Hepatitis B virus polymerase inhibits the interferon-inducible MyD88 promoter by blocking nuclear translocation of Stat1

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Previous studies have suggested that hepatitis B virus (HBV) blocks expression of the alpha interferon (IFN-α)-inducible myeloid differential primary response protein (MyD88) gene. To study the molecular mechanism(s) of the inhibition of MyD88 expression by HBV, MyD88 promoter reporter plasmids and vectors expressing different HBV viral proteins were constructed. Co-transfection experiments showed that IFN-induced MyD88 promoter activity was inhibited by HBV polymerase expression in a dose-dependent manner and that the terminal protein (TP) domain of HBV polymerase was responsible for this antagonistic activity. Analysis of site mutants showed that the region targeted by the polymerase protein contained the signal transducer and activator of transcription (Stat) binding site. Chromatin immunoprecipitation analysis showed that the IFN-induced DNA-binding activity of Stat1 was affected. Further study demonstrated that the HBV polymerase protein inhibited the Stat1 nuclear translocation induced by IFN-α, but did not induce Stat1 degradation nor interfere with its phosphorylation. In addition, HBV polymerase could inhibit the transcriptional activity of other IFN-stimulated response element-driven promoters and the expression of interferon-stimulated genes (ISGs), such as Stat1 and ISG15. In summary, these results indicate that HBV polymerase is a general inhibitor of IFN signalling and can inhibit IFN-inducible MyD88 expression by inhibiting the activity of the MyD88 promoter through blocking the nuclear translocation of Stat1.

INTRODUCTION

Alpha interferon (IFN-α) is a pleiotropic cytokine with immunomodulatory and antiviral activities. With regard to the latter, it induces a condition termed the antiviral state, in which host cells are especially resistant to infection by viral pathogens. This antiviral state is mediated through many IFN-regulated cellular proteins (Staeheli, 1990; Thomas et al., 2003). However, when patients suffering from chronic hepatitis B are treated with IFN-α, only 30–40% show clearance of hepatitis B virus (HBV) serum markers and normalization of liver function (Karayianis, 2003; Krastev, 2006). This indicates that HBV, as a successful pathogen, may have developed specific mechanisms that antagonize the IFN response.

In order to study the interplay between HBV and the IFN system, we previously used cDNA microarrays to examine the transcriptional changes in an HBV DNA-transfected cell line (HepG2.2.15 cells) and its parental cell line (HepG2 cells) after treatment with IFN-α. The results showed that the transcription of MyD88 was significantly reduced in HepG2.2.15 cells (Xiong et al., 2003). Using antiviral drugs (lamivudine or adefovir) to cure HepG2.2.15 cells of HBV infection, we were able to restore transcription of the MyD88 gene induced by IFN-α (data not shown). These results indicated that HBV might inhibit IFN-α-inducible MyD88 gene expression. MyD88 is a critical component in the signalling cascade through toll-like receptors (TLRs) (Oda & Kitano, 2006), and our further work has demonstrated that MyD88 has antiviral activity against HBV (Xiong et al., 2004). All of these results suggested that the interplay between MyD88 and HBV might contribute to the establishment of viral persistence, providing a rationale for further studies.

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In this study, we investigated the mechanism(s) of inhibition of IFN-inducible MyD88 expression by HBV viral protein(s). The results showed that HBV polymerase was the main suppressive factor, and the terminal protein (TP) domain was identified as the region responsible for this antagonistic activity. Deletion of the TP domain of HBV polymerase significantly weakened its ability to block IFN-induced MyD88 promoter activity. Further investigation showed that HBV polymerase reduced the IFN-induced DNA-binding activity of Stat1 and inhibited Stat1 nuclear translocation, but did not interfere with Stat1 phosphorylation or induce Stat1 degradation. Furthermore, HBV polymerase expression could inhibit both the transcriptional activity of IFN-stimulated response element (ISRE)-driven promoters and the expression of interferon-stimulated genes (ISGs) such as Stat1 and ISG15, indicating that the HBV polymerase could be a general inhibitor of IFN signalling by inhibiting Stat1 nuclear translocation.

