positive- and negative-strand HCV RNA. Both positive- and negative-strand HCV RNA were quantified by means of a real-time PCR assay using the LightCycler instrument and technology (Roche Applied Science, Indianapolis, Ind.) and SYBR green I dye for detection. The primer pair was located in the HCV 5' noncoding region and included antisense primer KY78 (5'-CTCGCAAGACCCCTCATGCCAGC-3', nt 869 to 849) and sense primer KY80 (5'-GCAGAAAGCGTCTAGCCATGGCGT-3', nt 68 to 91) (46). One microgram of cellular RNA was used for cDNA synthesis in a 20-μl reaction mixture containing 5 U of Tth polymerase and 1 μM RT primer. Primer HCV-I was used for positive-strand cDNA synthesis, and primer HCV-II was used for negative-strand cDNA synthesis. In addition, PCR amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed as an internal control for the quality of extracted cellular RNA, with RT using primer GAPDH-31 (5'-GCCCTGCTTCACCACCTTCTTG-3', nt 869 to 849). cDNA was synthesized at 70°C for 2 min in all instances, and generated cDNA was purified with the HighPure PCR product purification kit (Roche Applied Science) in a 50-μl reaction mixture. Positive- and negative-strand HCV PCR amplifications were performed with 3 μl of purified cDNA in a 10-μl reaction mixture containing 1 μl of LightCycler-FirstStart DNA Master SYBR green (Roche Applied Science) and 0.5 μM (each) HCV primer KY78 and KY80. PCR consisted of an initial denaturation step of 1 min at 94°C, 50 cycles, with 1 cycle consisting of 15 s at 94°C, 30 s at 58°C, and 30 s at 72°C; and (iii) a final extension step of 7 min at 72°C. PCR products were analyzed by agarose gel electrophoresis. The negative-strand HCV RNA assay was performed by the same procedure, except that the primers were used in reverse order.

**RESULTS**

**HCV replicates in primary cultures of healthy human hepatocytes infected in vitro.** We prepared primary cultures of healthy human hepatocytes from 15 HCV-seronegative donors (Table 2). Ferrini et al. previously showed that healthy hepatocytes retain a differentiated phenotype for at least 35 days under the conditions used here (6). Serum samples from nine patients chronically infected with HCV genotype 1 (subtype 1a or 1b) who had never been treated were used for in vitro infection of 13 of the 15 primary hepatocyte cultures (Table 1). Cultures were infected 3 days after plating and harvested at various times between 3 and 12 days of culture for extraction of total cellular RNA. Two complementary assays were used to study positive- and negative-sense HCV RNA strands in the inocula and infected hepatocyte cultures. The first assay is a highly sensitive qualitative (i.e., nonquantitative) detection assay, based on a modification of our previously described strand-specific rTth RT-PCR assay (7). As shown in Fig. 1a and b, the assay detects 0.1 fg of the correct RNA strand (i.e., 3 × 10⁵ molecules), whereas at least 1 to 10 pg of the incorrect RNA strand is required to obtain a detectable signal. The second assay is a quantitative assay based on real-time PCR with the LightCycler technology allowing quantification of positive- and negative-sense HCV RNA strands in both the inocula and hepatocyte cultures (Fig. 1c). As shown in Fig. 1c, the tested interval from 3.5 to 5.5 log HCV RNA copies per capillary was within the dynamic range of quantification of
The quantitative assay is less sensitive for HCV RNA strand detection than the qualitative assay, with a lower detection cutoff of 1 to 2 log HCV RNA copies per milliliter higher than the latter. The results are summarized in Table 1.

As expected, the positive-sense RNA strand was the only form of HCV RNA present in the inoculum. We thus used detection of the positive- and negative-sense RNA strands with the qualitative assay as a marker of HCV replication in hepatocyte cultures. Both positive- and negative-sense RNA strands were detected in the cultures. In culture FT147 infected with serum S26 (Fig. 2a), positive RNA strands were detected on day 1 postinfection and were still present in the last plate harvested. The negative-sense RNA strand was detected on day 2 postinfection and remained detectable up to day 10, proving viral replication in the culture. We observed the presence of positive-sense HCV RNA throughout the culture period and persistent expression of negative-sense HCV RNA in the other cultures infected with different sera (Table 1 and data not shown).

