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Structural and functional heterogeneity of naturally occurring hepatitis B virus variants

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Abstract

Most organisms have developed sophisticated machineries to preserve their genomic integrity. On the contrary hepatitis B virus (HBV), like a lot of other viruses can undergo rapid and drastic sequence changes, especially if the virus has to cope with natural or therapy induced antiviral mechanisms in the host. Here, we try to summarize possible implications for the molecular pathogenesis of HBV based on the extensive research on the genetic variants of HBV.

Keywords: Hepatitis B virus; Pathogenesis; Genetic variants

1. Introduction

Despite the availability of an effective vaccine, infection with hepatitis B virus (HBV) is still one of the major world health problems. This is mainly due to the fact that more than 350 million people are chronically infected with HBV worldwide, who are at high risk of developing liver cirrhosis and hepatocellular carcinoma (HCC). There is still little information about the factors, which determine the outcome of HBV infection. The discovery of a large number of HBV sequence variants in acutely infected patients, as well as in chronic carriers has raised the question of whether sequence variability plays a role in viral pathogenesis. In a recent comprehensive review, we have compiled epidemiological and sequence data from the extensive research done during the last decade on the genetic variability of HBV (Günther et al., 1999a). Here, we present some of the conclusions of this compilation, as well as data on the molecular biology and immunology of selected variants, in particular those which are relevant for antiviral treatment.

1.1. Molecular biology and structure of hepatitis B virus

Human HBV belongs to the *Hepadnaviridae* family. The virion is composed of a DNA-con-

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taining nucleocapsid that is surrounded by a lipid layer with the envelope proteins. The circular DNA is partially double-stranded, 3.2-kb in length and codes for four genes (C, S, X, P; Fig. 1). Base pairing of an overlapping region flanked by two direct repeats (DR1 and DR2) allows circularization of the genome with the P protein being covalently linked to the 5′-end of the minus strand. The C gene codes for the nucleocapsid/core protein and the secreted hepatitis B e antigen (HBeAg), and the S gene for the three envelope proteins (pre-S1, pre-S2 and S). The P gene codes a protein (P-protein), which has several functions in virus replication, namely RNA pregenome encapsidation, priming of DNA synthesis, reverse transcription, and plus strand DNA-polymerization. Finally, the X gene codes for the X protein, which is believed to have transactivating activity and a variety of other regulatory functions. The transcripts leading to expression of the viral proteins are initiated at four different promoters and coterminate at a common poly(A) signal (Nassal and Schaller, 1996).

Upon infection of a cell, the open circular HBV DNA is converted in the nucleus into the transcriptionally active, covalently closed circular (ccc DNA) form. Although HBV is a DNA virus, it replicates through an RNA intermediate, the 3.5-kb pregenomic/C mRNA. Its encapsidation into the core particle requires the P protein, the encapsidation signal on the pregenomic RNA, and the core protein. Reverse transcription of the RNA pregenome is performed by the P protein, which has to switch the template three times to accomplish the synthesis of the final virion HBV DNA.

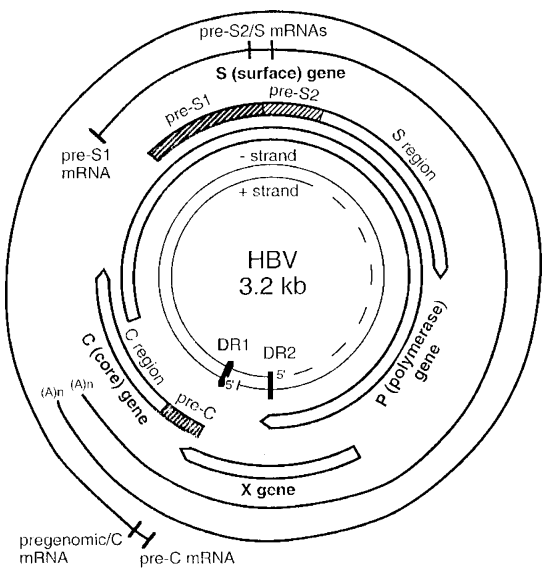

Fig. 1. Schematic representation of the HBV genome.

The C gene promoter drives the synthesis of the pre-C mRNA (initiates upstream of pre-C ATG) and the pregenomic/C mRNA (initiates upstream of C-ATG, i.e. in the pre-core region between the pre-C and C ATG). The pregenomic/C mRNA contains an encapsidation signal in the pre-core region, which is not functional in pre-C mRNA (Pollack and Ganem, 1993; Nassal et al., 1990). Translation beginning at the pre-C ATG yields the pre-core protein, which contains a signal peptide, which is encoded by the pre-core region and directs it to the endoplasmic reticulum (ER; Garcia et al., 1988). Cotranslational cleavage of the signal peptide yields the 22-kDa pre-core protein. This pre-core protein is cleaved C-terminally in the Golgi apparatus yielding a 17-kDa protein (Wang et al., 1991). This 17-kDa protein is secreted and detected in the circulation as HBeAg. Some experimental evidence suggests that the pre-core protein also has a regulating effect on virus production, e.g. by interfering with core protein assembly (Scaglioni et al., 1997a). HBeAg is speculated to downregulate the nucleocapsid-specific immune response during infection (Milich et al., 1997, 1998).

Translation beginning at the C-ATG yields core protein, which self-assembles into core particles. From crystal structure and cryoelectron microscopy data, it can be concluded that two core polypeptides form dimers that assemble into the core particle (Böttcher et al., 1997; Conway et al., 1997; Wynne et al., 1999). Each monomer consists of 183 or 185 amino acids, which form four α-helices. The inner α-helices form an antiparallel hairpin. The tip of this antiparallel hairpin is exposed to the surface, and contains the immunodominant HBcAg epitope. The core protein can be divided into an N-terminal assembly domain (amino acids 1–144) and an arginine-rich C-terminal domain (145–183), which is required

for binding of the pregenomic RNA, genomic replication, and nuclear transport.