**METHODS**

**Cell lines.** Huh7 human hepatocellular carcinoma cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10 % fetal calf serum and penicillin (100 IU ml⁻¹) and streptomycin (100 µg ml⁻¹) (Gibco-BRL), in a humidified atmosphere at 37 °C with 5 % CO₂. When indicated, cell cultures were treated with 1000 IU recombinant human IFN-α (Calbiochem).

**Plasmids and antibodies.** Plasmid pHBV3.8, encoding the whole transcript of HBV DNA (adr subtype), was constructed as a 1.2-copy insert of the full-length HBV genome into vector pBS+ (Stratagene) (kindly provided by Professor Wang Yuan, Institute of Biochemistry, Academia Sinica, Shanghai). Coding regions of the HBV core (HBc) and X (HBx) proteins were generated by PCR amplification of plasmid pcDNA3.1-3Flag-pol (expressing Flag-tagged HBV polymerase) and cloned into the pEGFP-C2 mammalian expression vector. Plasmid pcDNA3.1-3Flag-pol (expressing Flag-tagged HBV polymerase) was a gift from Professor Y. M. Wen (Fudan University, Shanghai). For truncated polymerase plasmids, the corresponding DNA fragments were obtained from pHBV3.8 by PCR using primers specific for the fragments, and then subcloned in frame to a Flag-tagged pcDNA3.1 vector. Reporter plasmid pISRE-Luc was purchased from Stratagene. All of the constructs were handled using standard molecular cloning techniques and verified by DNA sequencing.

Antibodies to Stat1, phospho-Stat1(Tyr701), phospho-Stat1(Ser727), anti-ISG15 and anti-lamin A/C were obtained from Cell Signalling; anti-ISG15 and pMyD-mStat, respectively (Fig. 1b).

**MyD88 promoter plasmids.** Homology analysis of the binding sites of known transcription factors in this promoter sequence revealed that the fragment from −70 to −50 bp had the following sequence: GCTTCTCCGAAAGCGGAAGC. The Stat motif is shown in bold letters; the interferon regulatory factor (IRF) motif is underlined (Fig. 1a). This sequence was generated by oligonucleotide synthesis of four subfragments with a KpnI sequence at the extreme 5’ end and an XhoI site at the extreme 3’ end. The four fragments were annealed and ligated into the pGL3 luciferase vector (Promega) upstream of the simian virus 40 promoter to obtain the pMyD-Luc plasmid.

Mutations were obtained and controlled based on the TRANSFAC database. For the IRF site, the sequence GAAA was replaced by CTCT, whilst for mutation of the Stat site, the sequence TTCCTC was replaced by AAGAC. The mutant promoter constructs were again generated by having oligonucleotide subfragments synthesized, annealed, ligated, and cloned into the pGL3 luciferase vector; these were named pMyD-mIRF and pMyD-mStat, respectively (Fig. 1b).

**Reporter gene assays.** Transient transfection of Huh7 cells was performed using FuGENE 6 (Roche) according to the manufacturer’s instructions. Cell monolayers, 60–70 % confluent, in 24-well plates were transfected with 100 ng MyD88 promoter-driven firefly luciferase reporter plasmid pMyD-Luc, 10 ng constitutive expression plasmid Renilla luciferase reporter vector pRL-tk (Promega) and the indicated amounts of the HBV protein expression plasmids together. At 36 h post-transfection, cells were stimulated with or without (mock-treated control) 1000 U human IFN-α 10⁻¹¹ M. Twelve hours after IFN treatment, cells were harvested and analysed for luciferase activity. Luciferase assays were performed using the Promega Dual-Luciferase assay system according to the manufacturer’s instructions. Relative firefly luciferase activity was normalized to relative Renilla luciferase activity.

**Western blot analysis.** Cell lysates were prepared in SDS sample buffer [62.5 mM Tris/HCl (pH 6.8), 2 % SDS, 10 % glycerol, 50 mM dithiothreitol, 0.1 % bromophenol blue] containing a cocktail of protease inhibitors (Roche). Similar amounts of protein were loaded onto the gel, separated by SDS-PAGE and transferred to a nitrocellulose membrane (Roche). The membrane was blocked with PBS (0.05 % Tween 20 in PBS) containing 5 % skimmed milk and then incubated overnight with the primary antibody. Membranes were washed three times in PBS and then incubated with horseradish peroxidase-conjugated secondary antibody for 3–4 h.