HCV replication in hepatocyte cultures was further supported by the accumulation of HCV RNA strands, as measured by real-time quantitative RT-PCR. Indeed, we observed a significant increase in the amount of both positive- and negative-sense HCV RNA strands in culture FT172 infected with serum S42 (Fig. 3a). A similar increase in the amount of positive-sense HCV RNA was observed in cultures FT189 and FT195, both infected with S155, but the total amount of positive-sense HCV RNA was smaller in these cultures at the various time points, suggesting less replication than that in culture FT172 (Fig. 3b and c). The negative-sense HCV RNA also accumulated in culture FT172, but the amount of negative-sense HCV RNA was consistently smaller than the amount of positive-sense HCV RNA on days 3 and 5 (Fig. 3a). This explains why, in cultures FT189 and FT195, negative-sense HCV RNA was not detected with the quantitative assay, whereas it was detected with the more sensitive qualitative assay; i.e., its amount was below the detection cutoff of the quantitative assay. Similar results were obtained with culture...
FT168 infected with serum S34 (data not shown). Finally, neither positive- nor negative-strand HCV RNA was detected in culture supernatants by the sensitive qualitative assay.

In order to prove that HCV replicated in primary hepatocyte cultures, i.e., that HCV RdRp synthesized both negative- and positive-sense HCV RNA strands, we examined the accumulation of mutations on HCV genomes in five cultures. A 300-bp fragment located within the NS5A gene was chosen for this study. In all instances, nucleotide mutations accumulated on positive-strand HCV genomes during the culture period. Comparison of NS5A quasispecies sequences in the inoculum and after 8 days of culture (20 clones per time point) showed significantly higher between-sample genetic distances (calculated by pairwise comparison of NS5A quasispecies sequences in the inoculum versus the culture) than within-sample genetic distances (calculated by pairwise comparison of NS5A quasispecies sequences in the inoculum). In all instances, accumulation of synonymous mutations per synonymous site was significantly greater than accumulation of nonsynonymous mutations per nonsynonymous site (data not shown), indicating that the accumulation of mutations on HCV genomes in culture resulted from random nucleotide misincorporations by RdRp, in the absence of positive selection forces driving genetic evolution.

Together, these findings demonstrated unequivocally that HCV replicated in the primary cultures of healthy human hepatocytes.
hepatocytes as a result of viral RdRp function. Complete infectious virions did not appear to be secreted in the medium. Primary cultures of healthy human hepatocytes exhibit a biological response to IFN-α. To determine whether primary hepatocyte cultures are equipped to respond appropriately to IFN-α stimulation, uninfected cultures were treated with 5,000 U of IFN-α per ml. We then extracted total cellular protein at various times from 0 to 24 h and analyzed the expression of IRF-1 and double-stranded PKR by Western blotting. These two proteins are encoded by two prototypic IFN-α-regulated genes: IRF-1 is a transcription factor induced as a primary response to IFN-α, while PKR induction is a secondary response, necessitating prior synthesis of IRF-1. IFN-α-stimulated Daudi cells were used as positive controls for these experiments. IFN-α induced the expression of both IRF-1 and PKR in cultured hepatocytes by factors of approximately 4 and 3, respectively (Fig. 5). As expected, IRF-1 expression preceded PKR expression by approximately 8 h. Similar experiments were carried out after 8 days of culture, with both uninfected and HCV-infected hepatocytes. Identical results were obtained, indicating that the response to IFN-α is maintained for more than a week and is not eliminated by HCV infection (Fig. 5).

IFN-α is not toxic for primary cultures of healthy human hepatocytes at the concentrations used in this study. As an effect of IFN-α on markers of intracellular HCV replication might merely reflect cytotoxicity rather than inhibition of viral RdRp, we studied IFN-α toxicity in our primary hepatocyte culture system. Phase-contrast microscopy revealed no signs of cellular toxicity. Furthermore, culture of hepatocytes from three different donors (FT154, FT155, and FT156), treated for 5 days with 5,000 U of IFN-α per ml, revealed no significant

![Graphs showing accumulation of positive- and negative-strand HCV RNA in hepatocyte cultures](image-url)
reduction in total de novo protein synthesis (not shown), a sensitive marker of cytotoxic stress in cultured hepatocytes (6).

