Trans-complementation among naturally occurring deletion mutants of hepatitis B virus and integrated viral DNA for the production of viral particles with mutant genomes in hepatoma cell lines

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Cultured hepatoma cells (HepG2) were cotransfected with two different plasmids carrying a head-to-tail dimer of recombinant hepatitis B virus (HBV) DNA cloned from deletion mutants isolated from the circulation of persistently infected hosts. They were tested for the secretion of viral particles with mutant genome encapsidation. A recombinant plasmid defective in the S gene and one defective in both the C and P genes complemented in trans for the production of viral particles. Mutant genomes from either of the recombinants were encapsidated. Similarly, a recombinant defective in the C gene and another defective in the P gene trans-complemented for the production of viral particles containing mutant genomes. A hepatoma cell line with integrated HBV DNA sequences defective in the C and P genes (PLC/PRF/5) when transfected with a recombinant defective in the S gene produced viral particles with the HBV genome from the transfecting recombinants. These results confirm the expected trans-complementation among the S, C and P genes of HBV, when either episomal or integrated into chromosomes, for the maintenance of defective HBV mutants in persistently infected hosts.

Introduction

Various mutations of hepatitis B virus (HBV) such as point mutations, insertions and deletions have been observed in isolates from the circulation of persistently infected hosts. Point mutations in the S gene coding for the envelope protein, commonly referred to as hepatitis B surface antigen (HBsAg), induce subtype changes (Okamoto et al., 1987c). Point mutations as well as insertions and deletions in the preC region, preceding the C gene coding for the viral nucleocapsid, induce changes from a hepatitis B e antigen (HBeAg)-positive to -negative phenotype (Okamoto et al., 1990b). HBV mutants with these changes are replication-competent, and can survive longer than the wild-type HBV against the selective force of host immune responses.

Various deletion mutants are observed in the circulation of HBV carriers (Okamoto et al., 1987a; Gerken et al., 1991; Tran et al., 1991; Wakita et al., 1991), some of which are replication-competent due to defects in the S or C gene, or the polymerase (P) gene encoding DNA polymerase/reverse transcriptase. All of them co-occur with replication-competent HBV which can trans-complement the replication of deletion mutants. Complementation may not necessarily come from predecessor wild-type viruses, however. It may be from other mutants or even from HBV DNA sequences integrated in host chromosomes with complementary defects.

To evaluate this possibility, plasmids carrying head-to-tail dimers of recombinant HBV DNA were constructed, the viruses having been cloned from deletion mutants occurring in the circulation of persistently infected hosts. They were tested for the ability to assemble viral particles and to encapsidate mutant genomes, by means of trans-complementation, in a transient expression system with cultured hepatoma (HepG2) cells (Aden et al., 1979). They were also tested for their capacity to recruit gene products, encoded by HBV DNA sequences integrated in PLC/PRF/5 cells (Alexander et al., 1976), for the production of viral particles.

Methods

Recombinant plasmids. The following four plasmids carrying recombinant HBV DNA that was defective in one or two of the S, C and P genes due to deletions (as illustrated in Fig. 1) were used. pN342-63(S-) had been cloned from HBV DNA in core particles produced by human hepatoma cells (PLC/342) serially propagated in nude mice (Matsui et
al., 1986). It had a total nucleotide length of 3158 base pairs (bp) with two deletions in the preS region (Imai et al., 1987). In addition, the preS region and the S gene each had a premature termination codon. It could not, therefore, code for HBsAg. The C gene was kept intact, while the P gene had two in-phase deletions at locations overlapping the preS region and was capable of coding for a product of 824 amino acids (aa). pODW404(C−P−) was isolated from the plasma of a Japanese blood donor who carried HBsAg subtype of adw asymptptomatically (Okamoto et al., 1987a). Both the C and P genes were defective due to a 718 bp deletion spanning nucleotides (nt) 1957 to 2674. pRTB840(C−) was isolated from the plasma of an Indonesian blood donor who carried HBsAg/adw, and had a deletion in the C gene (Okamoto et al., 1987a). pJDW55(P−) was isolated from the plasma of a Japanese blood donor who carried HBsAg/adw. It had a deletion in the P gene which overlapped the 5′ terminus of the preS1 region (Okamoto et al., 1987a).