![Image](https://via.placeholder.com/150)

**Fig. 1.** (a) Promoter sequence of the MyD88 gene. The translation start site is in bold and underlined. Potential transcription regulatory sequences are in italic. (b) Schema of the cloned MyD88 promoter and mutants.
After further washing with PBST, proteins were visualized using an ECL Western blotting system (Western Lightning; Perkin Elmer).

**ELISA.** Cell culture supernatants were collected and the levels of HBV surface antigen (HBsAg) and e antigen (HBeAg) in the supernatants were measured using a standard ELISA (Sino-American Biotechnology).

**Chromatin immunoprecipitation (ChiP) assay.** ChiP analysis was carried out using a commercially available kit (Upstate Biotechnology) and polyclonal antibodies against Stat1 (Santa Cruz Biotechnology). Briefly, cells were cross-linked with 1% formaldehyde and lysed, and the chromatin was sheared by sonication. Soluble chromatin was then incubated overnight at 4°C with or without antibody against Stat1. After reversing the cross-linking, DNA isolated from immunoprecipitated material was amplified by PCR using primers specific for the MyD88 promoter: 5'-CTTTTGCTGGGGGCTCCAGATT-3’ and 5’-CTCTGCCAGGCTCCTCTTTCT-3’ (105 bp product). Negative-control primers flanked a region of genomic DNA between the glyceraldehyde-3-phosphate dehydrogenase gene and the chromosome condensation-related SMC-associated protein (CNAP1) gene. The sequences were: 5’-ATGGTTTGCCACTGGGGATCT-3’ and 5’-TGCAAGACGCCCTAGGGGAAGA-3’ (174 bp product). One per cent of the sheared chromatin was used as a control to quantify the amount of DNA present in different samples. The PCR products were visualized on an ethidium bromide-stained gel.

**Immunofluorescence.** Huh7 cells were mock transfected or transfected with plasmids expressing the indicated virus proteins. Cells were then treated with IFN-α (1000 U ml⁻¹) for 1 h at 36 h post-transfection. The cells were fixed and permeabilized. After incubation with primary antibodies (rabbit anti-Stat1 antibody or mouse anti-Flag antibody), the cells were incubated with fluorescently labelled secondary antibodies: goat anti-rabbit–Cy3 (Jackson) and goat anti-mouse IgG–FITC (Santa Cruz). The expression and location of endogenous Stat1 (red) and polymerase proteins (green) were observed under a fluorescence microscope.

**Subcellular fractionation assay.** Cytoplasm and nuclear fractions were obtained as described by Rahmouni et al. (2005). A total of 5 x 10⁶ cells was resuspended in ice-cold hypotonic buffer [42 mM KCl, 10 mM HEPES (pH 7.4), 5 mM MgCl₂, 1 mM Na₃VO₄ and EDTA-free protease inhibitor cocktail] and incubated on ice for 15 min. Cells were then sheared by five passes through a 30-gauge needle. The lysates were centrifuged at 500 g for 10 min. The supernatant (cytosol) was collected and the pellet of nuclear material was washed three times in hypotonic buffer and then collected. Extracts were analysed by Western blotting using anti-Stat1, anti-Flag (polymerase), anti-lamin (nuclear) and anti-La (cytoplasmic) antibodies.

**Statistical analysis.** All results were confirmed in multiple independent experiments in duplicate or triplicate within each experiment. Densitometry data were analysed using Student’s t-test and expressed as mean ± SEM. A value of P<0.05 was considered to be statistically significant.

