Transcription of the Human Beta Interferon Gene Is Inhibited by Hepatitis B Virus

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The manner by which the *trans*-acting factor encoded by the 1,828-base-pair (bp) *Bam*HI DNA fragment of hepatitis B virus (HBV) suppresses the production of human beta interferon was determined. Steady-state levels of RNA specific for human beta interferon were decreased in cells that contained the 1,828-bp *Bam*HI DNA fragment of HBV. The reduced accumulation of interferon-specific RNA was due to an inhibition of transcription of the interferon gene by the HBV *trans*-acting moiety. The expression of the interferon gene that is under the control of a heterologous promoter such as the simian virus 40 early promoter was not altered by the presence of the 1,828-bp *Bam*HI HBV DNA fragment. In contrast, the HBV moiety inhibited the expression of the *cat* gene, whose expression is controlled by the regulatory DNA region of the human beta interferon gene. These results indicate that the HBV *trans*-acting moiety suppresses the expression of the human beta interferon gene at the transcriptional level by interacting with the regulatory DNA sequences 5' to the coding sequences for beta interferon.

Hepatitis B virus (HBV) is a small enveloped DNA virus that is an etiological agent of acute and chronic hepatitis in humans (10, 34). The HBV genome, which is partially double stranded, consists of four open reading frames (ORF). The largest ORF encompasses most of the HBV genome and encodes the DNA polymerase (2, 35). The smallest ORF, designated X, has the potential to encode a protein of 154 amino acids that may be a regulatory protein for gene expression, since it has the capability to transactivate both viral and cellular enhancers (30, 33, 38). Another ORF encodes the HBV surface antigens that presumably function in the attachment of the virus to cell surfaces (5, 39). Two proteins, the core antigen and the e antigen, are encoded by ORF C (20). The core antigen appears to be retained intracellularly for the assembly of virions, whereas the e antigen is secreted (24, 26). The function of the e antigen in infection by HBV is unknown, but this antigen does not appear to be crucial for viral replication (4).

The factors that play a role in the development and maintenance of chronic HBV infections are unclear but could involve defects in natural host defenses, such as interferon. Although active viral replication occurs in chronic HBV carriers, serum interferon has not been detectable (17, 19, 32, 36, 42). Interferon-inducible responses, such as enhanced expression of class I human leukocyte antigens on hepatocyte membranes and increased production of cellular 2',5'-oligoadenylate synthetase, are not observed in many chronic HBV carriers (18, 25, 27). Lastly, peripheral blood lymphocytes from individuals with chronic hepatitis produce less interferon than do such lymphocytes from uninfected controls when stimulated in vitro with viruses (36). These observations have led to the speculation that a defect in the ability to produce interferon may be involved in the development of chronic HBV infections (9).

Recently, we have demonstrated that the 1,828-base-pair (bp) *Bam*HI HBV DNA fragment that contains ORF C

suppresses the production of cloned human beta interferon (37). The suppression of the synthesis of the beta interferon occurs via an HBV *trans*-acting factor and is alleviated by the introduction of a frameshift mutation within the HBV core antigen gene. However, the stage of synthesis of the beta interferon that is inhibited by the *trans*-acting HBV factor is unclear. We now report that the HBV *trans*-acting inhibitory factor suppresses the induction of the human beta interferon gene by poly(I)-poly(C) at the level of transcription by interacting, directly or indirectly, with a 353-bp sequence containing the regulatory elements that function in the induction of the human beta interferon gene.

MATERIALS AND METHODS

Plasmids. Various cloned fragments of HBV subtype adw (8) (Fig. 1) were cloned into the *Bam*HI site of either the bovine papillomavirus (BPV) vector pDBPV-1 or pBI, a BPV plasmid containing the 1.6-kilobase (kb) *Hind*III human beta interferon gene fragment (37). Details of the construction of these plasmids are described elsewhere (37). Briefly, the cloning of the 1,828-bp *Bam*HI HBV DNA fragment (map position 1400 to 28) into both pDBPV-1 and pBI yielded pBHC and pBHCI, respectively. The 2,755-bp *Bgl*II HBV DNA fragment (map position 2429 to 1984) was cloned into the *Bam*HI site of pBI, creating pBHSI. Plasmid pBIHCd-TaqI is identical to pBHC except for an insertion mutation at the *Taq*I site (map position 2014) that is within the HBV core antigen gene was created as described elsewhere (37). The insertion mutation resulted in the destruction of the *Taq*I site in the HBV core antigen gene and in a shift in the ORF.