The four HBV DNA clones had been ligated at the BamHI site of plasmid vector pSP65 (Promega). Recombinant plasmids were prepared, each carrying a head-to-tail dimer of a defective HBV genome at the BamHI site.

Transient expression of HBV DNA in human hepatoma cell lines. The HepG2 (Aden et al., 1979) and PLC/PRF/5 (Alexander et al., 1977) cell lines were used for the expression of HBV genes. Recombinants were introduced into cells by calcium phosphate precipitation. Cells (1 x 10⁶) in a 25 cm² Petri dish (Miles Laboratories) received 12.5 μg of plasmid DNA, or 6.25 μg each of two different DNA preparations in cotransfection studies. They were harvested from a dish 2 to 5 days after transfection. Cells harvested from a dish were suspended in 0.5 ml of Tris·HCl buffer (20 mm, pH 7.5) containing 150 mm-NaCl, 10 mm-EDTA and 0.5 mm-PMSF (Sigma) and were lysed by freezing and thawing.

Serological and biochemical tests. Spent culture medium and cell lysate were tested for serological markers of HBV by ELISA with monoclonal antibodies (MAbs) to the common or subtypic determinants of HBsAg (Usuda et al., 1986) or to the product of the preS1 or preS2 region (Takai et al., 1986). Hepatitis B core antigen (HBcAg), representing viral core particles, was determined by ELISA by sandwiching it between two MAbs of distinct specificities (Takahashi et al., 1983). Similarly, HBeAg was determined by sandwiching it between two MAbs of different specificities (Imai et al., 1982). HBV DNA was determined by dot blot hybridization and the activity of the endogenous DNA polymerase of HBV was determined by the incorporation of [³H]TTP (Du Pont NEN Products).

Sucrose density fractionation. Five days after transfection the medium was harvested and 40 ml was centrifuged at 1200 g for 30 min. Viral and subviral particles in the supernatant were spun down in an SW-28 rotor (Beckman Instruments) at 83000 g for 12 h. The pellet was suspended in 1 ml of Tris·HCl buffer (10 mm, pH 7.2), and centrifuged at 83000 g for 20 h in a gradient of sucrose density from 15 to 60% (w/v) in an SW-28 rotor. After centrifugation, the tube was pierced at the bottom and 0.5 ml fractions were collected.

Immune electron microscopy. Fractions positive for HBV DNA were incubated with MAbs to either the preS1 region product, preS2 region product, S gene product or HBcAg. Immune complexes were stained with 2 % (w/v) phosphotungstic acid and observed in a type EM-002A electron microscope (Akashi Beam Technology).

Southern blot analysis. Viral particles in culture media or sucrose density fractions were centrifuged in an SW60Ti rotor (Beckman) at
270000 g for 1 h. The pellet was suspended in 1 ml of Tris-HCl buffer (50 mM, pH 7.8) containing 150 mM-NaCl and 0.5 mM-EDTA. To the suspension were added, in order, 10 μl of magnesium acetate and 20 μl of digestion buffer (50 mM Tris-HCl, pH 8.0). The digestion mixture was made 0-5 % (w/v) NaCl and 0.5 mM-EDTA. To the digestion mixture were added 150 mM-EDTA and 0.5 mM-EDTA. The digestion mixture was made 0-5 % (w/v) NaCl and 0.5 mM-EDTA. The reaction mixture was made 0-5 % (w/v) NaCl and 0.5 mM-EDTA. The DNA obtained, before or after digestion with endonucleases, was subjected to electrophoresis in 1% (w/v) agarose and blotted to a nitrocellulose filter membrane (Schleicher & Schuell). Hybridization was carried out, under stringent conditions, with cloned HBV DNA radiolabelled with [α-32P]dCTP (Amersham) at a specific activity of 1.0 x 10⁹ to 1.5 x 10¹⁰ c.p.m./μg.