**RESULTS**

**HBV inhibits IFN-α-inducible MyD88 expression**

To investigate the possible mechanisms by which HBV interacts with the IFN system, we first studied the expression of MyD88 protein induced by IFN-α in the presence or absence of HBV viral replication and protein expression. The kinetics of IFN-α-induced expression of the MyD88 gene in Huh7 was analysed using 1000 IU IFN-α ml⁻¹. As shown in Fig. 2(a), upon induction with IFN-α, an increase in the expression of MyD88 was observed, which peaked at 12 h. The protein expression of MyD88 in Huh7 cells was dose-dependent: increasing the dose of IFN-α led to increased expression of MyD88 with a maximal response at 2000 IU IFN-α ml⁻¹ (Fig. 2b).

To examine the effect of HBV on the expression of MyD88 protein, we transiently transfected Huh7 cells with different.

![Fig. 2. HBV inhibits IFN-inducible MyD88 expression in Huh7 cells. (a, b) Huh7 cells were treated with 1000 IU IFN-α ml⁻¹ for the indicated time periods (a), or treated with the indicated dose of IFN-α for 12 h (b). Western blot analysis was carried out to detect the expression of MyD88 protein using anti-MyD88 antibody. The level of total protein in the cell lysates was monitored using anti-actin antibody. (c) Cells (2x10⁶) were transfected with 0 μg (lanes 1 and 2), 1 μg (lanes 3 and 4), or 3 μg (lanes 5 and 6) of pHBV3.8 plasmid. After 24 h of incubation, cells were stimulated with 1000 IU IFN-α ml⁻¹, as indicated, for an additional period of 12 h. The cells were then harvested and lysed to analyse the expression of MyD88 by Western blotting with specific antibodies as described. The bands in the autoradiograms were scanned and semi-quantified by densitometry in arbitrary units (AU).](image-url)
amounts of pHBV3.8 plasmid and treated them with 1000 IU IFN-α ml⁻¹. Results showed that transfection of Huh7 cells with increasing amounts of HBV DNA plasmid resulted in a marked reduction in the expression of MyD88 protein (Fig. 2c), suggesting that HBV replication and viral protein expression could inhibit IFN-inducible MyD88 expression. To ensure the correct expression of viral protein, we measured the levels of HBsAg and HBeAg in the supernatants of transfected Huh7 cells. Increases in the amounts of HBsAg and HBeAg were observed and correlated with the increase in transfected HBV DNA (data not shown).

**HBV inhibits IFN-α-induced MyD88 promoter activity**

MyD88 was initially characterized as an interleukin-6 primary response gene isolated in M1 cells, and the 5′-upstream sequence of the murine MyD88 gene has been mapped (Harroch et al., 1995). As the MyD88 protein is highly conserved between humans and mice (Bonnert et al., 1997), it has been postulated that inhibition of MyD88 expression is due to the effect of the virus on its promoter activity. To confirm this hypothesis, we constructed a plasmid expressing the luciferase reporter gene under the control of the four tandem repeats of the MyD88 promoter gene fragments, designated pMyD-Luc. To assay its functionality, Huh7 cells were transfected with pMyD-Luc and treated with IFN-α. As shown in Fig. 3(a), a marked induction of luciferase activity by IFN-α was observed.

To investigate whether the inhibition of IFN-α-induced MyD88 expression was associated with a viral effect on MyD88 promoter activity, both transiently and persistently HBV-expressing cells were used. pMyD-Luc was co-transfected with increasing amounts of the pHBV3.8 plasmid. Results showed that the luciferase activity conferred by the MyD88 promoter was inhibited by HBV in a dose-dependent manner (Fig. 3b). Similar results were observed in HepG2.2.15 cells persistently expressing HBV: the IFN-induced MyD88 promoter activity was significantly reduced in HepG2.2.15 cells compared with HepG2 cells (data not shown). All of the above results support the hypothesis that the inhibition of IFN-α-induced MyD88 expression is due to the effect of the virus on MyD88 promoter activity.