IFN-α inhibits the expression of positive- and negative-sense HCV RNA strands in primary cultures of healthy human hepatocytes infected in vitro. We tested eight primary hepatocyte cultures infected in vitro for the effects of continuous incubation with 5,000 and 10,000 U/ml of IFN-α by means of the qualitative HCV RNA assay. In culture FT147 infected with serum S26 (Fig. 2b), the positive-sense RNA strand was detected from day 1 but disappeared after day 10 in the presence of IFN-α, whereas the negative-sense RNA strand remained undetectable throughout the culture period (i.e., until day 12). Similarly, the negative-sense RNA strand was never detected in any other IFN-α-treated culture (not shown). In contrast, when IFN-α treatment was started 3 days after HCV infection, the positive-sense RNA strand was detected throughout the culture period, whereas the negative-sense RNA strand was detected from infection through day 5 before disappearing (Fig. 2c).

The effect of increasing IFN-α concentrations (500 to 10,000 U/ml) on the detection of positive- and negative-sense HCV RNA strands was studied 5 or 8 days after infection. In cultures FT147 and FT161 (Fig. 6a), the negative-sense HCV RNA strand was never detected, whatever the IFN-α concentration.
used, whereas the positive-sense HCV RNA strand was always detected, even at the maximum IFN-α concentration used, i.e., 10,000 U/ml. Inhibition of viral replication in the culture was confirmed by using the less sensitive, but quantitative, real-time RT-PCR assay. Indeed, this assay did not detect positive-strand HCV RNA at IFN-α concentrations higher than 500 U/ml, meaning that the intracellular amount of positive-strand HCV RNA was below the detection cutoff level (Fig. 6b).

Altogether, these results suggested potent concentration-dependent inhibition of positive-sense HCV RNA strand accumulation in response to IFN-α treatment.

**IFN-α inhibits the accumulation of mutations on the HCV genome during replication in primary cultures of healthy human hepatocytes infected in vitro.** In order to confirm that IFN-α inhibited HCV replication, we studied its effect on the accumulation of mutations on HCV genomes. In the absence...
of IFN-α, significant HCV genetic evolution was always observed, due to random accumulation of mutations (Fig. 4). In contrast, when 5,000 or 10,000 U of IFN-α per ml was added daily before testing for positive-strand RNA on day 8 of infection (i.e., several days before it otherwise became undetectable), no significant genetic evolution was observed in two of the five cultures tested (Fig. 4). In addition, the between-sample genetic distances (calculated by pairwise comparison of the quasispecies sequences in culture on day 8 versus that in the inoculum) were significantly lower in the presence of IFN-α.
IFN-α than in the absence of IFN-α in three of the five cultures, again suggesting that IFN-α significantly inhibited the accumulation of HCV genome mutations. No significant difference was seen in the remaining two cultures. In one culture, phylogenetic analysis followed by phylogenetic tree plotting showed a trend toward distinctive clustering of postculture and inoculum quasispecies sequences, respectively, in the absence of IFN-α; this clustering was abolished in the presence of IFN-α (data not shown). No such trend was clearly visible in the remaining four cultures.

**DISCUSSION**

This study shows that HCV can replicate in primary cultures of human hepatocytes infected in vitro, as a result of viral RdRp function. As previously described (7), in vitro infection resulted in the production of negative-sense HCV RNA strands, an HCV replication intermediate. In addition, real-time RT-PCR quantification showed a significant accumulation of positive-sense HCV RNA strands and, when present in sufficient amounts, of negative-sense HCV RNA strands during hepatocyte culture. Significant accumulation of random mutations on the HCV genome showed that the viral RdRp, an error-prone RNA polymerase with no proofreading activity, was responsible for the accumulation of positive-strand HCV RNA genomes during culture. The random accumulation of mutations in the region studied, in the absence of positive pressure toward amino acid changes, was not surprising in this in vitro culture system. However, the same region of the NS5A gene displays a similar pattern of genetic evolution in HCV-infected patients (33). As NS5A is part of the replication complex and is most likely involved in regulating RdRp function (13, 43), the conservation constraints on NS5A evolution occurring in vivo might also be present in our hepatocyte culture system.