The S gene encodes the three surface proteins of HBV. The S gene has three start codons, which divide the gene into the pre-S1, pre-S2 and S region. Transcription of pre-S1 region is initiated at the pre-S1 promoter upstream of the S gene, whereas transcription of the pre-S2 and S regions is directed by the S promoter, which is located in the pre-S1 region. The pre-S1 protein is encoded by the pre-S1, pre-S2 and S region, the pre-S2 protein is encoded by the pre-S2 and S region; and finally the S protein by the S region. The pre-S1 and pre-S2 regions overlap with the non-essential spacer domain of P protein, while the S region overlaps with the reverse transcriptase (RT) domain of the P protein. The surface proteins are cotranslationally inserted into the ER membrane, and, after virus maturation and secretion, are partially exposed on the outside of the virion. The pre-S domain is believed to interact with cellular receptor proteins during infection (Neurath et al., 1986; Klingmüller and Schaller, 1993). The bulk of surface proteins end up as components of non-infectious subviral HBsAg particles lacking core protein and nucleic acids.

The P protein mediates encapsidation of the pregenomic RNA into the core particle and synthesizes the HBV DNA genome therein (Bartenschlager and Schaller, 1992). It has three enzymatic activities: DNA synthesis priming activity, RNA-dependent (RT) and DNA-dependent DNA polymerase activity, and RNase H activity. The primer activity is coded N-terminally, followed by a non-essential spacer region and the polymerase activity domain. The RNase H domain is located C-terminally (Radziwill et al., 1990). In the active site of the RT domain, the HBV polymerase shares conserved residues (YMDD) with other RTs.

For details on the molecular biology of HBV, see recent reviews (Ganem, 1996; Nassal and Schaller, 1996).

1.2. Clinical manifestations of hepatitis B virus

HBV can cause acute and chronic hepatitis. Acute hepatitis can either resolve spontaneously (acute self-limited hepatitis), lead to liver failure (fulminant hepatitis) or can become chronic. Acute hepatitis develops mainly after horizontal transmission (Seeff et al., 1987). In contrast, chronic hepatitis develops mainly after vertical transmission (Stevens et al., 1975; Okada et al., 1976). In particular, if HBV is transmitted perinatally, the following stages of chronic infection can be identified: in the beginning of infection, HBsAg, HbeAg, as well as a high virus load are present in serum, but there are no or only weak signs of inflammatory liver disease (immune tolerance phase). After this phase an inflammatory liver disease can develop, which is frequently followed by seroconversion from HBeAg to anti-HBe. This is commonly associated with low virus load, low inflammatory liver disease (Lee et al., 1990; Chan et al., 1994) and a good prognosis (de Franchis et al., 1993). Resolution of disease is usually accompanied by seroconversion to anti-HBs. Patients with long-standing active liver disease are prone to develop end-stage liver disease (ESLD) and HCC (Beasley et al., 1981; Fattovich et al., 1988).

2. Hepatitis B virus sequence variation

2.1. Phylogenetic evolution of hepatitis B virus genome

Historically, HBV strains were categorized by different antigenic determinants of the HBsAg (Le Bouvier, 1971; Bancroft et al., 1972). This led to the definition of four serological subtypes: adw, adr, ayw, ayr, with d/y depending on a lysine/arginine at position 126, and w/r on a lysine/arginine at position 160 (Okamoto et al., 1987). More recently, HBV strains were categorized by sequence similarities into six genetic groups, the so-called genotypes A–F (Okamoto et al., 1988; Orito et al., 1989; Norder et al., 1992). Between these genetic groups, the sequence differences range from 8.8 to 14.5%, while the intra-type sequence differences range from 1.5 to 4.2% (Norder et al., 1994). A geographical clustering of these genotypes is observed: A is prevalent in the US, Middle and Northern Europe and South

Africa; B and C in the Far East; D in the Mediterranean area and in the Near and Middle East; E in Africa; and F in Latin America (Mangia et al., 1996; Gray et al., 1997; Minami et al., 1996; Naumann et al., 1993; Moraes et al., 1996). Recent data suggest an influence of the genotype on the course of hepatitis (Mayerat et al., 1999).

2.2. Generation of hepatitis B virus variants

Several mechanisms can lead to mutations in the HBV genome. The P protein probably lacks proofreading function and is, together with the cellular RNA polymerase II, the major driving force for the emergence of point-mutations in the HBV genome.

Deletions and insertions could be created by template switching during reverse transcription, splicing of pregenomic RNA, topoisomerase I cleavage/ligation or non-homologous recombination of linear HBV DNA molecules (Su et al., 1989; Suzuki et al., 1989; Wang and Rogler, 1991; Yang and Summers, 1995).

3. Pre-C variants

3.1. Pre-C variants prevent HBeAg synthesis

Three types of mutations are found in the pre-C region, which prevent HBeAg synthesis (designated as pre-C defective variants): first, point mutations inactivating the pre-C ATG; second, insertions/deletions resulting in a frameshift; third, point mutations introducing a stop codon into the pre-C region. These mutations affect only the translation of the pre-core protein, but not that of the core protein (Tong et al., 1991a). The absence of coinfecting HBV without a pre-C defect in serum and liver of patients suggests that HBeAg is not essential for HBV replication in vivo (Ackrill et al., 1992; Dienes et al., 1995; Villari et al., 1995). This view is supported by in vivo infection studies with HBeAg-antigen-negative variants of the related duck hepatitis B virus (DHBV) and woodchuck hepatitis virus (WHV; Chen et al., 1992; Chang et al., 1987; Schneider et al., 1991; Schlicht et al., 1987). In addition, transfection of pre-C defective HBV into hepatoma cells demonstrated that in the absence of HBeAg synthesis does not lead to a defect in replication (Ulrich et al., 1990; Tong et al., 1991b, 1992, 1993; Kim et al., 1992; Yuan et al., 1995). The most common pre-C defective mutation is a G–A change at position 1896, which leads to a stop codon in the penultimate codon of the pre-C region. However, in genotype A, this mutation severely interferes with the function of the RNA encapsidation signal (Li et al., 1993). Because of this restriction at the molecular level, a pre-C defect is rarely observed in regions, where genotype A is prevalent.