Amplification of HBV DNA fragments by PCR. An HBV DNA sequence was amplified by PCR with primers 3′ TTCAAGGCTTCC-AAGCTTGTCG 3′ (sense: nt 1863 to 1882 of the plus strand) and 5′ TCCCAAGAATATGGTGACCC 3′ (antisense: nt 2814 to 2833 of the minus strand). The PCR amplified an HBV DNA fragment of 971 bp from pN342-63(S−), while it amplified a 235 bp fragment from pODW404(C−P−) owing to the deletion (Fig. 1). For detecting contamination with recombinants, a pSP65 sequence of 510 bp (nt 101 to 610) was amplified with primers 3′ CCGCTCACAATTCCAGGAGAGC 3′ (antisense: nt 101 to 120) and 5′ CAGGGTCGGAAC-AAGGAGAGC 3′ (antisense: nt 591 to 610).

PCR was performed with a mixture of HBV and pSP65 primers for 25 cycles with a Gene Amp DNA amplification reagent kit (Perkin-Elmer Cetus) with denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1.5 min in each cycle. The products of PCR were electrophoresed in a composite agarose gel made of 1% (w/v) each of NuSieve and SeaKem (FMC BioProducts), stained with ethidium bromide and observed under u.v. light.

Results

Transient expression of the four HBV mutants in cultured hepatoma cells

HepG2 cells were transfected with recombinant plasmid carrying a tandem dimer of each of the four deletion mutants pN342–63(S−), pODW404(C−P−), pRTB840(C−) and pJDW55(P−). Medium was separated from cells 2 to 5 days after transfection. Both medium and cell lysate were tested for antigenic and biochemical markers of HBV (lines 1 to 4 in Table 1; lanes 1 to 4 in Fig. 2). HepG2 cells transfected with the S− recombinant did not secrete HBsAg into the culture medium. The remaining three recombinants, C−P−, C− and P− caused HepG2 cells to direct the secretion of HBsAg particles into the medium; their subtypes were all adw, reflecting those of the transfectants. They all possessed an intact preS2 region, but only two of them, C−P− and C−, had an intact preS1 region. Secretion of HBsAg particles with the preS1 and/or preS2 region product demonstrated that the preS regions and the S gene had been preserved in the three recombinants.

The medium of cells transfected with S− or P− showed some HBCAg activity, albeit much lower than that detected in lysates of these cells (Table 1). A high DNA polymerase activity was found in the medium of cells transfected with S−, and dsDNA as well as replicative intermediates were observed by Southern hybridization (Fig. 2, lane 1). Neither DNA polymerase activity nor HBV DNA was detected in media of cells transfected with C−P−, C− or P−. S− and P− had an intact preC/C gene and, therefore, HBeAg was detected in media of cells transfected with them. Neither HBCAg nor HBeAg was detectable in the medium or lysates of cells transfected with C−P− or C−.

Morphological forms resembling Dane particles (hepatitis B virions) were observed in the medium of cells transfected with P− but not in that of cells transfected with S−, C− or C−P−, as shown by immune electron microscopy with MAb to the product of the S gene or the preS2 region (data not shown).

Complementation of two recombinants transfecting HepG2 cells for the production of viral particles

The results are shown in Table 1 (lines 5 to 7) and Fig. 2 (lanes 5 to 7). The culture medium of HepG2 cells cotransfected with S− and C−P−, as well as that of HepG2 cells transfected with C− and P−, showed some HBCag activity and strong DNA polymerase activity. Dane particles were observed in media of these cells by immune electron microscopy (data not shown). Double stranded HBV DNA and replicative intermediates were detected in media of these cells. Neither DNA polymerase activity nor HBV DNA was detected in the medium from cells cotransfected with C−P− and P−.

Complementation of recombinants and HBV DNA sequences integrated in PLC/PRF/5 cells (C−P−) for the production of viral particles

PLC/PRF/5 cells have integrated HBV DNA sequences with an intact preS/S gene and defective C and P genes (Ziemer et al., 1985). When they were transfected with the S− recombinant, strong HBCag, HBeAg and DNA polymerase activities, as well as HBV DNA, were detected in the medium (Table 1, line 8; Fig. 2, lane 8). When they were transfected with the C− recombinant, however, no such activities were detectable in the medium.

Viral and subviral particles were harvested from the medium of PLC/PRF/5 cells (C−P−) 5 days after they had been transfected with the S− recombinant. They were separated by sucrose density gradient centrifugation (Fig. 3). HBCag was observed in two fractions with HBV DNA at high sucrose densities, and HBsAg was distributed more widely in fractions at lower densities.
The high density fraction (no. 3) when incubated with a MAb to HBcAg revealed aggregates of core particles in immune electron microscopy (Fig. 4a). The following fraction (no. 4), when incubated with a MAb to HBsAg, was found to contain aggregates of Dane particles and tubular forms of HBsAg (Fig. 4b).

