Hepatitis delta virus replication in vitro is not affected by interferon-α or -γ despite intact cellular responses to interferon and dsRNA

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The hepatitis delta virus (HDV) genome consists of circular ssRNA which has extensive intramolecular complementarity and can form a dsRNA rod-like structure. If such RNA species were to exist in an unmasked form in cells, they would be expected to induce interferon (IFN) expression and activate two IFN-inducible dsRNA-dependent enzymes with antiviral activity, namely the dsRNA-dependent protein kinase (PKR) and 2',5' oligoadenylate (2',5'A) synthetase. Since the virus replicates to high copy number for prolonged periods in infected cells it is apparently able to evade these antiviral mechanisms. The RNA genome may be masked and fail to induce or activate the antiviral response, or the virus may inhibit such a response. Treatment of a hepatoma cell line, Huh7, and a fibrosarcoma cell line, HT1080, stably transfected with a trimeric HDV eDNA construct, with IFN-α or IFN-γ for up to seven days failed to influence the level of expression of genomic or antigenomic HDV RNA, or delta antigen (Ag). This is consistent with either failure of activation or inhibition of the IFN response. However the induction of several IFN-responsive genes, including PKR, 2',5'A synthetase and class I MHC is normal and cotransfection of a construct expressing delta Ag did not affect expression from an IFN-inducible chloramphenicol acetyltransferase construct. In addition, the activation of PKR is not inhibited in HDV-expressing cells and antiviral assays suggest that the ability of these cells to mount an antiviral response to at least two cytopathic viruses is unaffected. IFN-β is inducible normally by dsRNA in cells transfected with the delta cDNA trimer. We conclude that HDV replication is not inhibited by IFN-α or IFN-γ, even though the responses of cells expressing HDV RNA and antigen to IFN and dsRNA are intact.

Introduction

The hepatitis delta virus (HDV) causes acute and chronic liver disease in association with the hepatitis B virus (HBV); HDV requires the HBV surface antigens as an essential coat component (Bonino et al., 1986; Rizzetto et al., 1980). Co-infection or superinfection with HDV increases the severity of liver disease compared to infection with HBV alone (Rizzetto, 1983). Although HDV is unique among the known viral agents of animals, its genome has certain properties in common with the plant viroid and viroid-like satellite RNAs: (i) the HDV genome is 1679 nucleotides in length and so is relatively small; (ii) it consists of covalently closed, circular ssRNA with extensive regions of intramolecular complementarity, theoretically allowing the RNA to adopt a collapsed, ds rod-like secondary structure, in which approximately 70% of bases are paired (Kuo et al., 1988a; Wang et al., 1986); (iii) it replicates by a rolling circle mechanism to give oligomeric RNA intermediates of opposite polarity, which self-cleave into circular monomers (Kuo et al., 1988b; Sharmeen et al., 1988). Cells infected with HDV have been estimated to contain around 300000 copies of the viral genome (Chen et al., 1986). Unlike viroids, which do not encode any proteins, HDV expresses a single protein, the delta antigen (Ag). This protein is encoded by an open reading frame on the antigenomic strand and, as a result of an RNA editing event, it exists in two forms with Mₚs of 24K and 27K, the larger protein possessing an additional 19 amino acids at its C terminus (Bergmann & Gerin, 1986; Luo et al., 1990; Zheng et al., 1992). The delta Ag is a nuclear phosphoprotein with RNA-binding activity (Chang et al., 1988) which plays a central role in the HDV replication cycle. The small antigen (P24) is responsible for transport of the RNA into the nucleus (MacNaughton et al., 1991) and is essential for HDV RNA...
replication in transfected cells in culture (Kuo et al., 1989). The large antigen (P27) inhibits RNA replication (Chao et al., 1990; Glenn & White, 1991) and by interacting with HDV RNA and the HBV envelope proteins it participates in assembly of virus particles (Chang et al., 1991).