3.2. Emergence of pre-C defective hepatitis B virus during chronic infection

Pre-C defective HBV is largely absent from HBeAg-positive patients with normal ALT levels and minimal histological signs of liver inflammation (Lindh et al., 1996). It emerges after activation of hepatitis and in particular during seroconversion to anti-HBe. Therefore, pre-C defective HBV is frequently the predominant or exclusive virus population in anti-HBe-positive patients in regions, where genotype A is not prevalent. Regardless of this event, the compilation of clinical and sequence data from several longitudinal and cross-sectional studies revealed no evidence that the accumulation or presence of pre-C defective HBV is related to a specific outcome of hepatitis. Similarly, the presence of pre-C defective HBV seems not to have a major influence on the success of interferon-alpha therapy (reviewed in Günther et al. (1999a)). The reason for the molecular selection of pre-C defective variants is speculative. While some experimental studies indicate a replication advantage of these viruses, other studies suggest that they are selected by the HBeAg-specific immune response (Zhang and Summers, 1999)

4. C gene variants

4.1. Variants with HBc/e amino acid changes

Seventy five per cent of all amino acids changes occur in 20% of the amino acid sequences of core protein and HBeAg (HBc/e sequence). Sequence

changes occur rarely in domains that are important for core protein dimerization and particle assembly.

4.2. Emergence of HBc/e amino acid changes during chronic infection and their clinical relevance

The prevalence of HBc/e amino acid changes observed during the different phases of chronic infection is very similar to that of pre-C defective variants. During the initial immune tolerance phase, no or very few changes occur in HBc/e. After activation of hepatitis and appearance of anti-HBe about five amino acid changes are introduced into the HBc/e sequence.

Taking into account all currently published data, there is no convincing evidence that the accumulation of these variants significantly influences the outcome of hepatitis. Patients go into remission, have ongoing disease, or develop ESLD or HCC independent of the presence or emergence of amino acid changes in the HBc/e sequence (Uchida et al., 1994; Asahina et al., 1996; Bozkaya et al., 1996; Carman et al., 1997a; Karasawa et al., 1997; Fujiwara et al., 1998).

4.3. Do HBc/e variants allow the virus to escape the immune response?

A large number of HBc/e mutations affect T and B-cell epitopes of the HBc/e protein. A cluster of mutations overlaps with the dominant Th-cell epitope between positions 50 and 69, which is recognized by virtually all patients irrespective of MHC class II background (Ferrari et al., 1991). Whether these mutations reduce the Th cell response is not known. The overlapping HBe1 and HBc B-cell epitopes (Salfeld et al., 1989) also are often affected by one or more amino acid changes during the course of hepatitis (Günther et al., 1998). However, it is currently unclear how mutations in HBc/e B-cell epitopes could interfere with virus clearance, because HBeAg and its precursors are not an integral part of the virion and the nucleocapsid is surrounded by the lipid layer of the virion. Mutations reducing or even hindering the CTL response in vitro have been observed in a chronic carrier, whose CTL response was narrowly focussed on the corresponding epitope (Penna et al., 1991). Another study indicated that this type of response as well as the escape of HBV from CTL recognition is a rare event (Rehermann et al., 1995).

4.4. C gene deletion variants

Most deletions occur in the central part of the C gene and affect the expression and structure of core protein, HBeAg and P protein. The corresponding mutants are defective in replication, because the mutant core protein is unstable and/or cannot assemble into particles (Okamoto et al., 1993; Yuan et al., 1998a Günther et al., 2000). However, the replication of C gene deletion variants can be rescued by complementation with functional core protein (Okamoto et al., 1993; Yuan et al., 1998a), which explains the co-occurrence of HBV genomes with and without deletions in vivo. Although these variants are defective, they can accumulate both in vivo and in vitro. Enhanced replication as well as suppression of wild-type replication may be reasons for their accumulation (Yuan et al., 1998b Günther et al., 2000).

4.5. C gene deletion variants during chronic infection

In contrast to variants with pre-C defects or amino acid changes in HBc/e, C gene deletion variants are most prevalent in HBeAg-positive patients and usually disappear on appearance of anti-HBe (Marinos et al., 1996). Independent of whether C gene deletion variants disappear on seroconversion to anti-HBe, remission of liver disease and decrease of viremia is observed (Marinos et al., 1996; Nakayama et al., 1995), indicating that deletion variants in immunocompetent patients are usually associated with a favourable outcome. In contrast, C gene deletion variants also appear during long-term immunosuppressive treatment, which seems to be associated with a progression of liver disease and a poor outcome (Günther et al., 1996).

5. Core promoter variants

5.1. Functional elements of core promoter

The core promoter consists of a basic core promoter (BCP) and upstream regulatory sequences (Yuh et al., 1992). The BCP contains a major binding site for a variety of transcription factors including hepatocyte nuclear factor 4 (HNF4) and chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1; Raney et al., 1997; Yu and Mertz, 1997).

5.2. The 1762/64-T/A core promoter variants

Most of the promoter mutations affect the major binding site of the BCP. The most frequent change in this binding site is an A→T nucleotide exchange at position 1762 combined with a G→A nucleotide exchange at position 1764. These exchanges result in reduced binding of the COUP-TF1 (Buckwold et al., 1997) and the acquisition of a novel binding site for the liver-specific transcription factor HNF1 (Li et al., 1999). These changes in transcription factor binding probably account for the decreased level of pre-C mRNA and of HBeAg observed in transfection studies with 1762/64-T/A variants (Buckwold et al., 1996; Moriyama et al., 1996; Scaglioni et al., 1997b). Some though not all studies observed an increase of replication due to the 1762/64-T/A mutations (Buckwold et al., 1996; Scaglioni et al., 1997b). In contrast to pre-C defective mutations, 1762/64-T/A mutations are most prevalent in patients with active hepatitis before and after seroconversion to anti-HBe. The reason for selection of the 1762/64-T/A mutant is unclear. As speculated for the pre-C defective mutants, the 1762/64-T/A mutants may be selected, because they produce less HBeAg than HBV without these mutations.