**Inhibition of IFN-α-induced MyD88 promoter activity by HBV polymerase**

To identify the HBV protein(s) responsible for blocking the IFN-α-induced MyD88 promoter activity, three HBV expression plasmids were generated to express core and HBx protein individually tagged with enhanced green fluorescent protein (EGFP) and Flag-tagged HBV polymerase. pMyD-Luc was co-transfected with plasmids expressing various HBV proteins into Huh7 cells, which were then stimulated with IFN-α. Plasmid pHBV3.8 was used as a positive control. Results showed that IFN-induced MyD88 promoter activity was inhibited by both HBx and polymerase protein, with polymerase having the greatest effect (Fig. 4a, b). Co-transfection of Huh7 cells with pMyD-Luc and increasing amounts of the pcDNA3.1-3Flag-pol plasmid showed that inhibition of IFN-induced luciferase activity increased with an increase in HBV polymerase expression (Fig. 4c), further suggesting the
specificity of the inhibitory effect. However, HBx did not show this dose-dependent inhibition activity, indicating that HBx might act in an indirect way (data not shown).

**Determination of the region of the HBV polymerase required for the inhibitory effect on the MyD88 promoter**

To define the minimum region of the HBV polymerase required to block MyD88 promoter activity, a series of N-terminal and C-terminal truncated mutants of the polymerase were constructed (Fig. 5a). The expression of full-length and truncated polymerase proteins with the expected molecular sizes was verified by Western blotting (Fig. 5b). The ability of these truncated polymerase proteins to block MyD88 promoter activity was then tested by co-transfection with pMyD-Luc. Results showed that the N-terminal residues of the polymerase comprising 691, 347 and even 178 aa all showed an inhibitory effect similar to that of the full-length polymerase protein (Fig. 5c). However, polymerase proteins without the N-terminal 178 residues, the TP domain, showed no antagonistic activity (Fig. 5c). The above results suggested that the TP domain of HBV polymerase plays an important role in inhibiting IFN-α-induced MyD88 promoter activity and expression.

**HBV polymerase interferes with Stat signalling**

Homology analysis of the potential transcription regulatory sequences of the MyD88 promoter has identified binding sites for both Stat and IRF (Harroch et al., 1995). To examine the importance of the Stat or IRF site for IFN-α-induced trans-activation, we constructed reporter plasmids pMyD-mStat and pMyD-mIRF (Fig. 1). When the Stat motif was mutated, IFN-induced trans-activation was markedly reduced (Fig. 6a), suggesting that the Stat site is essential for IFN-α-induced trans-activation of the MyD88 promoter.

To investigate whether the polymerase protein could interfere with Stat signal transduction, Huh7 cells were transfected with polymerase protein expression vectors...
together with pMyD-mIRF reporter genes, which contained only the Stat-binding site. As shown in Fig. 6(b), the polymerase protein inhibited IFN-α-induced luciferase activity of pMyD-mIRF in a dose-dependent manner, indicating that the HBV polymerase protein can be an efficient inhibitor of the Stat signalling pathway.

As the HBV polymerase could inhibit the Stat signal pathway, it was speculated that Stat1 DNA binding activity would also be reduced under these conditions. To investigate this possibility, chromatin immunoprecipitation analysis was carried out (Fig. 6c). Stat1 was observed to be associated with the MyD88 promoter in vivo in an IFN-dependent fashion (Fig. 6c, lanes 2 and 3). Interestingly, expression of the polymerase protein significantly reduced the binding intensity of Stat1 to the MyD88 promoter (Fig. 6c, lanes 4 and 5).

To examine the effect of HBV polymerase on phosphorylation of Stat1, total cell extracts from Huh7 cells transfected with pcDNA3.1-3Flag-pol were analysed for Stat1 and phosphorylated Stat1 (pStat1) levels by Western blot. As shown in Fig. 6(d), levels of tyrosine and serine phosphorylation of Stat1 were not affected by expression of the polymerase protein.

To investigate whether HBV polymerase could interfere with IFN-α-induced Stat1 nuclear translocation, we examined the subcellular localization of endogenous Stat1 proteins after stimulation with IFN-α. In mock-transfected cells, IFN-α treatment led to a redistribution of Stat1 from the cytoplasm to the nucleus (Fig. 6e), whereas cells expressing HBV polymerase maintained the phenotype of untreated cells, with the major portion of Stat1 in the cytoplasm (Fig. 6e). In addition, expression of the TP domain alone had an inhibitory effect similar to that of the full-length polymerase protein. However, polymerase protein without the TP domain lost its inhibiting ability.