Double-stranded RNA is known to be capable of directly inducing the expression of a number of cellular genes, the best characterized of which is interferon IFN-β (for a review see De Maeyer & De Maeyer-Guignard, 1988). It also independently activates two enzymes with antiviral activity: 2′,5′ oligoadenylate (2′,5′A) synthetase (reviewed in Kerr, 1987; Lengyel, 1982) and dsRNA-dependent protein kinase (PKR; Clemens et al., 1993). Both these enzymes are present in the cell constitutively at low but detectable levels and are strongly induced following treatment with IFN-α or IFN-β and, to a lesser extent, IFN-γ. In cells infected with virus, dsRNA is commonly produced as an intermediate of the viral replicative cycle. This in turn induces the expression of IFN-β and activates the two enzymes. Activation of PKR is accompanied by autophosphorylation on several serine and threonine residues (Samuel, 1979). The α subunit of eIF2 is then phosphorylated, leading to the inhibition of protein synthesis (Hovanessian, 1989). Although PKR inhibits translation from both viral and cellular mRNA, there is some evidence that it may act preferentially on viral RNA as a result of localized activation within infected cells (De-Benedetti & Baglioni, 1984). Activated 2′,5′A synthetase produces 2′,5′ oligoadenylates from ATP. These bind to and activate an endogenous ribonuclease, RNase L, which breaks down viral and cellular RNA.

All animal viruses, irrespective of their structure and mode of replication, can induce IFN expression under appropriate conditions (Ho, 1984), but their IFN sensitivity is variable. Many viruses have evolved mechanisms of inhibiting the IFN system (for a review see McNair & Kerr, 1993). Examples include the inhibition of induction of IFN in cells expressing HBV core protein (Twu et al., 1988; Whitten et al., 1991) and interruption of the post-receptor signalling pathway by adenovirus 12S E1A protein (Ackrill et al., 1991; Gutch & Reich, 1991; Kalvakolanu et al., 1991). In addition, certain viruses produce specific protein or RNA species which are capable of inhibiting the function of PKR or 2′,5′A synthetase. Since HDV replicates to high copy number for prolonged periods in infected cells, the possibility arises that this virus may also have evolved a means of evading the antiviral actions of IFN.

To establish whether HDV is able to inhibit the induction or actions of IFN, cell lines transiently and stably expressing delta Ag and RNA have been examined.

### Methods

**Plasmids.** The delta Ag- and RNA-expressing constructs used the expression vector pSVL (Pharmacia) and were kindly provided by Dr J. Taylor (Fox Chase Cancer Center, Philadelphia, Pa., U.S.A.). As previously described (Kuo et al., 1989), cloned HDV cDNAs were inserted into the vector to create an infectious trimer of unit-length HDV, pSVL/D3 and a cDNA, of less than unit length, expressing antigenic RNA that synthesized the small delta Ag, pSVL/Ag-S. Stable transfection of cells with pSVL/D3 leads to the expression of genomic and antigenic HDV RNAs and both the small and large forms of delta Ag (Kuo et al., 1989). Constructs expressing an mRNA containing the full-length HDV RNA, but not expressing the delta Ag, were derived from pSVL/D2M, which contains a dimer of the complete genome except for the deletion of 2 bp at positions 1434 and 1435 [using the notation for the 1679 base genome previously described by Kuo et al. (1988b)]. A unit-length fragment was excised with XbaI and cloned into the expression vectors pRC.CMV and pRC.RSV (Invitrogen) to give plasmids pCMV.D2M and pRSV.D2M respectively.

**Cells, transfections and assays.** Human hepatoma cells (Huh7), fibrosarcoma cells (HT1080), mouse fibroblast (C127) and HeLa cells were grown as monolayers in Dulbecco's modified MEM supplemented with 10% fetal calf serum, 1 mM-glutamine and penicillin/streptomycin and grown at 37 °C in an atmosphere of 5% CO₂. Cultures were split at a ratio of 1:3 and the medium was changed twice weekly. DNA transfections, using a calcium phosphate procedure, were performed as previously described (Porter et al., 1988). Stable transfectants were generated by cotransfection of pTKneo and subsequent selection in a medium containing 400 μg/ml G418. Colonies were ring-cloned and clones expressing delta Ag and/or HDV RNA were then identified by immunoblotting and RNase protection assay (see below). Chloramphenicol acetyltransferase (CAT) assays were performed in HeLa cells as previously described (Porter et al., 1988). Briefly, cells were plated on six-well plates at a concentration of 10⁴ cells/well and 24 h later were cotransfected with an IFN-inducible CAT construct, 474.1.I.CAT, which contains the IFN-stimulated response element from the IFN-α-inducible 6-16 gene (1 μg/well), and either pSVL.Ag-S or the vector pSVL (2 μg/well). IFN-α and IFN-γ at 1000 IU/ml were applied after 12 h. Cell lysates were harvested and assayed 36 h later using the method of Sleigh (1986).

