Hepatitis delta virus proteins repress hepatitis B virus enhancers and activate the alpha/beta interferon-inducible MxA gene

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection is an important worldwide cause of acute and end-stage liver diseases, including cirrhosis and hepatocellular carcinoma. Two billion individuals have been infected with HBV and more than 400 million are chronic carriers. Hepatitis delta virus (HDV) is a naturally defective RNA virus, a satellite of HBV, which requires HBV surface antigen (HBsAg) for packaging and transmission. Between ten and twenty million HBV-infected individuals are thought to be co-infected with HDV, leading to disease chronicity and worsening histological lesions.

Concomitant HBV/HDV infection often results in inhibition of HBV replication both in human patients (Arribas et al., 2005; Colombo et al., 1991; Govindarajan, 1990; Jardi et al., 2001; Lee et al., 1987; Pastore et al., 1990) and in animals (Negro et al., 1989). One in vitro transient co-transfection model of HBV DNA and HDV-expressing plasmids in Huh-7 cells has been described (Wu et al., 1991). In that study, the authors showed remarkably reduced levels of 3.5 and 2.1 kb HBV RNAs involving the delta proteins. However, the mechanisms of such inhibition remain unknown and, to our knowledge, have not been explored further.
The HBV genome consists of a 3.2 kb circular, partially double-stranded DNA genome. HBV replication is governed by four promoters (pre-S, S, core and X) and two enhancer elements, Enh1 and Enh2 (Lo & Ting, 1994; Moolla et al., 2002; Park et al., 1997; Yuh & Ting, 1990). It has been hypothesized that the two enhancers, each regulating a different set of transcripts, are functional at different stages of the HBV life cycle. Enh1 is thought to be active at an early stage in infection, while Enh2 is activated later, concomitant with the silencing of Enh1, providing strong evidence for the role of Enh1 in regulating global and temporal HBV gene expression (Doitsh & Shaul, 2004). Enhancer activity results from the combined action of both ubiquitous and liver-enriched transcription factors, including hepatocyte nuclear factors (HNF)-3 and -4, the signal transducer and activator of transcription (STAT)-3 and the CCAAT/enhancer binding protein (C/EBP) (Kosovsky et al., 1996; Lai & Ting, 1999; Moolla et al., 2002; Park et al., 1997; Waris & Siddiqui, 2002).

The HDV genome is a 1679 nt single-stranded, negative-sense, circular RNA that encodes two proteins from one open reading frame: the small (or p24) and large (or p27) delta antigen. p27 is synthesized later in the viral cycle, after an editing event affecting the RNA template that is carried out by adenosine deaminase acting on RNA-1 (George & Samuel, 1999; Hartwig et al., 2006; Patterson & Samuel, 1995; Wong & Lazinski, 2002). p24 is required for HDV genome replication, while p27 inhibits RNA replication and directs virion assembly and secretion (for reviews see Lai, 2005; Taylor, 2006 and references therein). Several in vitro studies have shown that p27, but not p24, was able to transactivate expression of a variety of heterologous promoters, including the serum response factor/sacura response element (Goto et al., 2000, 2003), N-myc2 promoter and several upstream stimulatory elements such as activating transcription factor, CAAT box, specificity factor 1, activating protein 1 and even the HBV promoters preS, S and C (Wei & Gamem, 1998).

In an attempt to analyse the mechanisms of HBV inhibition by HDV, we questioned whether HDV proteins could act on HBV enhancers, and/or on one or several transcription factors involved in enhancer function. Indeed, such a mechanism has been described in hepatitis C virus (HCV)/HBV co-infection. HCV capsid protein was shown to have a direct trans-suppressive effect on HBV transcription and replication, depending on its phosphorylation state on serine residues 99 and 116 (Schuttler et al., 2002; Shih et al., 1993). Moreover, we had earlier demonstrated that the alpha interferon (IFN-α)-inducible MxA protein is involved in the inhibitory action of IFN-α towards HBV replication (Gordien et al., 2001; Peltekian et al., 2005). Therefore, we wondered whether p27 could activate the MxA gene as an additional mechanism of HBV inhibition by HDV.