Similar results were observed in the subcellular fractionation assay. As shown in Fig. 6(f), the level of Stat1 in the cytoplasmic fraction was similar in each group and IFN-α caused an increased accumulation of nuclear Stat1. However, in HBV polymerase-transfected cells, the quantity of Stat1 present in the nucleus following IFN-α stimulation was significantly reduced. Furthermore, cells expressing the TP domain alone exhibited a similar inhibitory effect, whilst the polymerase protein without the TP domain was not inhibitory. All of the above results indicated that HBV polymerase can inhibit IFN-α-induced Stat1 nuclear translocation and that the TP domain plays an important role in the inhibitory activity of HBV polymerase. It is interesting to observe that the polymerase and its mutant proteins were detected in both the cytoplasm and nucleus.

**HBV polymerase inhibits the expression of several ISGs**

Considering the important role of the Stat1 signalling pathway in inducing the expression of ISGs, we speculated that the inhibitory effect of HBV polymerase on Stat1 nuclear transduction might not merely lead to the downregulation of MyD88 expression, but also to global downregulation of Stat1-mediated transcription. To confirm this hypothesis, the effects of HBV polymerase on the transcriptional activity of the ISRE reporter plasmid and the expression of ISGs were examined. The results showed that both the transcriptional activity of the ISRE reporter plasmid (Fig. 7a) and the expression of ISGs such as Stat1 and ISG15 (Fig. 7b) were inhibited by HBV polymerase expression, indicating that the HBV polymerase might be a general inhibitor of IFN signalling by specific inhibition of Stat1 nuclear transduction.

**DISCUSSION**

IFN-α has been used for the treatment of HBV infection for two decades. However, over 60% of patients continue to suffer from chronic active hepatitis B despite IFN-α therapy (Guan, 2000; Manns, 2002). The molecular mechanisms responsible for the ineffectiveness of IFN-α treatment in chronic active hepatitis B are still unclear. In recent years, several studies have been undertaken to unveil the mechanisms responsible for HBV antagonism of the IFN-α response. Expression of the TP region of HBV has been showed to inhibit cellular responses to IFN-α and IFN-γ, but the detailed mechanisms remain unknown (Foster et al., 1991). HBV precore/core proteins can downregulate IFN-inducible MxA expression through direct interaction with the MxA promoter (Fernandez et al., 2003). Here, we have shown that HBV polymerase can inhibit expression of the IFN-α-inducible protein MyD88 by interfering with target promoter activity through blockage of Stat1 nuclear import. In addition, transcriptional activity of the ISRE promoter and expression of ISGs such as Stat1 and ISG15 were also inhibited.

With a genome of only 3 kbp, HBV expresses a very limited repertoire of proteins. The core and polymerase proteins are essential for viral DNA replication, and the envelope proteins are essential for envelopment of nucleocapsids. Two additional gene products, HBx and HBeAg, which are expressed during natural infections, are of unknown function (Seeger & Mason, 2000). In this study, we found that both HBx and polymerase protein could inhibit IFN-α-induced pMyD-Luc activity. As HBx did not show a dose-dependent inhibition activity, we focused on the polymerase for further study. HBV polymerase is a virally encoded reverse transcriptase that catalyses reverse transcription within cytoplasmic particles composed of the viral core protein. Recent studies have shown that HBV polymerase can accumulate to appreciable levels in the cytoplasm outside the core particles, in a similar way to that of duck hepatitis B virus (DHBV) (Yao et al., 2000; Yao & Tavis, 2003; Cao & Tavis, 2004). These observations indicate that the polymerase may have additional roles in virus replication or pathology beyond synthesizing DNA within the core particles, such as regulating cellular or viral processes. Previous reports have
found that DHBV polymerase can modestly reduce accumulation of mRNAs (Cao & Tavis, 2006). In addition, polymerase can interfere with the cellular response to IFN-α by inhibiting activation of the ISGF3 complex (Foster et al., 1991). Consistent with the above result, we found that HBV polymerase could inhibit the expression of IFN-induced MyD88 as well as other ISGs. Therefore, HBV polymerase might act as a dual-function protein that replicates the viral genome when encapsulated and inhibits the IFN response when free in the cytoplasm or nucleus.