**IFN treatment.** Cells were treated with a highly purified mixture of α-IFNs (Wellferon, 1·5 × 10⁶ IU/mg protein; provided by Wellcome Research Laboratories) at 1000 IU/ml for the time stated. Recombinant IFN-γ (4 × 10⁷ IU/mg protein) was supplied by Dr G. Adolf (Ernst Boehringer Institut für Arzneimittelforschung, Vienna, Austria).

**Poly(rI).poly(rC) treatment.** Cells were treated by a modification of the method of Zinn et al. (1983). Monolayers were washed twice in serum-free medium and then poly(rI). poly(rC), at a final concentration of 0·1 mg/ml, was applied in serum-free medium for 2 to 6 h. The cells were then harvested for RNA preparation.

**RNase analyses.** Total cellular RNA was prepared from monolayer cells by NP40 lysis and phenol-chloroform extraction. RNase protection (Sambrook et al., 1989) was performed, using probes labelled with [α-³²P]UTP and synthesized from SP6 transcription vectors, containing PKR, 2′,5′A synthetase and γ-actin cDNA inserts, and gave protected fragments of 300, 220 and 130 nucleotides respectively. An SP6 construct containing a cDNA to IFN-β protected 270 nucleotides and was kindly provided by Dr S. Goodbourn (Imperial Cancer Research Fund). Probes for HDV RNA protected 95 bases of antigenomic RNA (from the EcoRI site at position 1427 to the SstI site at 1332) and 155 bases of genomic RNA (from XhoI at 1272 to EcoRI at 1427). Probes were labelled to an activity of 2 × 10⁶ c.p.m. per
µg of input DNA. An activity of 3 × 10^6 c.p.m. for the probe was used in each assay, along with 10 µg of cytoplasmic RNA. Probes were present at 100- to 1000-fold excess, demonstrated by running a lag of input DNA. An activity of 3 × 10^5 c.p.m. for the probe was used.

Sample preparation was performed as previously described (Porter et al., 1988).

**Immunostaining.** Cells from Huh7 clones transfected with pSVL-D3 were fixed with acetone for 20 min at −20 °C. They were then incubated for 30 min with polyclonal rabbit antiserum raised against a truncated delta Ag (amino acids 13 to 64) fused with the first 98 amino acids of MS2 polymerase (Saldanha et al., 1990) at a dilution of 1:50 in PBS containing 5% fetal calf serum. After washing three times in PBS, the second layer of swine anti-rabbit immunoglobulin conjugated with fluorescein isothiocyanate (Dako) was applied at a 1:20 dilution in PBS and incubated at 37 °C for 30 min. The stained cells were washed twice in PBS and once in water and mounted.

**Immunoblotting.** Protein samples were subjected to electrophoresis on 12% acrylamide gels by the method of Laemmli (1970) and transferred electrophoretically to PVDF membrane (Immobilon-P; Millipore) according to manufacturer's instructions. The delta Ag was detected using a rabbit polyclonal antiserum, a peroxidase-labelled goat anti-rabbit antiserum (Jackson Immunoresearch) and 3,3'-diaminobenzidine (Sigma) as the peroxidase substrate.

**Antiviral assays.** Cells seeded in 96 well microtitre plates at a concentration of 5 × 10^4 per well were incubated for 18 h at 37 °C. They were then treated with serial dilutions of IFN-α and IFN-γ for 24 h and challenged with serial dilutions of encephalomyocarditis virus (EMC) and Semliki Forest virus (SFV). Protection from the cytopathic effects of each virus was scored after 24 to 48 h. Each assay was repeated three times.

**PKR assays.** These were performed as previously described (Silverman et al., 1982b). Briefly, 3 µl extracts from cells treated with IFN-α at 1000 IU/ml for 24 h were incubated in a final volume of 10 µl containing 10 mM-MgCl₂, 100 µM-ATP (unlabelled) and 1.0 µCi [γ-³²P]ATP (> 5000 Ci/mmol), with or without 1 µg/ml poly(rI), poly(rC). The samples were incubated at 30 °C for 15 min and analysed on 12% SDS–polyacrylamide gels.