Other plasmids used in this study were pTWU54, containing the *cat* structural gene that is controlled by the 353-bp regulatory elements involved in the expression of the human beta interferon gene (β -IFN_R-*cat*) (38), and pSVI911, containing the *Pvu*II-*Hind*III simian virus 40 (SV40) DNA fragment including the early promoter fused to the *Hind*II human beta interferon fragment that carries the coding sequence and the 3' nontranslated region up to the poly(A) site (21).

Cells. Vero cells and WISH cells were cultured at 37°C in

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FIG. 1. Diagrammatic representation of HBV constructs used in transfections. Shown are the plus strand of a linearized HBV genome and known genes (S, major surface protein gene; Pre S₁, preS₁ surface antigen gene; Pre S₂, preS₂ surface antigen gene; P, polymerase gene; X, X antigen gene; Pre C, precore gene; C, core gene). The scale at the top shows the number of bases.

Eagle minimum essential medium supplemented with 10% fetal calf serum, whereas murine C127 cells were propagated in Dulbecco modified Eagle medium containing 10% fetal calf serum. C127 cells transformed with various BPV plasmids containing the cloned human beta interferon gene and various HBV DNA fragments (Fig. 1) were grown as described previously in Dulbecco modified Eagle medium containing 400 μ g of G418 per ml (37).

Transfection of cells with DNA. DNA transfections were performed by the calcium phosphate precipitation technique by the modified procedure of Wigler et al. (43) as described previously (37, 38). For transient transfections, 10 μ g of various plasmids containing HBV DNA and 10 μ g of the plasmid containing the β -IFN_R-cat construct were precipitated with calcium phosphate and added to a 100-mm plate in which 10^6 cells had been seeded 1 day prior to transfection. After incubation with the added DNA for 4 to 8 h, the cells were treated with 15% glycerol for 1.5 min at room temperature. The cells were washed with phosphate-buffered saline, and 10 ml of fresh medium was added.

To obtain stable transformants, we cotransfected C127 cells with 1 μ g of pSVI911 and 1 μ g of either pBHC or pdBPV-1 in the presence of 0.2 μ g of a plasmid carrying the neomycin resistance gene. After 4 to 6 h of exposure to DNA, the medium was replaced and the cells were incubated for 20 h. The cells were diluted, and G418 (400 μ g/ml) was added. After 14 to 21 days, well-separated foci were picked and stable transformants were grown to confluence in the presence of G418.

Treatment of transfected cells with poly(I)-poly(C) and interferon assay. Transfected Vero cells were treated with poly(I)-poly(C) by the method of Havell and Vilček (16) as described by Twu and Schloemer (38). Control cells were treated in the same manner, except that poly(I)-poly(C) was omitted. Interferon was assayed by determining antiviral activity (inhibition of cytopathic effect) against vesicular stomatitis virus (37). Assays for human interferon were conducted with human WISH cells as the indicator cells.

CAT assays. Cells were harvested and chloramphenicol acetyltransferase (CAT) assays were performed as described by Gorman et al. (14, 15). Briefly, cells were pelleted, suspended in 0.25 M Tris hydrochloride (pH 7.8), and disrupted by freezing-thawing. The protein concentrations of cell lysates were determined, and CAT assays were per-

formed with the same amount of total protein for all samples in an individual experiment. CAT activity was determined as described previously and is expressed as percent acetylation (38).