The principal finding of this study is the effect of IFN-α on HCV replication in primary hepatocyte culture. IFN-α was recently shown to inhibit the replication of dengue virus, another member of the Flaviviridae family, in hepatoma cell lines infected in vitro (5). Mathematical modelling of HCV dynamics during human IFN-α treatment recently suggested that IFN-α blocks HCV virion production or release as a result of its direct, nonspecific antiviral effect (29, 30). The capacity of IFN-α to directly inhibit HCV replication in healthy human hepatocytes had not previously been demonstrated.

We show that IFN-α blocks HCV genome synthesis by the HCV RdRp in cultured healthy human hepatocytes in a concentration-dependent manner. This effect is probably mediated by IFN-α-induced cellular pathways supporting nonspecific antiviral actions. Indeed, we observed the following. (i) IFN-α-induced genes were expressed in primary hepatocyte cultures treated with IFN-α, and their expression was not altered by HCV infection. (ii) Expression of negative-strand HCV RNA was always suppressed in IFN-α-treated cultures. (iii) IFN-α significantly inhibited the accumulation of mutations on the HCV genome in three of five hepatocyte cultures. The concentrations of IFN-α used here were relatively high (500 to 10,000 U/ml), but we showed that IFN-α toxicity could not explain the inhibitory effect on HCV replication. An earlier report suggested that IFN-α could act by inhibiting de novo infection of hepatocytes (48). If IFN-α effectively prevents HCV entry into hepatocytes in vivo, this effect would probably be mediated by IFN-α-induced humoral responses (neutralizing antibodies), which are not present in hepatocyte cultures. We did not examine whether IFN-α could affect virus entry in our model, in addition to inhibiting viral replication. It is conceivable that the reduction of positive-strand HCV RNA accumulation in IFN-α-treated cells could be enhanced by receptor down-regulation or by decreased internalization or membrane fusion.

Interestingly, despite the disappearance of negative-strand HCV RNA from all IFN-α-treated cultures, positive-strand RNA always persisted for several days, suggesting that the kinetics of HCV RNA strands in cell culture differ from those in the peripheral circulation. This finding was not surprising, because most of the mechanisms governing viral clearance are absent in vitro, especially when only intracellular HCV RNA is concerned, whereas the estimated mean half-life of free HCV virions is only 2.7 h in vivo (30). In contrast, the apparent lack of IFN-α inhibition of mutation accumulation on HCV genomes in two cultures, despite a clear effect on negative-strand HCV RNA production, was surprising. The inhibitory effect of IFN-α may have been weaker in these two cultures, permitting low-level viral replication, while negative-strand HCV RNA was undetectable, even with our sensitive qualitative strand-specific HCV RNA assay. Such variability in the effect of IFN-α might be explained by partial hepatocyte resistance to IFN-α stimulation or by partial viral resistance to IFN-α, possibly mediated by viral proteins inhibiting antiviral effectors induced by IFN-α (32). It is noteworthy that all the cultures were infected with HCV genotype 1, a genotype that displays various levels of IFN-α sensitivity based on initial IFN-α blocking efficacy (16, 30), possibly owing to differences in the sequences of viral proteins and, thus, in their structure and function. Unfortunately, data on early viral dynamics during IFN-α therapy in the patients whose blood samples were used for in vitro infection are not available to confirm this hypothesis.

In conclusion, we show that primary cultures of healthy human hepatocytes can be infected in vitro by HCV and support its sustained replication. We further show that IFN-α is able to block HCV replication in this culture model, which is close to the HCV-infected human liver. These results strengthen the hypothesis that IFN-α acts primarily through its nonspecific antiviral effects and suggest that primary cultures of human hepatocytes may provide a good model to study intrinsic HCV resistance to IFN-α. However, clearance of infected cells resulting from IFN-α-modulated immune responses probably plays a major role in permanent HCV RNA clearance during therapy (16, 30), emphasizing the need for both in vitro and in vivo studies to understand IFN-α treatment failure in HCV-infected patients.

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