**METHODS**

**Plasmids and oligonucleotides.** Plasmids expressing p24 and p27 were constructed using HDV p24 and p27 sequences derived from a cloned cDNA isolated from an HDV genotype-1-infected patient. We subcloned this cDNA into BamHI and HindIII sites of the pCDNA-3 plasmid (Invitrogen) and obtained pCDNA-3-p24 (or p24) and pCDNA-3-p27 (or p27). Plasmid pSVL-D3 was kindly provided by John Taylor (Fox Chase Cancer Center, Philadelphia, USA). Plasmid pTHBV1.1 has been described previously (Guidotti et al., 1995). Luciferase constructs driven by Enh1 (PENS-Luc) or control (PS-Luc), pFPV3Luc, pFPV‘4Luc and p’SLuc (Kosovsky et al., 1996) were gifts from Aleem Siddiqui (University of Colorado Health Sciences Center, USA). Enh2 plasmid constructs PEII-1.Luc, PEII-2.Luc and TATA-Luc were gifts from Hisashi Ishida (Osaka University, Japan) (Ishida et al., 2000). pSpLuc, SpLuc, CpLuc and XpLuc were obtained from Alan McLachlan (The Scripps Research Institute, USA) (Raney et al., 1990). The pMxA 550 Luc has been described elsewhere (Rossmorduc et al., 1999). The commercial pCH110 plasmid expressing β-galactosidase (Pharmacia biotech) was used to monitor transfection efficiency. STAT-3-specific oligonucleotide and mutant forms were purchased from Proligo (Sigma) according to footprint five (FPV)/STAT-3 DNA sequences (Kosovsky et al., 1996; Leong et al., 2003).

**Cell culture and transfection.** Huh-7 cells (2 × 10⁵ per six-well tissue culture dish or 2.4 × 10⁶ per 10 cm dish) were cultured at 37 °C in Dulbecco’s modified Eagle’s medium (Gibco) supplemented with 10% fetal bovine serum and penicillin (100 U ml⁻¹)–streptomycin (50 U ml⁻¹). Transfections were performed using the superfect transfection reagent (Qiagen) according to the manufacturer’s instructions. In a typical transfection experiment, the total DNA transfection complex was 2 µg per six-well dish or 10 µg per 10 cm dish. Transfections with pSVL-D3 were performed as follows: Huh-7 cells (0.45 × 10⁶) were seeded in 10 cm dishes and transfected with 7 µg pSVL-D3 plasmid and 3 µg of the TATA-Luc plasmid (as transfection internal control). The medium was changed every 3 days.

**MTT-based cytotoxicity assays.** The MTT colorimetric assay using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide was performed to evaluate a possible cytotoxic effect of delta protein expression in transfected cells. Briefly, Huh-7 cells were plated at 30 000 per well in 24-well plates, then transfected with 1 µg DNA, 0.9 µg pCDNA-3-, p24- or p27-expressing plasmids and 0.1 µg pCH110 plasmid (as transfection internal control). Two days post-transfection (p.t.), cells were treated with MTT (400 µl per well of a 0.5 mg ml⁻¹ solution of MTT). Cells were incubated further for 1 h at 37 °C. After discarding the supernatant, cells were solubilized with 400 µl per well of DMSO. The absorbance in each 24-well dish was measured at 570 nm using an automatic microplate spectrophotometer (MRX, Dynex Technologies).

**Analysis of p24 and p27 expression by Western blot.** Briefly, at days 3, 6, 9 and 12 after transfection with pSVL-D3 construct or at day 3 after transfection with p24- or p27-expressing plasmids, cells were lysed in RIPA buffer (50 mM Tris pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM NaF, 1 mM orthovanadate) containing protease inhibitors (Complete Protease Inhibitors Cocktail Tablets, Roche). The protein content was measured by using the Bradford assay (Bio-Rad). Protein (40 µg) was boiled in 2 × Laemmli buffer and resolved by SDS-PAGE (12%). After transfer onto a nitrocellulose membrane, blots were incubated with anti-delta antibodies (1:1000) obtained from the plasma of an infected patient (provided by Camille Sureau, Institut National de la Transfusion Sanguine, Paris, France). Stripped membranes were reprobed using a monoclonal anti-actin antibody (1:1000) (Cell Signaling) for normalization. Detection was carried out using a horseradish peroxidase-conjugated secondary antibody (1:8000) and subsequent chemiluminescent development (ECL Kit, Amersham).
Analysis of HBV-related antigens. HBsAg and HBV e antigen (HBeAg) were semi-quantified on diluted cell lysates and culture supernatants by ELISA (Ortho Vitro, Ortho Clinical Diagnostic or Enzygnost 5.0, Dade Behring).