Isolation of DNA. Total cellular DNA was extracted from cells by the modified method of Colbere-Garapin et al. (7). Cells were lysed with 0.2 M Tris hydrochloride (pH 8.5) containing 100 mM EDTA, 100 mM NaCl, 1% sodium dodecyl sulfate, and 100 μ g of proteinase K per ml. The cell lysate was extracted with phenol-chloroform. After dialysis against TE buffer (22), the DNA preparation was treated for 30 min at 37°C with DNase-free RNase at a final concentration of 10 μ g/ml. After extraction with phenol-chloroform, the DNA was precipitated by ethanol and suspended in TE buffer.

Isolation and analysis of RNA. Total RNA was isolated from cells by using guanidium thiocyanate and by pelleting through a pad of 5.7 M CsCl as described by Sachstein and Colten (29). RNA was stored as a precipitate in ethanol until use. RNA was analyzed by either Northern (RNA) blotting or dot blotting. For Northern blots, RNA was electrophoresed in a 1.5% formamide-agarose gel and transferred to Nytran membranes. Alternatively, RNA samples were applied to Nytran membranes with a Bio-Dot apparatus (Bio-Rad Laboratories). Hybridizations were accomplished by standard procedures with DNA probes labeled with 32 P by nick translation (22). DNA probes were the 1.6-kb *Hind*III human beta interferon gene (37), murine interferon cDNA (13), or actin cDNA (6).

Nuclear transcription assays. Cells (2×10^7) were incubated on ice for 5 min in 10 mM Tris hydrochloride (pH 7.4) containing 10 mM NaCl, 3 mM MgCl₂, and 0.5% (vol/vol) Nonidet P-40 (lysis buffer). The mixture was centrifuged at $500 \times g$ for 5 min, and the pellet containing nuclei was washed with lysis buffer. The pellet was suspended in 50 mM Tris hydrochloride (pH 8.3) containing 5 mM MgCl₂, 0.1 mM EDTA, and 40% glycerol.

Transcription of nuclei with 0.2 mCi of [α - 32 P]UTP was performed essentially as described by Velcich and Ziff (40), whereas isolation of resulting labeled RNA was performed as described by McKnight and Palmiter (23). Nuclei (5×10^6) were mixed with 100 μ l of reaction buffer (10 mM Tris hydrochloride [pH 8.0], 5 mM MgCl₂, 300 mM KCl, 0.5 mM each ATP, CTP, and GTP, and 200 μ Ci of [α - 32 P]UTP) and

incubated at 30°C for 30 min. The reaction was terminated by the addition of 25 μ l of 10% sodium dodecyl sulfate and 5 μ l of proteinase K (10 mg/ml). Labeled RNA was isolated by hot acid-phenol extraction and treated with RNase-free DNase. Nytran filters containing 5 μ g of a 1.6-kb *Hind*III fragment of human beta interferon DNA were prepared and hybridized with the 32 P-labeled nuclear run-on RNA. After hybridization the blots were treated with RNase A (10 μ g/ml) at 37°C for 20 min. The radioactive RNA that had bound to the filters was eluted and quantitated by liquid scintillation counting. The background (21 cpm), which represented hybridization to DNA-free Nytran filters, was subtracted from all values.

RESULTS

Inhibition of interferon-specific RNA synthesis by HBV. Murine C127 cells stably transformed with the BPV vector containing the cloned human beta interferon gene and the 1,828-bp *Bam*HI fragment of HBV DNA, which contains the gene for the HBV core antigen (pBHCl), produced considerably less human interferon than did C127 cells transformed with either a BPV plasmid containing the human beta interferon gene and the 2,755-bp *Bgl*II HBV DNA fragment, which contains the surface antigen gene (pBHSI), or a BPV plasmid containing the human beta interferon gene (pBI) (35). For determination of the step in the synthesis of human beta interferon that was being suppressed by HBV, total cellular RNA was prepared from these transformants, electrophoresed on agarose gels, and analyzed by Northern hybridization with the human interferon gene as the probe. As controls, RNAs from murine C127 cells and from MG63 cells, human osteosarcoma cells that are known producers of interferon (44), were also analyzed.