6. S gene variants

6.1. Pre-S1 deletion variants

Deletions have been observed in the 5′-end, central part, as well as at the 3′-end of the pre-S1 region. Nearly all of them are in-frame. Therefore, the corresponding variants can express shorter forms of pre-S1 and P protein with internal deletion. These variants are functional for replication in vitro (Melegari et al., 1994, 1997; Xu and Yen, 1996). Pre-S1 deletions, which result in removal of the CCAAT element of the pre-S2/S promoter, reduce the synthesis of pre-S2 and S mRNA and simultaneously increase the pre-S1 mRNA level. This results in a partial or complete block of HBsAg expression and in the relative overexpression of pre-S1 protein (Xu and Yen, 1996; Bock et al., 1997; Melegari et al., 1997; Okamoto et al., 1993; Melegari et al., 1994). Such dysregulation of surface protein expression has been shown to cause direct or immune-mediated liver cell injury in HBV transgenic mouse models (Chisari et al., 1987; Ando et al., 1993). The virus subpopulation with a deletion in the pre-S1 region may constitute a small fraction of the virus population or may represent the predominant population (Fiordalisi et al., 1994; Gerken et al., 1991; Melegari et al., 1994). Pre-S1 deletion variants have been observed in all stages during the natural course of chronic infection and under immunosuppressive conditions (Günther et al., 1999a; Takayanagi et al., 1993; Fiordalisi et al., 1994; Nakajima et al., 1994; Trautwein et al., 1996; Pult et al., 1997). There is no evidence that the liver disease observed in the animal models parallels the situation in immunocompetent humans infected with pre-S1 deletion variants.

6.2. Pre-S2 deletion variants

Pre-S2 deletions are usually in-frame and occur only in the 5′-end of the pre-S2 region. Corresponding variants can express internally deleted pre-S1, pre-S2, and P protein, and are competent for replication (Fernholz et al., 1993). They seem to emerge preferentially during the late stages of chronic infection and are most prevalent in HBsAg-negative or anti-HBs-positive patients.

6.3. Variants defective in pre-S2 protein expression

Expression of pre-S2 protein can be prevented

by pre-S1/2 deletions overlapping the pre-S2 ATG, deletions which exactly remove the pre-S2 ATG, and point mutations within the pre-S2 ATG. Pre-S2 deletion variants are often the only detectable virus strain indicating that these mutants can replicate autonomously, as shown by in vitro infection experiments for one pre-S2 defective HBV (Fernholz et al., 1993). Thus, pre-S2 protein seems neither essential for HBV replication nor for infectivity. Pre-S2 defective HBV is rare in HBeAg-positive patients, but prevalent in anti-HBe-positive patients and in patients with HCC or ESLD.

6.4. a Determinant mutations

The *a* determinant is the major antigenic site of HBsAg and is exposed on the surface of virions and subviral particles. It forms three loops between S protein positions 121 and 146 (Chen et al., 1996; Qiu et al., 1996). Several cysteine residues hold the structure in its conformation. The *a* determinant is the target of protective or neutralizing anti-HBs antibodies elicited during natural infection, as well as following vaccination with HBsAg particles (Brown et al., 1984; Ogata et al., 1993).

HBV with *a* determinant mutations can emerge during chronic infection and is most prevalent in patients negative for HBsAg or positive for anti-HBs. Vaccinated chimpanzees that were challenged with a specific *a* determinant did not develop an infection (Ogata et al., 1999). This argues for cross-protection against *a* determinant variants, at least in this animal model system. However, the *a* determinant variants also were found in children, who were simultaneously immunized with HBsAg vaccines and anti-HBs immunoglobulin preparations. These children became chronically infected despite having anti-HBs titres generally regarded as protective. In most breakthrough infections, the mothers were infected with wild-type virus, but the children were infected by a mutant (Carman et al., 1990; Fujii et al., 1992; Okamoto et al., 1992; Hino et al., 1995; Oon et al., 1995; Hsu et al., 1997; Lee et al., 1997; Ngui et al., 1997). The increasing selection of these variants by vaccination, their potential to establish a long-term carrier state, and their transmissibility may lead to the spread of *a* determinant variants in the human population, raising potential problems for successful global vaccination in the future. Indeed, data obtained during a 10-years mass-vaccination program indicate an increase of the prevalence of *a* determinant variants in children positive for HBV DNA (Hsu et al., 1999).

Anti-HBs antibodies are passively administrated as immunoprophylaxis following liver transplantation for HBV-related liver disease to prevent graft re-infection. Re-infection of the liver despite protective anti-HBs titres was found to be associated with mutations in the *a* determinant. However, only 36% of all patients treated with a polyclonal antibody showed *a* determinant mutations (Cariani et al., 1995; McNair et al., 1995; Carman et al., 1996; Brind et al., 1997; Sterneck et al., 1997; Ghany et al., 1998; Protzer-Knolle et al., 1998).

The epidemiology of *a* determinant variants strongly suggest that anti-HBs antibodies are responsible for their selection. Furthermore, mutations in the *a* determinant were shown to reduce binding of polyclonal and monoclonal anti-HBs antibodies (Okamoto et al., 1992; Waters et al., 1992; Wallace et al., 1994; Cariani et al., 1995; Carman et al., 1995, 1996; Hou et al., 1995; Kohno et al., 1996; Chiou et al., 1997) Since many diagnostic HBsAg assays use antibodies recognizing the *a* determinant, HBsAg can be falsely negative depending on the assay used and the type of *a* determinant mutation (Carman et al., 1995, 1997b; Hawkins et al., 1996; Jongerius et al., 1998; Weinberger et al., 2000).

7. Mutations affecting P protein

Mutations, which significantly alter the function of the P protein are selected during treatment with nucleoside analogs, such as are lamivudine and famciclovir. These drugs are used to treat patients with re-infection after transplantation and immunocompetent patients with chronic infection. Breakthrough infections

occur in both settings and are associated with the emergence of P protein mutations. Resistance to lamivudine therapy is associated with amino acid changes in the YMDD motif, namely, a change from methionine to valine or isoleucine (Ling et al., 1996; Tipples et al., 1996; Bartholomew et al., 1997; Allen et al., 1998; Buti et al., 1998; Chayama et al., 1998; Niesters et al., 1998) accompanied sometimes by mutations upstream of this motif (Ling et al., 1996; Bartholomew et al., 1997; Allen et al., 1998; Buti et al., 1998; Niesters et al., 1998). In vitro experiments showed that out of several different methionine replacement mutants only the valine and isoleucine mutants are able to replicate and to mediate resistance to lamivudine (Ona-Nita et al., 1999). However, these two mutations strongly decrease the polymerase activity in vitro (Fu and Cheng, 1998; Melegari et al., 1998). Resistance to famciclovir seems not to be associated with amino acid changes in the YMDD motif (Günther et al., 1999b; Pichoud et al., 1999). Instead, mutations upstream and downstream of the YMDD motif, in particular V555I and L528M were observed in breakthrough infections and primary non-responders. The V555I mutation mediates resistance to famciclovir, but not to lamivudine in cell culture (Pichoud et al., 1999).