Preparation of nuclear extracts. Transfected cells were lysed for 30 min on ice in hypotonic buffer [20 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM Na3VO4, 1 mM EDTA, 10 % glycerol, 1 mM PMSF, protease inhibitors, 1 mM dithiothreitol (DTT), 0.2 % NP-40]. After centrifugation at 15,000 g for 15 min at 4 °C, the nuclear pellets were resuspended in high-salt buffer (hypotonic buffer supplemented with 20 % glycerol and 420 mM NaCl) and incubated for 1 h at 4 °C. Supernatants were collected and stored at −80 °C until assayed.

Biotinylated oligonucleotide pull-down and immunoblot assays. The 5′-end-biotinylated oligonucleotide FPV/STAT-3 wild-type or mutated form was incubated with nuclear lysates from cells that were untreated (negative control) or treated with 30 ng interleukin (IL)-6 ml−1 for 30 min (positive control) or transfected with pCDNA-3, p24 or p27 plasmids. Nuclear extracts (200 μg) were incubated at 4 °C for 20 min in binding buffer [20 mM HEPES (pH 7.6), 200 μM EDTA, 0.1 M NaCl, 0.1 M KCl, 10 mM MgCl2, 8 mM spermidine, 4 mM DTT, 0.2 mg BSA ml−1, 5 % glycerol, 8 % Ficoll type 400-DL] with 2 μg biotinylated oligonucleotide. The protein–DNA complexes were captured with 50 μg avidin–Sepharose beads (Pierce) by incubation for 2 h at 4 °C, followed by washing three times in TBS and once in TBS−0.05 % Tween. Bound proteins were then separated by using SDS-PAGE (8 %) and subjected to immunoblotting using anti-STAT-3 or anti-delta antibodies.

 Luciferase assays. Two days after co-transfection with pCDNA-3, p24 or p27 and different luciferase constructs, cells were lysed in NP-40 buffer [10 mM Tris (pH 8), 100 mM NaCl, 1 mM EDTA and 1 % Nonidet P-40] containing protease inhibitors. A typical luciferase assay was performed on 50 μl clarified supernatant using an LB 9501 Lumat Berthold luminometer. Each lystate was assayed for β-galactosidase activity. All experiments were performed in duplicate and at least three times.

MxA RNA quantification. Two or three 10 cm dishes were seeded with 2.4 × 106 Huh-7 cells and transfected with 10 μg pCDNA-3−, p24- or p27-expressing plasmids, using Superfect reagent. Twenty-four hours after transfection, cells were washed and total RNA was extracted from cell pellets using the RNeasy kit (Qiagen) according to the manufacturer’s instructions. MxA RNA quantification was performed in a two-step method. Total RNA was quantified on Mx3000P (Stratagene) using the Quant-iT RNA assay kit (Invitrogen). Then, specific reverse transcription (using the AffinityScript QPCR cDNA synthesis kit, Stratagene) and amplification for the MxA gene (using the Brilliant SYBRgreen QPCR Master Mix, Stratagene) were performed. The 25 μl PCR mixture consisted of 2 × Master Mix, 30 nM DYER/ROX, specific MxA primers (2.5 μM each) and 2 μl cDNA. The amplification program on Mx3000P was as follows: 95 °C for 10 min and 40 cycles of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C. After amplification, dissociation curves were generated using the program recommended by the suppliers. A standard curve for MxA gene quantification was generated after reverse transcription and amplification of QPCR human reference total RNA (Stratagene). Final results are expressed as copy numbers of MxA gene per μl and converted into copy numbers of MxA gene per ng RNA given by the ratio (copy number of MxA gene per μl)/(ng of RNA per μl).

Virus yield reduction assays. Huh-7 cells (105) transfected with pCDNA-3, p24 or p27 were untreated or treated with 500 or 1000 IU ml−1 IFN-α-2a (referred to as 2aIFN; Roche Products) or IFN-β-1a (Shering-Plough) for 20 h and then infected with vesicular stomatitis virus (VSV) at an m.o.i. of 0.1 for 1 h. After discarding the viral inoculum, fresh medium was added and cells were further incubated for 16 h at 37 °C. The supernatants were collected and frozen at −80 °C until assayed. Vero cells were used for titration in plaque assays in 1 % methylcellulose containing medium. The p.f.u. ml−1 was counted for each point after fixation with 10 % formaldehyde in PBS for 15 min and subsequent staining with crystal violet (Rossmorduc et al., 1999).