Untreated C127-pBI cells contained low levels of human beta interferon RNA, which increased dramatically following treatment of the cells with poly(I)-poly(C) (Fig. 2A). Similar results were observed when RNA isolated from C127-pBHSI cells was analyzed. In contrast, C127-pBHCl cells, which contained the 1,828-bp *Bam*HI segment of HBV DNA, possessed vastly reduced levels of human beta interferon RNA when left untreated or when treated with poly(I)-poly(C). Murine C127 cells, which produced murine interferon upon treatment with poly(I)-poly(C) (37), did not synthesize RNA that hybridized to the human interferon gene probe. Poly(I)-poly(C)-treated MG63 cells contained RNA that reacted with the gene probe.

Figure 2B demonstrates the results of dot blots to detect RNA specific for murine beta interferon and murine actin in the various transformed C127 cell lines. In contrast to the inhibition of accumulated levels of RNA specific for human beta interferon, the levels of RNA specific for murine beta interferon and murine actin were not affected by the presence of either HBV DNA fragment.

Inhibition by HBV of transcription of the interferon gene. The reduced levels of steady-state human beta interferon RNA in the presence of the 1,828-bp *Bam*HI HBV DNA could be due to inhibition of the initiation of transcription or to a defect in the stability of the human beta interferon RNA. To distinguish between these possibilities, we performed nuclear transcription experiments with nuclei from C127-pBI cells and C127-pBHCl cells. In addition, nuclei were isolated from C127-pBIHCdTaql cells, which contain a plasmid that is identical to pBHCl except that a frameshift mutation has been introduced at the *Taq*I site within ORF C of the HBV DNA. Previous studies have shown that this frameshift

FIG. 2. Interferon transcripts in C127 cells transformed with various BPV plasmids. C127 cells that had been transformed with BPV plasmids containing (i) the human beta interferon gene (pBI), (ii) the human beta interferon gene and the 2,755-bp *Bgl*II HBV DNA fragment (pBHSI), or (iii) the human beta interferon gene and the 1,828-bp *Bam*HI HBV DNA fragment (pBHCl) were treated with poly(I)-poly(C) or mock treated. Total cellular RNA was extracted after 8 to 10 h. (A) Equal amounts of RNA (5 μ g) were electrophoresed in a 1.5% formamide-agarose gel and hybridized with a 32 P-labeled 1.6-kb *Hind*III fragment containing the human beta interferon gene. As controls, RNAs from C127 cells and from human MG63 osteosarcoma cells, a known producer of human interferon, were also analyzed. (B) Dot blot hybridization of equal amounts of RNA from the indicated cell lines with either 32 P-labeled murine beta interferon DNA or 32 P-labeled murine actin cDNA as a probe. Hu, Human; IFN, interferon.

mutation results in the loss of the ability of the HBV DNA fragment to inhibit the production of human beta interferon (35).

Treatment of C127-pBI cells with poly(I)-poly(C) resulted in a significant increase in the rate of transcription of the human interferon gene (Table 1). However, this increase was blocked by the presence of the HBV core antigen gene in C127-pBHCl cells. The ability of the HBV DNA fragment to inhibit the rate of transcription of the human interferon gene was abolished by the introduction of the frameshift mutation in ORF C.

Effect of the HBV trans-acting factor on the regulatory region of the human interferon gene. The 353-bp fragment that flanks the 5' end of the coding sequence for human beta interferon is responsible for the induction of this gene in response to viral infection of cells or to treatment of cells with poly(I)-poly(C) (11, 45). To determine whether the HBV trans-acting inhibitory factor could suppress the

TABLE 1. Inhibition of transcription of the human beta interferon gene by the 1,828-bp *Bam*HI HBV DNA fragment^a

Cell source of nuclei	cpm bound to interferon DNA
Uninduced C127-pBI	158
Induced C127-pBI	1,115
Uninduced C127-pBHCl	58
Induced C127-pBHCl	120
Uninduced C127-pBIHCdTaqI	184
Induced C1270-pBIHCdTaqI	1,272