Table 1. Markers of HBV in culture medium and lysates of HepG2 or PLC/PRF/5 cells transfected with recombinants

<table>
<thead>
<tr>
<th>Host cells and recombinants*</th>
<th>Culture medium†</th>
<th>DNA polymerase (c.p.m.)</th>
<th>Lysate‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbsAg (A452)</td>
<td>HBcAg (A452)</td>
<td>HBeAg (A452)</td>
</tr>
<tr>
<td>HepG2 cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) pN342-63(S⁻)</td>
<td>0-03</td>
<td>0-49</td>
<td>1-54</td>
</tr>
<tr>
<td>(2) pODW404(C⁻P⁻)</td>
<td>&gt; 2-00</td>
<td>0-01</td>
<td>0-01</td>
</tr>
<tr>
<td>(3) pRTB840(C⁻)</td>
<td>&gt; 2-00</td>
<td>0-03</td>
<td>0-02</td>
</tr>
<tr>
<td>(4) pJDW55(P⁻)</td>
<td>&gt; 2-00</td>
<td>0-20</td>
<td>1-64</td>
</tr>
<tr>
<td>(5) pN342-63(S⁻) plus ODW404(C⁻P⁻)</td>
<td>&gt; 2-00</td>
<td>0-21</td>
<td>1-03</td>
</tr>
<tr>
<td>(6) pRTB840(C⁻) plus JDW55(P⁻)</td>
<td>&gt; 2-00</td>
<td>0-23</td>
<td>1-10</td>
</tr>
<tr>
<td>(7) pODW404(C⁻P⁻) plus JDW55(P⁻)</td>
<td>&gt; 2-00</td>
<td>0-13</td>
<td>1-61</td>
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<td>Wild-type control§</td>
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<td>1-70</td>
</tr>
<tr>
<td>PLC/PRF/5 cells (C⁻P⁻)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8) pN342-63(S⁻)</td>
<td>&gt; 2-00</td>
<td>0-24</td>
<td>1-14</td>
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<tr>
<td>(9) pRTB840(C⁻)</td>
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<tr>
<td>Cell control§</td>
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<td>0-02</td>
<td>0-01</td>
</tr>
</tbody>
</table>

* Hepatoma cells were transfected with recombinant plasmids carrying tandem dimers of HBV genomes defective in one or two of the S, C and P genes.
† Culture media were tested for HbsAg, HBcAg and HBeAg and, after centrifugation, tested for endogenous DNA polymerase activity.
‡ Cell lysates were tested for HBcAg.
§ HepG2 cells were transfected with a wild-type, replication-competent recombinant [pPYW310 (Okamoto et al., 1990a)].
|| PLC/PRF/5 cells were cultured without being transfected with recombinants.

HBV DNA in viral particles secreted from HepG2 cells cotransfected with S⁻ and C⁻P⁻ recombinants and from PLC/PRF/5 cells (C⁻P⁻) transfected with the S⁻ recombinant

Digestion of the S⁻ recombinant with BstEII and BamHI gave rise to 1743 bp and 1415 bp fragments hybridizable with an HBV DNA probe; a 1800 bp fragment and a much shorter 697 bp fragment, owing to a 718 bp deletion spanning nt 1957 to 2674 (Fig. 1), were produced.
Trans-complementation of HBV mutants

Fig. 4. Immune electron microscopy of viral and subviral particles in culture medium of PLC/PRF/5 cells transfected with pN342-63(S'). Fraction no. 3 in Fig. 3 was incubated with MAb to HBCAg (a), and fraction no. 4 with MAb to HBsAg (b). Bar markers represent 100 nm.

by digesting the C-P- recombinant. HBV DNA in viral particles in the medium of HepG2 cells cotransfected with S- and C-P was analysed by Southern hybridization. Fragments characteristic for these two recombinants were detectable (Fig. 5, lane 4). The hybridization of DNA fragments characteristic for S- was much more intense than of those of C-P-, however.