8. Hepatitis B virus variants in fulminant hepatitis

Less than 1% of acutely infected patients develop fulminant hepatitis. In nearly all sporadic cases of fulminant hepatitis, as well as in the three reported common source epidemics of fulminant hepatitis, the chronically infected source patients were HBeAg-negative (reviewed in Günther et al. (1999a)). Accordingly, mutations, which are prevalent in chronically infected HBeAg-negative patients, such as pre-C defective mutations (in geographic regions with a high prevalence of these variants), HBc/e amino acid changes, 1762/64-T/A mutations, and mutations preventing pre-S2 protein expression also are frequent in patients with fulminant hepatitis. In contrast, these mutations are rare or absent in patients with acute self-limited HBV disease. In these situations, the source patients are frequently HBeAg-positive. Therefore, the transmitted HBV lacks the above mentioned mutations. In conclusion, fulminant hepatitis seems to be preferentially caused by transmission of strains from HBeAg-negative patients. Which of these mutations are responsible for the pathogenesis and which represent only an epiphenomenon is not known.

9. Conclusions

A large variety of mutations can accumulate in HBV genomes of patients during the course of chronic infection. It is unlikely that they emerge and accumulate simply by chance. If one considers the emergence of a variant with a point mutation, only by neutral sequence drift (3×10^{-5} nucleotide changes per position per year; Okamoto et al., 1987), replacement of 1% of the circulating genome pool by this variant would require more than 100 years. Since variants with a pre-C defect, HBc/e amino acid changes, or deletions can accumulate in short periods of time within the virus population, these variants should have a selective advantage over wild-type virus during particular stages of chronic infection. Replication advantage, as well as escape from immune response are possible mechanisms, which may trigger their selection. Each particular stage of chronic infection seems to be characterized by the emergence or presence of specific mutations. However, there is no convincing evidence that the accumulation of specific variants during the natural course of infection heralds the outcome of disease, especially in immunocompetent patients. Conversely, it is well demonstrated that variants selected due to antiviral treatments or vaccination may have a major impact on the success of the therapy and the course of disease.

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References

Ackrill, A.M., Naoumov, N.V., Eddleston, A.L., Williams, R., 1992. Comparison of pre-core/core hepatitis B virus region in liver tissue and serum from patients with chronic hepatitis B infection. J. Hepatol. 16, 224–227.

Allen, M.I., Deslauriers, M., Andrews, C.W., Tipples, G.A., Walters, K.A., Tyrrell, D.L., Brown, N., Condreay, L.D., 1998. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. Lamivudine Clinical Investigation Group. Hepatology 27, 1670–1677.

Ando, K., Moriyama, T., Guidotti, L.G., Wirth, S., Schreiber, R.D., Schlicht, H.J., Huang, S.N., Chisari, F.V., 1993. Mechanisms of class I restricted immunopathology. A transgenic mouse model of fulminant hepatitis. J. Exp. Med. 178, 1541–1554.

Asahina, Y., Enomoto, N., Ogura, Y., Kurosaki, M., Sakuma, I., Izumi, N., Marumo, F., Sato, C., 1996. Sequential changes in full-length genomes of hepatitis B virus accompanying acute exacerbation of chronic hepatitis B. J. Hepatol. 25, 787–794.

Bancroft, W.H., Mundon, F.K., Russell, P.K., 1972. Detection of additional antigenic determinants of hepatitis B antigen. J. Immunol. 109, 842–848.

Bartenschlager, R., Schaller, H., 1992. Hepadnaviral assembly is initiated by polymerase binding to the encapsidation signal in the viral RNA genome. Eur. Mol. Biol. Org. J. 11, 3413–3420.

Bartholomew, M.M., Jansen, R.W., Jeffers, L.J., Reddy, K.R., Johnson, L.C., Bunzendahl, H., Condreay, L.D., Tzakis, A.G., Schiff, E.R., Brown, N.A., 1997. Hepatitis-B-virus resistance to lamivudine given for recurrent infection after orthotopic liver transplantation. Lancet 349, 20–22.

Beasley, R.P., Hwang, L.Y., Lin, C.C., Chien, C.S., 1981. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22.707 men in Taiwan. Lancet 2, 1129–1133.

Bock, C.T., Tillmann, H.L., Maschek, H.J., Manns, M.P., Trautwein, C., 1997. A preS mutation isolated from a patient with chronic hepatitis B infection leads to virus retention and misassembly. Gastroenterology 113, 1976–1982.

Böttcher, B., Wynne, S.A., Crowther, R.A., 1997. Determination of the fold of the core protein of hepatitis B virus by electron cryomicroscopy. Nature 386, 88–91.

Bozkaya, H., Ayola, B., Lok, A.S.F., 1996. High rate of mutations in the hepatitis B core gene during the immune clearance phase of chronic hepatitis B virus infection. Hepatology 24, 32–37.

Brind, A., Jiang, J., Samuel, D., Gigou, M., Feray, C., Brechot, C., Kremsdorf, D., 1997. Evidence for selection of hepatitis B mutants after liver transplantation through peripheral blood mononuclear cell infection. J. Hepatol. 26, 228–235.

Brown, S.E., Howard, C.R., Zuckerman, A.J., Steward, M.W., 1984. Affinity of antibody responses in man to hepatitis B vaccine determined with synthetic peptides. Lancet 2, 184–187.

Buckwold, V.E., Xu, Z.C., Chen, M., Yen, T.S.B., Ou, J.H., 1996. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. J. Virol. 70, 5845–5851.