^a Nuclei were isolated from C127 cells transformed with the indicated plasmids 8 h after treatment of the cells with poly(I)-poly(C). RNA transcripts were elongated in vitro for 30 min with [³²P]UTP. The resulting labeled RNA was isolated and treated with DNase prior to hybridization to human beta interferon DNA that had been immobilized on Nytran membranes. The amount of radioactively labeled RNA that had bound to the membranes was quantitated by liquid scintillation counting.

expression of a heterologous gene whose expression is under the control of the human beta interferon regulatory region, we used a plasmid (pTWU54) that contains the *cat* gene driven by the interferon regulatory region. Previous studies have indicated that poly(I)-poly(C) treatment of cells transfected with pTWU54 results in increased expression of CAT activity (38). Vero cells were cotransfected with the test plasmid (pTWU54) and pUC9 derivatives containing various HBV DNA fragments. After transfection, cells were treated with poly(I)-poly(C) prior to analysis of CAT activity (Fig. 3 and Table 2).

The low levels of CAT activity in cells containing pTWU54 and pUC9 increased upon treatment with poly(I)-poly(C) by an average of 5.5-fold. Extracts of cells that contained pTWU54 and the entire *Eco*RI HBV DNA fragment (plasmid pUC9/HBV) exhibited decreased amounts of CAT activity when cells were left untreated or were treated

with poly(I)-poly(C). The ability to cause the inhibition of CAT activity resides in the 1,828-bp *Bam*HI segment of HBV DNA, since comparable levels of CAT activity were observed in cells cotransfected with either the entire HBV genome or the *Bam*HI HBV DNA fragment (pTWU22). The extent of inhibition by pTWU22 ranged from 4.4- to 13.8-fold. Moreover, the frameshift mutation at the *Taq*I site within the core antigen gene of HBV DNA (pTWU22dTaqI) abolished the ability of HBV DNA to inhibit the expression of the *cat* gene controlled by the interferon regulatory elements. These results indicate that the HBV *trans*-acting moiety encoded by the 1,828-bp *Bam*HI HBV DNA fragment interacts with the 353-bp region that flanks the 5' end of the human beta interferon-coding region. The inhibitory activity of the HBV *trans*-acting factor appears to be specific. The activity of the *cat* gene in SV₃CAT (38) was not affected by the presence of the 1,828-bp HBV DNA fragment (30.8 versus 26.7% acetylation).

Effect of HBV on the stability of interferon RNA. To investigate the possibility that the HBV *trans*-acting inhibitory factor can alter the levels of human beta interferon RNA by affecting the stability of the RNA, we examined the effect of the HBV *trans*-acting inhibitory factor on the expression of the human beta interferon gene when controlled by a heterologous promoter. pSVI911 contains the human beta interferon gene whose expression is governed by the SV40 enhancer and early promoter elements (21). C127 cells were cotransfected with pSVI911 and either the BPV plasmid containing the 1,828-bp *Bam*HI HBV DNA segment (pBHC) or the BPV vector alone (pDBPV-1). A plasmid carrying the gene encoding neomycin resistance was also present in each transfection. Clones of cells from each transfection were isolated on the basis of the transformed phenotype and resistance to the drug G418. Each focus was expanded and examined for the production of human beta interferon and for the presence of the interferon gene and the gene for the HBV core antigen (Table 3).

All of the clones contained the SV40-interferon fusion gene as well as the BPV plasmids. The SV40-interferon gene was integrated into the chromosome at approximately 2 to 5 copies per cell, whereas the BPV-containing plasmids were present at 20 to 25 copies per cell (data not shown). The presence of the 1,828-bp *Bam*HI HBV DNA fragment had no effect on the production of human beta interferon when this gene was controlled by the SV40 regulatory elements.

DISCUSSION

Individuals who are chronically infected with hepatitis B virus have a poor response in the synthesis of interferon and interferon-inducible enzymes (17-19, 25, 27, 36). Previously we demonstrated that the expression of cloned human beta interferon was affected by the presence of the HBV genome (37). Specifically, an as-yet-unidentified *trans*-acting factor encoded by the 1,828-bp *Bam*HI HBV DNA segment that contains ORF C inhibited the production of human beta interferon. The experiments reported here confirm and extend these observations by determining the basis for this inhibition.