**Results**

**Effect of IFN-α and IFN-γ on expression of HDV RNA and delta Ag in Huh7- and HT1080-derived clones**

Clones of cells selected in G418 following transfection with pSVL-D3 (see Methods) were ring-cloned and analysed, by immunostaining and immunoblotting, for expression of delta Ag. Several delta Ag-expressing Huh7-derived clones were identified. One of these, H7-D, (referred to originally as 1.2D; Cheng et al., 1992) and two similarly isolated HT1080-derived clones, HT-D3(1) and HT-D3(2), were used in this study. HDV genomic and antigenomic RNAs were detected by an RNase protection assay in both cell lines and shown to be expressed at a constant level over time. Similarly, the level of expression of the small and large forms of delta Ag was constant with time. The effect of treatment of H7-D with IFN-α and IFN-γ for between 1 and 7 days on the level of HDV RNA and delta Ag was determined (Fig. 1 and 2). There was no significant variation in the level of either following IFN treatment. Similarly, the level of HDV RNA and delta Ag in the fibrosarcoma-derived cell lines was not affected by IFN treatment (data not shown).

![Fig. 1. IFN treatment does not affect HDV antigenomic RNA expression. H7-D clone cells were grown for 2 days (lanes 1) or 4 days (lanes 2) with or without IFN treatment (1000 IU/ml) as indicated. RNA was extracted and assayed by RNase protection. Huh7 cell lysate (lane 1) was used as a negative control.](image1)

![Fig. 2. The lack of effect of IFN-α or IFN-γ on expression of delta Ag in H7-D cells. IFN treatment (1000 IU/ml) was for 1, 2, 4 and 7 days (lanes 3 to 6 respectively) and compared with untreated cells (lane 2). Cell lysates were analysed by immunoblotting. The blot was probed with a rabbit polyclonal anti-delta Ag serum. The positions of the P24 and P27 delta Ags are indicated. Huh7 cell lysate (lane 1) was used as a negative control. Sizes of Mᵦ markers (lane M) are indicated to the left.](image2)
Expression of IFN-inducible genes

The response of several IFN-inducible genes to IFN-α and IFN-γ in H7.D cells was determined by RNase protection assay or Northern transfer. The 2',5'A synthetase and PKR genes, which are both predominantly IFN-α-inducible, respond normally to IFN (Fig. 3a and b) and the induction of the IRF-1 gene (the IFN-γ response of which is known to be primary) was also normal in H7.D (Fig. 3c). The 6-16, 9-27 and β2-microglobulin genes, which are IFN-α- and IFN-γ-inducible, also responded normally to IFN (data not shown). For the HDV-expressing fibrosarcoma cell lines, inducibility of the 6-16 and 9-27 genes by IFN-α and IFN-γ was also normal.

To determine the effect of the delta Ag on expression from an IFN-inducible reporter gene construct, HeLa cells were transfected with the plasmid pSVL.Ag-S and the IFN-α/γ-inducible CAT construct 474.1.1.CAT in a molar ratio of 4:1. As a control, the CAT construct was cotransfected with the expression vector pSVL in the same molar ratio. Experiments were performed in triplicate. Compared to controls, the delta Ag-expression construct had no effect on IFN-induced CAT expression; IFN-α treatment resulted in an 8- to 10-fold induction of CAT activity with both control and test constructs and IFN-γ resulted in a 2.5- to 3-fold induction. Delta Ag expression in the transfected HeLa cells was confirmed by immunoblotting.

Antiviral assays

To assess the integrity of the antiviral response in HDV-transfected hepatoma and fibrosarcoma cells, antiviral assays were performed using EMC and SFV. For EMC at a titre of 10 p.f.u./cell, IFN-α at 6 IU/ml protected 50% of cells from lysis in both HT.D3 and parental HT1080. Huh7 and H7.D both required IFN-α at 50 IU/ml for 50% protection. Assays on SFV also showed that the ability of the parental and HDV-expressing cell lines to mount an antiviral response to IFN-α was unaltered by HDV (Table 1).

Interferon induction

Attempts to induce the expression of IFN-β in Huh7 or Huh7-derived clones with dsRNA and Sendai virus were unsuccessful. Cells were treated with poly(rI).poly(rC) at the standard concentration of 0.1 mg/ml in serum-free medium for 1 to 6 h but no IFN-β expression was evident.
**HDV and the interferon response**

Fig. 4. Response of HDV-transfected fibrosarcoma cell lines to dsRNA. 
(a) The induction of IFN-β and IRF-1 mRNAs in HT. D3(1), HT. Ag-S and the parental HT1080 fibrosarcoma cells. Cells were either untreated (lanes 1), or treated with the ds poly(rI).poly(rC) at 0.1 mg/ml in serum-free medium for 2 h (lanes 2). (b) A longer exposure of the above assay. (c) Kinetics of induction of IFN-β mRNA in HT1080 cells. Cells were either untreated (lane 1) or treated with poly(rI). poly(rC) for 1, 2, 4 and 6 h (lanes 2 to 5 respectively). Lane 6, on RNase protection analysis. Priming the cells with IFN-α at 100 IU/ml overnight and cycloheximide treatment, both of which normally enhance the response to poly(rI).poly(rC), were without effect. Treatment of HT1080 cells under the same conditions successfully induced IFN-β mRNA (data not shown).