Statistical analysis. Data are shown as mean ± SD. Statistical analysis was performed using Student’s t test. A P value <0.05 was considered to be statistically significant.

RESULTS

Expression of p24 and p27
In order to analyse the level of expression of our p24 and p27 plasmid constructs, we compared p24 and p27 production by these plasmids to that from the well-described pSVL-D3 plasmid by using Western blot analysis at days 3, 6, 9 and 12 p.t. As shown in Fig. 1, similar levels of p24 and p27 were obtained at days 3 and 12 p.t. with plasmids expressing HDV proteins and pSVL-D3, respectively. Using the MTT-based cytotoxicity assay, no cytotoxic effect was observed in cells expressing p24, p27 or pCDNA-3 (data not shown).

Fig. 1. Detection and comparison of p24 and p27 levels in Huh-7-transfected cells. Immunoblot analysis of protein lysates from Huh-7 cells transfected with pSVL-D3 plasmid at days 3, 6, 9 and 12 p.t. and with pCDNA-3, p24 and p27 at day 3 p.t. Human antibodies against HDV were used and secondary antibodies were detected with ECL. Arrows indicate specific protein bands. Results are representative of at least two different experiments.
p24 and p27 inhibit HBV protein synthesis in vitro

Co-transfection experiments were performed using pTHBV1.1 plasmid along with p24- or p27-expressing plasmids. Synthesis and accumulation of HBsAg and HBeAg were monitored by ELISA of cytoplasmic extracts and culture supernatants of transfected cells. Kinetic analyses performed from day 2 to day 10 showed a four- to fivefold reduction in synthesis and secretion of HBsAg (Fig. 2). Identical results were obtained for HBe/c antigen (data not shown).

p24 and p27 strongly repress HBV Enh1 and Enh2

To analyse the mechanisms of inhibition of HBV replication, we investigated the action of p24 and p27 on HBV enhancers. We co-transfected p24 or p27 plasmids with Enh1 and Enh2 luciferase constructs (see schematic diagrams in Fig. 3). In order to eliminate a possible direct effect of p24 or p27 on simian virus 40 early promoter or TATA promoters of these constructs, the results shown were calculated as the ratio of normalized luciferase values obtained with constructs containing and lacking the specific enhancer sequence. Luciferase values obtained with plasmids lacking enhancer-specific sequence were taken to be 1. We found a strong reduction in Enh1 activity to 40 and 20 % by p24 and p27, respectively (Fig. 3a). Likewise, repression of Enh2 activity down from 60 to 50 % and 40 to 20 % was observed with p24 and p27, using, respectively, pEII.1 and pEII.2 [lacking the negative regulation element (NRE) motif] (Fig. 3b). Both p24 and p27 repressed Enh2 activity independently of the NRE.

Involvement of FPV within the Enh1 core region in Enh1 inhibition

It has been shown that, upon infection, Enh1 plays a dominant role in regulating global and temporal HBV gene expression and that it is also involved in Enh2 activation (Bock et al., 2000; Doitsh & Shaul, 2004). Enh1 function is exerted through binding of both ubiquitous and liver-enriched transcription factors, mainly in the central ‘core’ region. In particular, binding of STAT-3/HNF-3 to FPV in this region (Waris & Siddiqui, 2002) has been described as playing a major role. Thus, we performed co-transfection experiments with two target luciferase reporter vectors: pFPV3Luc (comprising three FPV motifs in front of the luciferase gene) and p’SLuc or pFPV’4Luc mutated, respectively, in a STAT-3 or HNF-3 motif. Surprisingly, we observed completely opposite results by p24 and p27 (Fig. 4). p27 activated pFPV3Luc 10-fold. This activation was reduced by half with pFPV’4Luc plasmid sharing a mutation on the HNF-3 motif (data not shown), indicating that the HNF-3 site was partly involved in this activation. No significant activation was seen with p’SLuc, confirming the specificity of observed effects involving the FPV/STAT-3 binding element. However, no activation was found with p24. To further investigate these mechanisms, oligonucleotide in vitro pull-down experiments were performed using the 5’ biotinylated FPV/STAT-3 sequence. We found that p27, and p24 to a lesser extent, induced STAT-3 binding on FPV. These latter results are specific as there was no or very weak binding when we used a mutated STAT-3 probe. Moreover, after stripping and reprobing the blots with anti-HDV antibodies, we found that both p24 and p27 were present in the protein complex bound to either FPV/STAT-3 wild-type or mutant (Fig. 5). Next, we co-transfected p24- or p27-expressing plasmids with the Xp luciferase plasmid comprising the overlapping X promoter/3’ end of Enh1, but lacking both the 5’ end modulator and the central core region. We observed 2.5-fold activation by p27 but not by p24 (data not shown).
p27 activates transcription of the MxA gene and potentiates an IFN-α/β effect