The suppression of the production of human beta interferon by the HBV *trans*-acting moiety is due to reduced steady-state levels of interferon-specific RNA. This reduction in RNA levels, which is specific for human beta interferon RNA, is a result of inhibition of the transcription of the interferon gene. In support of this contention are the following observations. Results of nuclear transcription experi-

FIG. 3. Effect of HBV DNA on the activity of the human beta interferon regulatory DNA region. Vero cells were cotransfected with 10 μ g of pTWU54, which contains the hybrid β -IFN_R-*cat* gene, and 10 μ g of the indicated pUC9 derivative. After 36 to 48 h of transfection, cells were either mock treated (odd-numbered lanes) or treated with poly(I)-poly(C) (even-numbered lanes). Cells were harvested after an additional 16 to 24 h of incubation, and CAT activity was determined. CM, Chloramphenicol; 1-AcCM, 1-acetyl chloramphenicol; 3-AcCM, 3-acetyl chloramphenicol.

TABLE 2. Suppression of the human beta interferon regulatory region by HBV as detected in CAT assays^a

Plasmid	No. of assays	% Acetylation (range)		Fold reduction (range)	
		Uninduced	Induced	Uninduced	Induced
pUC9	7	3.24 (3.0–3.8)	17.66 (14–24.6)		
pUC9/HBV	3	0.77 (0.6–1.1)	1.57 (1.2–2.1)	4.5 ± 1.1 (3–5.8)	10.9 ± 2.1 (9–13.8)
pTWU22	5	0.92 (0.7–1.2)	2.32 (1.3–3.4)	3.5 ± 0.8 (2.5–4.2)	8.1 ± 3.2 (4.4–13.8)
pTWU22dTaqI	3	2.8 (2.4–3.2)	15.2 (14.7–16.2)	1.08 ± 0.28 (0.97–1.25)	1.14 ± 0.14 (1.03–1.29)

^a Transfections were performed as described in the legend to Fig. 3 with equal amounts of the indicated plasmids and of pTWU54.

ments indicated that very little human beta interferon RNA was transcribed in poly(I)-poly(C)-treated C127-pBHCI cells, which contained the 1,828-bp *Bam*HI HBV DNA fragment. Moreover, the HBV *trans*-acting factor appeared to have no effect on the stability of RNA specific for beta interferon, since the production of beta interferon in the presence of this *trans*-acting factor was normal when the expression of this gene was controlled by a heterologous enhancer-promoter (the SV40 regulatory elements). Lastly, the HBV *trans*-acting factor was able to inhibit the expression of a heterologous gene, the bacterial *cat* gene, that was driven by the regulatory region of DNA that borders the 5' end of the coding sequence of the human beta interferon gene. This inhibition was observed when either the entire 3.2-kb HBV DNA fragment or the 1,828-bp *Bam*HI HBV DNA fragment was used as the source of the HBV *trans*-acting inhibitory factor.

It is of interest to note that the expression of the *cat* gene under the control of the interferon regulatory elements is inhibited by the presence of the entire HBV genome. In addition to containing the 1,828-bp *Bam*HI segment, which encodes the inhibitory factor, the HBV genome contains ORF X. We showed previously that the product of ORF X has the ability to transactivate the expression of the *cat* gene whose expression is controlled by the interferon regulatory

elements (38). The observation that inhibition of CAT activity occurs in the presence of the entire HBV genome would indicate that the negative effect of the *trans*-acting factor encoded by the 1,828-bp *Bam*HI fragment is dominant over the positive effect of the X antigen. Another possibility that is consistent with the above-described finding is that the HBV *trans*-acting inhibitory factor inhibits or down-regulates the expression of the X gene product, an event that would result in the production of mainly the *trans*-acting inhibitory factor. Experiments to distinguish between these possibilities are currently being performed.