Buckwold, V.E., Xu, Z., Yen, T.S., Ou, J.H., 1997. Effects of a frequent double-nucleotide basal core promoter mutation and its putative single-nucleotide precursor mutations on hepatitis B virus gene expression and replication. J. Gen. Virol. 78, 2055–2065.

Buti, M., Jardi, R., Cotrina, M., Rodriguez-Frias, F., Esteban, R., Guardia, J., 1998. Transient emergence of hepatitis B variants in a patient with chronic hepatitis B resistant to lamivudine. J. Hepatol. 28, 510–513.

Cariani, E., Ravaggi, A., Tanzi, E., Romano, L., Fiordalisi, G., Bellati, G., Caccamo, L., Galmarini, D., Albertini, A., Zanetti, A., 1995. Emergence of hepatitis B virus S gene mutant in a liver transplant recipient. J. Med. Virol. 47, 410–415.

Carman, W.F., Zanetti, A.R., Karayiannis, P., Waters, J., Manzillo, G., Tanzi, E., Zuckerman, A.J., Thomas, H.C., 1990. Vaccine-induced escape mutant of hepatitis B virus. Lancet 336, 325–329.

Carman, W.F., Korula, J., Wallace, L., MacPhee, R., Mimms, L., Decker, R., 1995. Fulminant reactivation of hepatitis B due to envelope protein mutant that escaped detection by monoclonal HBsAg ELISA. Lancet 345, 1406–1407.

Carman, W.F., Trautwein, C., Vandeursen, F.J., Colman, K., Dornan, E., McIntyre, G., Waters, J., Kliem, V., Müller, R., Thomas, H.C., Manns, M.P., 1996. Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. Hepatology 24, 489–493.

Carman, W.F., Boner, W., Fattovich, G., Colman, K., Dornan, E.S., Thursz, M., Hadziyannis, S., 1997a. Hepatitis B virus core protein mutations are concentrated in B cell epitopes in progressive disease and in T helper cell epitopes during clinical remission. J. Infect. Dis. 175, 1093–1100.

Carman, W.F., Van Deursen, F.J., Mimms, L.T., Hardie, D., Coppola, R., Decker, R., Sanders, R., 1997b. The prevalence of surface antigen variants of hepatitis B virus in Papua New Guinea, South Africa, and Sardinia. Hepatology 26, 1658–1666.

Chan, C.Y., Lee, S.D., Yu, M.I., Wang, Y.J., Tsai, Y.T., Lo, K.J., 1994. Long-term follow-up of hepatitis B virus carrier infants. J. Med. Virol. 44, 336–339.

Chang, C., Enders, G., Sprengel, R., Peters, N., Varmus, H.E., Ganem, D., 1987. Expression of the precore region of an avian hepatitis B virus is not required for viral replication. J. Virol. 61, 3322–3325.

Chayama, K., Suzuki, Y., Kobayashi, M., Kobayashi, M., Tsubota, A., Hashimoto, M., Miyano, Y., Koike, H., Kobayashi, M., Koida, I., Arase, Y., Saitoh, S., Murashima, N., Ikeda, K., Kumada, H., 1998. Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. Hepatology 27, 1711–1716.

Chen, H.S., Kew, M.C., Hornbuckle, W.E., Tennant, B.C., Cote, P.J., Gerin, J.L., Purcell, R.H., Miller, R.H., 1992. The precore gene of the woodchuck hepatitis virus genome is not essential for viral replication in the natural host. J. Virol. 66, 5682–5684.

Chen, Y.C.J., Delbrook, K., Dealwis, C., Mimms, L., Mushahwar, I.K., Mandecki, W., 1996. Discontinuous epitopes of hepatitis B surface antigen derived from a filamentous phage peptide library. Proc. Natl. Acad. Sci. USA 93, 1997–2001.

Chiou, H.L., Lee, T.S., Kuo, J., Mau, Y.C., Ho, M.S., 1997. Altered antigenicity of 'a' determinant variants of hepatitis B virus. J. Gen. Virol. 78, 2639–2645.

Chisari, F.V., Filippi, P., Buras, J., McLachlan, A., Popper, H., Pinkert, C.A., Palmiter, R.D., Brinster, R.L., 1987. Structural and pathological effects of synthesis of hepatitis B virus large envelope polypeptide in transgenic mice. Proc. Natl. Acad. Sci. USA 84, 6909–6913.

Conway, J.F., Cheng, N., Zlotnick, A., Wingfield, P.T., Stahl, S.J., Steven, A.C., 1997. Visualization of a 4-helix bundle in the hepatitis B virus capsid by cryo-electron microscopy. Nature 386, 91–94.

de Franchis, R., Meucci, G., Vecchi, M., Tatarella, M., Colombo, M., Del Ninno, E., Rumi, M.G., Donato, M.F., Ronchi, G., 1993. The natural history of asymptomatic hepatitis B surface antigen carriers. Ann. Intern. Med. 118, 191–194.

Dienes, H.P., Gerken, G., Goergen, B., Heermann, K., Gerlich, W., Meyer zum Büschenfelde, K.H., 1995. Analysis of the precore DNA sequence and detection of precore antigen in liver specimens from patients with anti-hepatitis B e-positive chronic hepatitis. Hepatology 21, 1–7.

Fattovich, G., Brollo, L., Alberti, A., Pontisso, P., Giustina, G., Realdi, G., 1988. Long-term follow-up of anti-HBe-positive chronic active hepatitis B. Hepatology 8, 1651–1654.

Fernholz, D., Galle, P.R., Stemler, M., Brunetto, M., Bonino, F., Will, H., 1993. Infectious hepatitis B virus variant defective in pre-S2 protein expression in a chronic carrier. Virology 194, 137–148.

Ferrari, C., Bertoletti, A., Penna, A., Cavalli, A., Valli, A., Missale, G., Pilli, M., Fowler, P., Giuberti, T., Chisari, F.V., Fiaccadori, F., 1991. Identification of immunodominant T cell epitopes of the hepatitis B virus nucleocapsid antigen. J. Clin. Invest. 88, 214–222.

Fiordalisi, G., Ghiotto, F., Castelnuovo, F., Primi, D., Cariani, E., 1994. Analysis of the hepatitis B virus genome and immune response in HBsAg, anti-HBs positive chronic hepatitis. J. Hepatol. 20, 487–493.