As HDV replication involves the action of IFN-α-induced proteins, such as PKR (Chen et al., 2002), and as we showed that the MxA protein was involved in inhibition of HBV replication (Gordien et al., 2001), we questioned whether p24 and/or p27 could interfere with this pathway. Co-transfection experiments were performed using the pMxA 550 luciferase construct. We found a threefold induction of the MxA promoter gene by p27, while p24 had no effect. In addition, after IFN-α treatment of transfected cells, we also found a threefold induction of IFN-α stimulation upon the MxA promoter in p27-expressing cells (Fig. 6). These results were further confirmed by MxA RNA quantification. We found a fourfold increase in the MxA RNA level and a 1.5- to 2-fold induction after treatment with 500 or 1000 IU IFN-α/β ml⁻¹ in p27-transfected cells (Fig. 7). Moreover, using a virus yield reduction assay, we found that p27 potentiated a protective effect against lysis of IFN-α and -β on VSV-infected cells. Indeed, after IFN-α or -β treatment (500 or 1000 IU ml⁻¹) of cells expressing p27, the p.f.u. ml⁻¹ decreased to less than 20% compared with that obtained in cells transfected with pCDNA-3 (considered to be 100%) (Fig. 8). Similar, although weaker, results were observed with p27 without IFN treatment (data not shown). Taken together, these results clearly show the involvement of MxA
and IFN-α/β in the mechanisms of inhibition of HBV replication by p27.

**DISCUSSION**

Inhibition of HBV replication by HDV is frequently observed in co- or superinfection in patients and animal and cellular models via mechanisms not yet elucidated. Wu et al. (1991) clearly demonstrated this inhibition in an in vitro cell culture system, in which both HBV and HDV are able to replicate. Moreover, the authors clearly showed that HDV delta antigen alone could suppress expression of HBV RNAs (3.5 and 2.1 kb) as well as HBV virion release into the culture medium (Wu et al., 1991). However, the mechanisms of this inhibition have not been described.

In this study, we sought to address possible mechanisms for this inhibition by the delta proteins. We used an in vitro model of transient transfection in Huh-7 cells with plasmids expressing the small (p24) or large (p27) delta protein. First of all, we showed that p24 and p27 strongly inhibit HBV enhancers. This repression was more pronounced for p27 and higher against Enh1 (60 to 80 % for Enh1 versus 40 to 60 % for Enh2, for p24 and p27, respectively), which plays a predominant role in the high level of replication of HBV (Doitsh & Shaul, 2004).

We then tried to explore the level of this inhibition against Enh1. We searched for action of the delta proteins on the Enh1 central core region, which is thought to be responsible for its main activity, notably via binding of STAT-3 and HNF-3 transcription factors to the FPV element within this region (Waris & Siddiqui, 2002). We co-transfected the FPV luciferase construct (pFPV3Luc) or its mutated forms with either p24 or p27 plasmid. We found that p27 activated pFPV3Luc (wild-type) and pFPV4Luc (mutated at the HNF-3 site) luciferase constructs by 10- and 5-fold, respectively, while little or no action was seen with p9SLuc (mutated on both STAT-3 and HNF-3 sites). In contrast, p24 showed no effect.