In order to define the structural domain of HBV polymerase required for its inhibitory activity, we constructed a series of C-terminal truncated mutants of the polymerase. Results showed that expression of the TP domain alone exhibited an inhibitory effect similar to that of the full-length polymerase protein. Next we constructed an N-terminal truncated mutant that expressed the polymerase without the TP domain. The results showed that the polymerase protein without the TP domain showed a loss of antagonistic activity, in contrast to a previous report (Foster et al., 1995) that the TP region of
the polymerase is not responsible for this inhibition. We have repeated the reporter gene assays more than three times and the results were consistent. We found that the TP domain alone exhibited the inhibitory effect and, furthermore, that deleting the TP domain produced a loss of antagonistic activity, all of which supports the essential role of the TP domain in the inhibitory activity of HBV polymerase. The discrepancy may be due to the different cell lines and methods used in the two studies. However, we also observed that deletion of the TP domain did not completely abolish the inhibitory activity of the polymerase protein, suggesting that another domain of HBV polymerase, for example the spacer, might also be involved in the inhibitory activity. More N-terminal truncated mutants need to be constructed to test this speculation.

We also found that the polymerase interfered with MyD88 expression by inhibiting Stat1 nuclear translocation, but did not interfere with Stat1 phosphorylation or induce Stat1 degradation. It has been reported that several viruses can inhibit IFN-induced Stat1 nuclear translocation without affecting its stability or phosphorylation, including rabies virus, dengue virus and measles virus (Munoz-Jordan et al., 2003; Palosaari et al., 2003; Brzozka et al., 2006). Ebola virus VP24 has been found to bind karyopherin x1 and block Stat1 nuclear accumulation (Reid et al., 2006). The V proteins of Nipah virus and Hendra virus have been demonstrated to bind to cellular Stat1 and Stat2 proteins to form high-molecular-mass complexes that inhibit IFN-induced antiviral transcription by preventing Stat nuclear accumulation (Rodriguez & Horvath, 2004; Shaw et al., 2004). Recent reports have shown that HBV infection can reduce Stat1 methylation, which then reduces its binding to PIAS1 as well as its capacity to stimulate IFN-α target genes (Christen et al., 2007). It is interesting to note that HBV polymerase and its mutant proteins were distributed in both the cytoplasm and the nucleus, in agreement with reports that HBV polymerase could be detected in the nucleus (Choi et al., 2003). The expression of nuclear forms of polymerase suggests that the retention of Stat1 in the cytoplasm by HBV polymerase may not be the only mechanism involved in the inhibition of IFN signalling by HBV polymerase. It is possible that HBV polymerase may bind directly to Stat1, or other cellular proteins, to block Stat1 nuclear accumulation in order to inhibit IFN-induced antiviral transcription. Studies are under way to determine how HBV polymerase blocks Stat1 nuclear accumulation.

It was interesting to observe that HBV polymerase not only inhibited the expression of MyD88, but also interfered with the transcriptional activity of other ISRE-driven promoters and the expression of ISGs such as Stat1 and ISG15. This indicates global downregulation of Stat1-mediated transcription. Our results suggest that the inhibition of Stat1 nuclear translocation by HBV polymerase might be a general strategy used by HBV to antagonize the IFN response by interfering with the Jak/Stat signalling pathway used by IFNs. In addition, the role of MyD88 in linking innate and adaptive immunity of the host immune system leads us to speculate that the inhibitory effect of HBV on expression of MyD88 may not be limited to its anti-IFN antagonist effect, but may also be advantageous for the establishment of persistent infection and is worthy of further study.

In conclusion, our results demonstrate that HBV polymerase, especially the TP domain, can inhibit the expression of IFN-α-inducible MyD88, possibly by blocking Stat1 nuclear import and decreasing target promoter activity. Furthermore, HBV polymerase expression can also inhibit both the transcriptional activity of the ISRE promoter and the expression of ISGs. This result, together with previous
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REFERENCES