To confirm the encapsidation of the C-P- genome, an HBV DNA sequence corresponding to nt 1863 to 2833 was amplified by PCR, which produced a fragment of 971 bp for S- and of 235 bp for C-P-. Both of these fragments were demonstrated by amplification of DNA in viral particles secreted from HepG2 cells cotransfected with S- and C-P- (Fig. 6, lane 3). A DNA fragment of 510 bp, designed to detect the plasmid vector (pSP65), was not amplified by PCR with appropriate primers; contamination with either the S- or C-P- recombinant plasmid would have been negligible in this case.

HBV DNA from viral particles in the medium of PLC/PRF/5 cells (C-P-) transfected with S- was analysed for its restriction pattern. BstEII/BamHI fragments of 1415 bp and 1743 bp were observed exclusively, and these were characteristic of S- (Fig. 5, lane 2). The restriction pattern was the same as that of

Fig. 5. Restriction patterns of HBV DNA in viral particles from culture media of cells transfected with recombinant plasmids. Lanes 1 and 2 are for PLC/PRF/5 cells (C-P') transfected with pN342-63(S'). Lanes 3 and 4 are for HepG2 cells cotransfected with pN342-63(S') plus pODW404(C-P'). Lane 5 is for pN342-63(S') and lane 6 for pODW404(C-P') as controls. HBV DNA was analysed by Southern hybridization, without (lanes 1 and 3) or with (other lanes) prior digestion with BstEI and BamHI. A band at the position of 697 bp, slightly visible in lane 5, was visible also in lane 4 in the original blotting, but the visibility has been seriously diminished during multiple duplications.

Fig. 6. Products of amplification by PCR, with HBV and pSP65 primers, of HBV DNA in viral particles from the culture media. Viral particles were obtained from the culture media of HepG2 cells transfected with: lane 1, pN342-63(S'); lane 2, pODW404(C-P'); lane 3, pN342-63(S') plus pODW404(C-P'). As controls, products of PCR on pN342-63(S') are shown in lane 4 and those of pODW404(C-P') in lane 5. Molecular size markers (φX174-RF DNA HaeIII digest; Pharmacia LKB Biotechnology) are in the left-hand lane.
viral particles secreted from HepG2 cells transfected with the S- recombinant.

Discussion

Various deletion mutants of HBV with defects in one or two of the preS/S, preC/C and P genes have been reported in isolates from the circulation of carriers. Mutants with deletions in the C gene within singly coded regions are most frequently observed (Okamoto et al., 1987a; Tran et al., 1991). Wakita et al. (1991) reported deletion mutants with a defective C gene in seven of 11 patients with chronic hepatitis B. Another preferential site for deletion is the preS region which overlaps the P gene (Okamoto et al., 1987a; Gerken et al., 1991; Tran et al., 1991). The corresponding part of the P gene falls in an area between the coding region for the S'-binding protein and that for reverse transcriptase/DNA polymerase, which has been proposed to be a non-essential spacer/tether region (Randziwill et al., 1990).

All reported deletion mutants co-occur with wild-type HBV (Okamoto et al., 1987a; Tran et al., 1991; Wakita et al., 1991) which can help the mutants to replicate by trans-complementation. It is possible, nevertheless, that two or more HBV mutants with complementary gene defects would help each other for their maintenance in persistently infected hosts. We tested four deletion mutants with various gene defects, propagated from plasma of asymptomatic carriers, for the ability to trans-complement the production of viral particles and encapsidation of mutant genomes in HepG2 cells.

Head-to-tail dimers of the four recombinant HBV DNA plasmids with gene defects, pN342-63(S-), pODW404(C-P), pRTB840(C-) and pJDW55(P-), could not direct, by themselves, the secretion of viral particles with mutant genomes from HepG2 cells into culture media. Dane particles were observed by immune electron microscopy in the culture medium harbouring pJDW55(P-), but they would be empty because HBV DNA or DNA polymerase activity was not detectable in the medium.