In order to establish whether HDV RNA or delta Ag expression affects the induction of IFN-β, the HDV-expressing fibrosarcoma clones, HT. D3(1) and HT. D3(2), and an additional HT1080-derived clone (HT. Ag-S) which expressed delta Ag alone, were examined. Expression of HDV RNA and/or delta Ag was confirmed by RNase protection and/or immunoblotting. Treatment of HT. D3(1), HT. D3(2) and HT. Ag-S with poly(rI).poly(rC) at 0.1 mg/ml for 2 h induced the expression of IFN-β mRNA to the same level as in the parental HT1080 cells (Fig. 4a). The induction of another dsRNA-inducible gene, IRF-1, was also normal in the HDV-expressing cells. Unlike the parental and delta Ag-expressing cell lines, both HT. D3(1) and HT. D3(2) expressed a significant constitutive level of IFN-β [shown for HT. D3(1) in Fig. 4b]. This may reflect the presence of a low level of unmasked dsRNA in cells expressing HDV RNA or it may be due to lysis of some of the cells, with release of HDV RNA into the medium where it becomes ds. To examine the latter possibility, clones HT. D3(1) and HT. D3(2) were co-cultivated with mouse fibroblast C127 cells for 48 h. The induction of mouse IFN-β mRNA in the C127 cells was then determined by RNase protection. There was no evidence of IFN-β mRNA expression in the mouse cells. However, the concentration of dsRNA released from lysed HDV-transfected cells may be very low and mouse C127 cells are less sensitive to exposure to dsRNA than human fibrosarcoma cells.

Exposure of HT1080 to dsRNA for prolonged periods (6 h or more) was shown to render the IFN-β mRNA response refractory to further treatment with dsRNA (Fig. 4c). It would therefore be expected that the HT. D3 clones would fail to respond to poly(rI).poly(rC) if they were persistently expressing an RNA species which was unmasked and ds; this was clearly not the case (Fig. 4a).

To examine the possibility that HDV RNA is masked by binding of the delta Ag, HT1080 clones which expressed HDV RNA in the absence of delta Ag were derived by transfection with the constructs pCMV.D2M and pRSV.D2M. RNase protection confirmed that these clones expressed HDV RNA to a level comparable to cells treated for 6 h, allowed to recover for 2 h and re-treated with poly(rI).poly(rC) for 2 h. Assays were by RNase protection. A probe for γ-actin mRNA was included to provide a loading control. The size in nucleotides of the markers (lane M) is indicated to the left.
that in HT.D3(1) and HT.D3(2). IFN-β mRNA was not expressed constitutively and was normally inducible in these cells (data not shown).

**PKR assays**

The lack of effect of IFN-α and IFN-γ treatment on the level of expression of delta Ag in H7.D cells (Fig. 2) suggests that either PKR is not activated by the presence of HDV RNA or the virus is able to inhibit the function of this enzyme. *In vitro* PKR assays were therefore performed using cellular extracts from the HT.D3 cell lines. Autophosphorylation of the 68K PKR in response to dsRNA was unaltered in the HT.D3 cells, compared to the parental HT1080, and there was no consistent evidence of significant constitutive activity of this enzyme (Fig. 5). The slightly lower inducibility of PKR apparent in HT.D3(1) cells compared with HT1080 cells in the absence of IFN and the slightly higher level with IFN, but minus dsRNA (Fig. 5), were not consistently observed and are likely to reflect slight differences in loading. Phosphorylation of the 68K protein was much weaker in cell extracts from Huh7 and H7.D and the results in these cells were therefore inconclusive.