As noted previously, the identity of the HBV *trans*-acting factor that inhibits the expression of the human beta interferon gene is unknown. The 1,828-bp HBV DNA fragment used in these studies contains (i) the intact ORF C, (ii) the core promoter that is weakly active in nonhepatic cells, such as HS27 cells, rat fibroblast cells, and HeLa cells, and (iii) the modified polyadenylation signal, AUUAAU, at the end of ORF C (1, 28). Thus, it is conceivable that the HBV *trans*-acting inhibitory factor is a gene product of ORF C.

These data suggest that the HBV *trans*-acting inhibitory factor prevents transcription of the beta interferon gene by interacting directly or indirectly with the 353-bp DNA region that is responsible for the induction of beta interferon in response to either viral infection of cells or treatment of cells with synthetic double-stranded poly(I)-poly(C) (11, 45). A 40-bp region, designated the interferon regulatory element, is sufficient for the induction of the human beta interferon gene in C127 cells and is located within the 353-bp DNA region that was used to control the expression of the *cat* gene in pTWU54 (11–13, 45). The interferon regulatory element contains an enhancer that is composed of two positive regulatory domains and one negative regulatory domain (11, 12). DNase footprinting experiments show that a protein(s) binds to the negative regulatory domain (44). Presumably during induction of the interferon gene by poly(I)-poly(C) the protein binding to the negative domain is removed and other proteins then bind to the positive regulatory domains. It is unclear how the HBV factor prevents transcription of the interferon gene. The HBV *trans*-acting inhibitory factor may bind to the negative regulatory domain of the interferon regulatory element in an irreversible manner so as to prevent the binding of proteins to the positive regulatory domains during induction of the interferon gene by poly(I)-poly(C). Alternatively, the HBV *trans*-acting inhibitory factor may interfere with cellular processes that are involved in the removal of cellular proteins from the negative regulatory domain during induction of the gene by poly(I)-poly(C) or may inhibit the action of the positively acting cellular factors. Further experiments are needed to clarify the mechanism of inhibition of transcription by the HBV factor.

It is possible that a reduction in the amount of interferon synthesized during the acute stage of an HBV infection could be a predisposing factor for the establishment of a

TABLE 3. Effect of HBV DNA on the expression of the human beta interferon gene under the control of the SV40 promoter^a

Plasmid(s) used to transfect C127 cells	Clone	Presence (+) or absence (–) of genes for:		Human beta interferon (U/ml)
		Interferon	HBV core antigen	
None	1	–	–	<40
pSVI911 + pdBPV-1	1	+	–	320
	2	+	–	1,280
	3	+	–	640
	4	+	–	1,280
	5	+	–	640
pSVI911 + pBHC	1	+	+	640
	2	+	+	2,560
	3	+	+	1,280
	4	+	+	1,280
	5	+	+	320

^a C127 cells were cotransfected with 1 µg of pSVI911 and 1 µg of either pBHC or pdBPV-1 in the presence of 0.2 µg of a plasmid carrying the neomycin resistance gene. After 14 to 21 days, G418-resistant foci were picked and stable transformants were grown to confluence in the presence of G418. Total cellular DNA was isolated from cells and probed by dot blot hybridization with a ³²P-labeled 1,828-bp *Bam*HI HBV DNA fragment and a ³²P-labeled 1.6-kb human beta interferon DNA fragment. Results of dot blots are expressed on the basis of the presence or the absence of the DNA fragment. Interferon activity was assayed by determining antiviral activity (inhibition of cytopathic effect).

chronic HBV infection. Interferons are cellular proteins that represent the initial response to viral infections (31). In addition to their antiviral activity, interferons have several properties that may play a role, via immune mechanisms, in the recovery from acute viral infections. Interferons increase the expression of class I human leukocyte antigens on membranes of infected cells (25), activate natural killer cell activity (41), and enhance the response of cytotoxic T lymphocytes to virus-infected cells (3). Therefore, a defect in the synthesis of interferons during an acute HBV infection could hinder the activities of antigen-producing cells and of cytotoxic T lymphocytes to such an extent as to allow continued viral infection and the development of a chronic HBV infection. It is also possible that the HBV inhibitory factor could suppress these interferon-inducible responses.

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