Moreover, when we used the X promoter luciferase plasmid construct (XpLuc) (Raney et al., 1990) comprising the 3′ overlapping Enh1/X promoter region, but lacking both the 5′ end modulator and the central core regions of Enh1, we found that p27 induced a 2.5-fold activation, while p24 had no effect. This first set of results indicated that p24 and p27 acted by different mechanisms and at different sites of Enh1. According to these results, p27 might actually act on the 5′ end modulator and the central core regions of Enh1, because neither FPV nor the 3′ overlapping Enh1/X promoter seemed to be involved in this inhibition. To further address this issue, we performed oligonucleotide in vitro pull-down experiments using this FPV wild-type or mutated sequence. We repeatedly observed a binding of
STAT-3 to FPV sequences in p27-expressing cells, and also weakly in p24-expressing cells. Moreover, p24 and p27 were present in the protein complex bound to FPV. It might be hypothesized that p24, which is first synthesized after HDV infection, may block Enh1 activity by interfering with STAT-3/HNF-3 binding to its main central core region, thus disturbing this binding. p27 is synthesized later in the viral cycle and may maintain global HBV suppression, probably by acting on the 5' modulatory region of Enh1, while activating HBV promoters (repeatedly by two- to fourfold in our experiments; data not shown) in order to provide HBV envelopes necessary for HDV virion assembly. In some experiments, however, binding was also observed to the STAT-3-mutated oligonucleotides in oligonucleotide in vitro pull-down experiments. This could be due to delta proteins that are charged and contain a binding site for nucleic acids in their sequence (Taylor, 2006). Such a non-specific mechanism cannot be excluded in the overall mechanisms of enhancer inhibition by the delta proteins. Nevertheless, we failed to find direct interactions between HDV proteins and STAT-3 using classical immunoprecipitation methods. It remains to be demonstrated whether p24 or p27 binds to one or several proteins of the enhanceosome. Further experimental approaches are needed to fully address this issue.

Considering Enh2 inhibition, we found that the 5' NRE motif was not implicated in its repression, since pEII.2-lacking NRE was repressed to an even greater extent than pEII.1 (2.5-fold in our experiments). We thus hypothesize that p24 and p27 directly repress Enh2, possibly through its z-box, known to bind C/EBP proteins, a family of highly conserved leucine zipper-type (bZIP) DNA-binding proteins expressed at high levels in the liver (Lai & Ting, 1999). Among them, the E4BP4 protein was shown to repress Enh2 by binding to the z-box via its leucine zipper domain. Moreover, in another study, co-transfection of Huh-7 cells with E4BP4 and a more-than-unit-length HBV genome plasmid showed suppression of HBV replication (Lai & Ting, 1999). Interestingly, C/EBP motifs have also been described within Enh1: two in the 5' modulator region and one in the 3' domain (Moolla et al., 2002). This suggests a putative mechanism of inhibition by both p24 and p27 involving the C/EBP elements present in both Enh1 and Enh2. In line with this hypothesis, disturbance of interactions between Enh1 and C/EBP transcription factors was shown to be the mechanism of inhibition of HBV replication by the Phyllanthus amarus plant (Ott et al., 1997). Alternatively, p24 and p27 could act by recruiting E4BP4, known to bind and repress Enh2 (Lai & Ting, 1999). Such a mechanism needs to be further elucidated.

On the other hand, we showed that the IFN-α-inducible antiviral MxA protein was involved in mechanisms of HBV inhibition by HDV. We demonstrated elsewhere that MxA
inhibits HBV replication (Gordien et al., 2001; Peltekian et al., 2005) and we now show that p27 is able to activate transcription from the MxA gene promoter and to potentiate the IFN-α effect upon this promoter. Using a functional assay, we confirmed this action, since we observed a protective effect of p27 against VSV-mediated cell lysis. This global effect of p27 on IFN-α might also be involved in HBV suppression by HDV. Indeed, it had been demonstrated earlier, in a cellular model, that IFN-α inhibited HBV replication by reducing transcription of viral genes driven by the HBV enhancers (Tur-Kaspa et al., 1990). In a previous model, Baca et al. (1994) showed that HIV-1 was able to induce expression of antiviral IFN-α-stimulated genes 2′−5′ oligo-adenylate synthetase and MxA as a means of protecting cells against other superinfecting viral pathogens (Baca et al., 1994). Such a mechanism may be suggested in an HBV/HDV context, and the effects could be exerted in an autocrine or paracrine manner. Finally, our study provides an additional example of subversion of the immune system by a human virus.

In summary, we demonstrate here that HBV inhibition by HDV is exerted by at least two mechanisms: (i) direct trans-repression of HBV Enh1 and Enh2 by a mechanism yet to be precisely defined and (ii) trans-activation of the IFN-α-inducible anti-HBV MxA gene and potentiation of IFN-α/β effects. To our knowledge, this study is the first to provide approaches for understanding the mechanisms of inhibition of HBV by its satellite HDV.

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