Fu, L., Cheng, Y.C., 1998. Role of additional mutations outside the YMDD motif of hepatitis B virus polymerase in L(−)SddC (3TC) resistance. Biochem. Pharmacol. 55, 1567–1572.

Fujii, H., Moriyama, K., Sakamoto, N., Kondo, T., Yasuda, K., Hiraizumi, Y., Yamazaki, M., Sakaki, Y., Okochi, K., Nakajima, E., 1992. Gly145 to Arg substitution in HBs antigen of immune escape mutant of hepatitis B virus. Biochem. Biophys. Res. Commun. 184, 1152–1157.

Fujiwara, K., Yokosuka, O., Ehata, T., Chuang, W.L., Imazeki, F., Saisho, H., Omata, M., 1998. The two different states of hepatitis B virus DNA in asymptomatic carriers: HBe-antigen-positive versus anti-HBe-positive asymptomatic carriers. Dig. Dis. Sci. 43, 368–376.

Ganem, D., 1996. Hepadnaviridae and their replication. In: Fields, B.N., Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, third ed. Lippincott, Philadelphia, pp. 2703–2737.

Garcia, P.D., Ou, J.H., Rutter, W.J., Walter, P., 1988. Targeting of the hepatitis B virus precore protein to the endoplasmic reticulum membrane: after signal peptide cleavage translocation can be aborted and the product released into the cytoplasm. J. Cell Biol. 106, 1093–1104.

Gerken, G., Kremsdorf, D., Capel, F., Petit, M.A., Dauguet, C., Manns, M.P., Meyer zum Büschenfelde, K.H., Brechot, C., 1991. Hepatitis B defective virus with rearrangements in the preS gene during chronic HBV infection. Virology 183, 555–565.

Ghany, M.G., Ayola, B., Villamil, F.G., Gish, R.G., Rojter, S., Vierling, J.M., Lok, A.S., 1998. Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. Hepatology 27, 213–222.

Gray, A.H., Fang, J.W., Davis, G.L., Mizokami, M., Wu, P.C., Williams, R., Schuster, S.M., Lau, J.Y., 1997. Variations of hepatitis B virus core gene sequence in Western patients with chronic hepatitis B virus infection. J. Viral. Hepat. 4, 371–378.

Günther, S., Baginski, S., Kissel, H., Reinke, P., Krüger, D.H., Will, H., Meisel, H., 1996. Accumulation and persistence of hepatitis B virus core gene deletion mutants in renal transplant patients are associated with end-stage liver disease. Hepatology 24, 751–758.

Günther, S., Paulij, W., Meisel, H., Will, H., 1998. Analysis of hepatitis B virus populations in an interferon-alpha-treated patient reveals predominant mutations in the C-gene and changing e-antigenicity. Virology 244, 146–160.

Günther, S., Fischer, L., Pult, I., Sterneck, M., Will, H., 1999a. Naturally occurring variants of hepatitis B virus. Adv. Virus Res. 52, 25–137.

Günther, S., von Breunig, F., Santantonio, T., Jung, M.-C., Gaeta, G.B., Fischer, L., Sterneck, M., Will, H., 1999b. Absence of mutations in the YMDD motif/B region of the hepatitis B virus polymerase in famciclovir therapy failure. J. Hepatol. 30, 749–754.

Günther, S., Piwon, N., Jung, A., Iwanska, A., Schmitz, H., Will, H., 2000. Enhanced replication contributes to enrichment of hepatitis B virus with deletion in core gene. Virology 273, 286–299.

Hawkins, A.E., Gilson, R.J.C., Gilbert, N., Wreghitt, T.G., Gray, J.J., Ahlers-de Boer, I., Tedder, R.S., Alexander, G.M.J., 1996. Hepatitis B virus surface mutations associated with infection after liver transplantation. J. Hepatol. 24, 8–14.

Hino, K., Okuda, M., Hashimoto, O., Ishiko, H., Okazaki, M., Fujii, K., Hanada, H., Okita, K., 1995. Glycine-to-arginine substitution at codon 145 of HBsAg in two infants born to hepatitis B e antigen-positive carrier. Dig. Dis. Sci. 40, 566–570.

Hou, J., Karayiannis, P., Waters, J., Luo, K., Liang, C., Thomas, H.C., 1995. A unique insertion in the S gene of surface antigen-negative hepatitis B virus Chinese carriers. Hepatology 21, 273–278.

Hsu, H.Y., Chang, M.H., Ni, Y.H., Lin, H.H., Wang, S.M., Chen, D.S., 1997. Surface gene mutants of hepatitis B virus in infants who develop acute or chronic infections despite immunoprophylaxis. Hepatology 26, 786–791.

Hsu, H.Y., Chang, M.H., Liaw, S.H., Ni, Y.H., Chen, H.L., 1999. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. Hepatology 30, 1312–1317.

Jongerius, J.M., Wester, M., Cuypers, H.T., van Oostendorp, W.R., Lelie, P.N., van der Poel, C.L., van Leeuwen, E.F., 1998. New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. Transfusion 38, 56–59.

Karasawa, T., Shirasawa, T., Okawa, Y., Kuramoto, A., Shimada, N., Aizawa, Y., Zeniya, M., Toda, G., 1997. Association between frequency of amino acid changes in core region of hepatitis B virus (HBV) and the presence of precore mutation in Japanese HBV carriers. J. Gastroenterol. 32, 611–622.

Kim, S.H., Hong, S.P., Kim, S.K., Lee, W.S., Rho, H.M., 1992. Replication of a mutant hepatitis B virus with a fused X-C reading frame in hepatoma cells. J. Gen. Virol. 73, 2421–2424.

Klingmüller, U., Schaller, H., 1993. Hepadnavirus infection requires interaction between the viral pre-S domain and a specific hepatocellular receptor. J. Virol. 67, 7414–7422.

Kohno, H., Inoue, T., Tsuda, F., Okamoto, H., Akahane, Y., 1996. Mutations in the envelope gene of hepatitis B virus variants co-occurring with antibody to surface antigen in sera from patients with chronic hepatitis B. J. Gen. Virol. 77, 1825–1831.