**Discussion**

Prolonged IFN treatment of cell lines transfected with an HDV cDNA trimer does not influence the level of expression of HDV RNA or delta Ag (Fig. 1 and 2). This is consistent with a failure of activation of the IFN-inducible enzymes 2′,5′A synthetase and PKR. This could arise through an inhibition of the cellular response to IFN by a product of HDV, but the transcriptional induction of several IFN-inducible genes, including 2′,5′A synthetase and PKR, is normal (Fig. 3). The IFN-induced antiviral response of HDV-expressing cells to two cytopathic viruses, EMC and SFV, is intact (Table 1). Since IFN is known to protect cells against the cytopathic effects of EMC, at least in part, by activating 2′,5′A synthetase (Silverman *et al.*, 1982b), this assay also provides an alternative index of the activity of the 2′,5′A synthetase system. The results suggest that this system is unaffected by HDV expression. The activation of PKR is also unaffected by HDV (Fig. 5) as is the inducibility of IFN-β (Fig. 4). The expression of a low constitutive level of IFN-β in HDV-transfected cells may reflect the presence of a low level of unmasked dsRNA in these cells. Alternatively, release of HDV RNA from lysed cells may allow it to become ds in the medium and induce IFN-β in neighbouring cells. IFN-β was not expressed in co-cultivated mouse cells, but this may reflect the different sensitivity of these cells. It does not, therefore, exclude low level lysis as the reason for IFN induction in the HDV-transfected fibrosarcoma cells. Since cells exposed to dsRNA for prolonged periods fail to express IFN-β on re-exposure to dsRNA and the HDV-transfected cells retain their response to poly(rI), poly(rC), it is unlikely that they are expressing significant amounts of unmasked dsRNA.

The failure of HDV RNA to alter significantly the inducibility of IFN-β (Fig. 4) or the activation of PKR (Fig. 5) suggests that the HDV RNA is masked or does not have significant secondary structure in the cytoplasm, i.e. is predominantly ss. The delta Ag, which has been shown to bind to specific subregions of the genomic and antigenomic HDV RNAs (Chao *et al.*, 1991), may be able to prevent the RNA interacting with the dsRNA-dependent enzymes. However, expression of HDV RNA in the absence of the delta Ag does not induce IFN-β nor affect its induction by exogenous dsRNA. It is possible that HDV RNA exists in a ds form only in cellular compartments that are separated from factors responsible for IFN induction.

The exact structure of HDV RNA in vivo remains in doubt. It is unlikely that the high degree of nucleotide complementarity of the HDV genome has been retained unless it is indeed ds at some stage in the viral life cycle, although it may only exist in this form when packaged into viral particles. Examination of HDV-infected human and woodchuck liver by *in situ* hybridization suggested that the genomic RNA is around 30 times more abundant than antigenomic. Unlike the genomic RNA, the antigenomic RNA was only detectable using conditions that would denature dsRNA (Gowans *et al.*, ...
mainly in a ds form as a result of base-pairing with genomic RNA, presumably representing replicative forms of HDV RNA. Gowans et al. (1988) concluded that extensive self-annealing of HDV RNA does not occur in vivo. A separate study (Chen et al., 1986) found that a significant proportion of antigenomic RNA was resistant to digestion with RNase and again concluded that this was due to complexing with genomic RNA rather than self-annealing, since the protected RNA released a heterogeneous distribution of fragments isolated in these studies are artefacts of RNA preparation.

There is indirect evidence to suggest that limited base-pairing of the RNA is essential for successful viral replication. The RNA editing of nucleotide 1012, which changes the stop codon in the open reading frame of the small delta Ag to allow production of the long delta Ag, does not occur if mutations are created which disrupt base-pairing in this region (Casey et al., 1992; Zheng et al., 1992). Of course it remains possible that only small regions of the genome are ds and that base-pairing elsewhere is somehow disrupted.

The above data suggest that a direct effect of endogenous or therapeutic IFN on HDV RNA replication or delta Ag production is unlikely. This may reflect the failure of activation of 2', 5'A synthetase and PKR, or could conceivably be due to the absence in HDV-infected (or transfected) cells of other IFN-inducible antiviral factors. However, treatment of patients suffering from chronic hepatitis delta with IFN-z is associated with a reduction in the level of HDV RNA in the serum (Farci et al., 1989; Porres et al., 1989). The mechanism of this reduction remains unclear. It has been shown in animal models that immunosuppression with cyclosporin A leads to increased levels of HDV RNA, suggesting that the immune system is important in the control of HDV expression (Karayiannis et al., 1992). Interferon therapy may therefore act on HDV by enhancing the cytotoxic T cell response against HDV-infected cells. It is also possible that it acts through inhibition of the helper virus HBV or through an effect on early and late events in the viral life cycle, such as viral entry or release. Work to examine these possibilities is in progress.

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References