An unexpected finding was a high DNA polymerase activity, along with some HBeAg activity, observed in the medium of HepG2 cells transfected with pN342-63(S-). The patterns of hybridization in the Southern blot, with replicative intermediates such as covalently closed circular DNA and intact single strands, were different from those of DNA from virions detectable in the serum of persistently infected individuals. These patterns indicated that viral cores would have been leaking from the cytoplasm into the culture medium. The level of DNA polymerase activity in the medium of the S- recombinant, however, was comparable with that in the medium of cells transfected with wild-type HBV, and too high to be accounted for by the release from few, if any, perishing cells. Core particles may have an intrinsic propensity to be secreted, as is observed for an adenovirus-transformed human embryo cell line transfected with a plasmid harbouring recombinant HBV DNA (Jean-Jean et al., 1989).

When HepG2 cells were cotransfected with S- and C-P- recombinants, or with C- and P-, viral particles associated with high DNA polymerase activity were secreted into the culture media. A high efficiency of viral replication, comparable to the wild-type efficiency, would speak against in vitro recombination of two defective genomes for the assembly of viral particles. Rather, they would have arisen by means of trans-complementation between recombinants with defective genes. This trans-complementation among mutants with defective genes has been used to study gene functions (Chang et al., 1989; Schlcht et al., 1989; Bartenschlager et al., 1990; Hirsch et al., 1990; Horwich et al., 1990; Junker-Niepmann et al., 1990; Randziwill et al., 1990). Such complementation occurs among mammalian hepadnaviridae and this extends beyond the species barrier (Okamoto et al., 1990a).

The origin of encapsidated, defective genomes was looked for in the viral particles secreted from HepG2 cells cotransfected with S- and C-P- recombinants. When an HBV DNA sequence (nt 1863 to 2833) including the deletion in C-P- (nt 1957 to 2674) was amplified by PCR, both a normal 791 bp fragment from S- and a shorter 235 bp fragment from C-P- were produced. Both the S- and C-P- genomes, therefore, were capable of being encapsidated. The cis-acting encapsidation signal (e) in HBV DNA of 85 bp spanning nt 1852 to 1936 (Bartenschlager et al., 1990; Junker-Niepmann et al., 1990) was preserved in both S- and C-P-, which would have enabled their encapsidation.

The efficiency of encapsidation, as judged by restriction patterns on Southern blot analysis, was much higher for the S- than for the C-P- genome, however. This finding suggests an action occurring primarily in cis of the P gene products for the packaging of viral genomes, as has been observed by others (Bartenschlager et al., 1990; Hirsch et al., 1990; Junker-Niepmann et al., 1990). A low efficiency of translation by internal initiation at the P gene AUG (Chang et al., 1989; Schlcht et al., 1989) would have decreased the chances of the P gene product encountering heterologous genomes.

Viral particles were produced and secreted from PLC/PRF/5 cells transfected with the S- recombinant. The trans-complementation would be accomplished, therefore, between integrated and episomal forms of HBV DNA. The preS/S gene is kept intact, but the C and P genes are defective, in all seven HBV DNA sequences found integrated in PLC/PRF/5 cells (Ziemer...
et al., 1985). The terminally redundant region with the DR1 and poly(A) signal required for reverse transcription of the pregenome (Will et al., 1987) is not preserved even in a single HBV DNA sequence with the encapsidation signal (Ziemer et al., 1985). HBV DNA encapsidated in viral particles, therefore, would most likely have been from the S gene recombinant. This view was supported by the restriction pattern of packaged HBV DNA which was identical to that of the S recombinant.

Replication-incompetent, defective mutants frequently occur in persistent viral infections (Huang & Baltimore, 1977). They can interfere with the wild-type virus and attenuate its virulence, supporting the survival of both host and the virus itself, and are called defective interfering particles. It remains to be seen, however, whether or not HBV mutants would play a substantial role, acting as defective interfering particles, in maintaining the persistent infection. Defective HBV mutants rarely replace the wild-type virus, and they account for up to 90% of circulating viruses (Okamoto et al., 1987a). None of three HBV DNA clones that had been propagated from the plasma of an individual who had carried HBV for 54 years were found to be defective (Okamoto et al., 1987b). A defective woodchuck hepadnavirus, coinfecting hosts with the wild-type virus, does not increase the frequency of persistent infection in the primary infection (Miller et al., 1990).

It is possible, nevertheless, that there would be interference between defective mutants and the wild-type HBV, which could attenuate its virulence. A mutant of duck hepatitis B virus, with truncated core and envelope genes, suppresses the production of coinfecting wild-type virus in HuH7 cells (Horwich et al., 1990).

References


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