Le Bouvier, G.L., 1971. The heterogeneity of Australia antigen. J. Infect. Dis. 123, 671–675.

Lee, P.I., Chang, M.H., Lee, C.Y., Hsu, H.Y., Chen, J.S., Chen, P.J., Chen, D.S., 1990. Changes of serum hepatitis B virus DNA and aminotransferase levels during the course of chronic hepatitis B virus infection in children. Hepatology 12, 657–660.

Lee, P.I., Chang, L.Y., Lee, C.Y., Huang, L.M., Chang, M.H., 1997. Detection of hepatitis B surface gene mutation in carrier children with or without immunoprophylaxis at birth. J. Infect. Dis. 176, 427–430.

Li, J.S., Tong, S.P., Wen, Y.M., Vitvitski, L., Zhang, Q., Trepo, C., 1993. Hepatitis B virus genotype A rarely circulates as an HBe-minus mutant: possible contribution of a single nucleotide in the precore region. J. Virol. 67, 5402–5410.

Li, J., Buckwold, V.E., Hon, M.W., Ou, J.H., 1999. Mechanism of suppression of hepatitis B virus precore RNA transcription by a frequent double mutation. J. Virol. 73, 1239–1244.

Lindh, M., Horal, P., Dhillon, A.P., Furuta, Y., Norkrans, G., 1996. Hepatitis B virus carriers without precore mutations in hepatitis B e antigen-negative stage show more severe liver damage. Hepatology 24, 494–501.

Ling, R., Mutimer, D., Ahmed, N., Boxall, E.H., Elias, E., Dusheiko, G.M., Harrison, T.J., 1996. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. Hepatology 24, 711–713.

Mangia, A., Chung, Y.H., Hoofnagle, J.H., Birkenmeyer, L., Mushahwar, I., Di Bisceglie, A.M., 1996. Pathogenesis of chronic liver disease in patients with chronic hepatitis B virus infection without serum HBeAg. Dig. Dis. Sci. 41, 2447–2452.

Marinos, G., Torre, F., Günther, S., Thomas, M.G., Will, H., Williams, R., Naoumov, N.V., 1996. Hepatitis B virus variants with core gene deletions in the evolution of chronic hepatitis B infection. Gastroenterology 111, 183–192.

Mayerat, C., Mantegani, A., Frei, P.C., 1999. Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? J. Viral. Hepat. 6, 299–304.

McNair, A.N., Viaggi, P., Smith, H.M., Tibbs, C.J., Naoumov, N.V., Williams, R., 1995. Hepatitis B envelope protein mutants. Lancet 346, 318–319 Letter.

Melegari, M., Bruno, S., Wands, J.R., 1994. Properties of hepatitis B virus pre-S1 deletion mutants. Virology 199, 292–300.

Melegari, M., Scaglioni, P.P., Wands, J.R., 1997. The small envelope protein is required for secretion of a naturally occurring hepatitis B virus mutant with pre-S1 deleted. J. Virol. 71, 5449–5454.

Melegari, M., Scaglioni, P.P., Wands, J.R., 1998. Hepatitis B virus mutants associated with 3TC and famciclovir administration are replication defective. Hepatology 27, 628–633.

Milich, D.R., Schödel, F., Hughes, J.L., Jones, J.E., Peterson, D.L., 1997. The hepatitis B virus core and e antigens elicit different Th cell subsets: antigen structure can affect Th cell phenotype. J. Virol. 71, 2192–2201.

Milich, D.R., Chen, M.K., Hughes, J.L., Jones, J.E., 1998. The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. J. Immunol. 160, 2013–2021.

Minami, M., Poussin, K., Kew, M., Okanoue, T., Brechot, C., Paterlini, P., 1996. Precore/core mutations of hepatitis B virus in hepatocellular carcinomas developed on noncirrhotic livers. Gastroenterology 111, 691–700.

Moraes, M.T.B., Gomes, S.A., Niel, C., 1996. Sequence analysis of pre-S/S gene of hepatitis B virus strains of genotypes A, D, and F isolated in Brazil. Arch. Virol. 141, 1767–1773.

Moriyama, K., Okamoto, H., Tsuda, F., Mayumi, M., 1996. Reduced precore transcription and enhanced core-pregenome transcription of hepatitis B virus DNA after replacement of the precore-core promoter with sequences associated with e antigen-seronegative persistent infections. Virology 226, 269–280.

Nakajima, E., Minami, M., Ochiya, T., Kagawa, K., Okanoue, T., 1994. PreS1 deleted variants of hepatitis B virus in patients with chronic hepatitis. J. Hepatol. 20, 329–335.

Nakayama, J., Nakanishi, T., Arataki, K., Obatake, T., Moriya, T., Kajiyama, G., 1995. Changes in the hepatitis B virus precore and core regions in association with the disappearance of hepatitis B e antigen in patients with chronic hepatitis B. Intern. Med. 34, 319–325.

Nassal, M., Junker-Niepmann, M., Schaller, H., 1990. Translational inactivation of RNA function: discrimination against a subset of genomic transcripts during HBV nucleocapsid assembly. Cell 63, 1357–1363.

Nassal, M., Schaller, H., 1996. Hepatitis B virus replication-an update. J. Viral. Hepat. 3, 217–226.

Naumann, H., Schaefer, S., Yoshida, C.F., Gaspar, A.M., Repp, R., Gerlich, W.H., 1993. Identification of a new hepatitis B virus (HBV) genotype from Brazil that expresses HBV surface antigen subtype adw4. J. Gen. Virol. 74, 1627–1632.

Neurath, A.R., Kent, S.B., Strick, N., Parker, K., 1986. Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. Cell 46, 429–436.

Ngui, S.L., O'Connell, S., Eglin, R.P., Heptonstall, J., Teo, C.G., 1997. Low detection rate and maternal provenance of hepatitis B virus S gene mutants in cases of failed postnatal immunoprophylaxis in England and Wales. J. Infect. Dis. 176, 1360–1365.

Niesters, H.G., Honkoop, P., Haagsma, E.B., de Man, R.A., Schalm, S.W., Osterhaus, A.D., 1998. Identification of more than one mutation in the hepatitis B virus polymerase gene arising during prolonged lamivudine treatment. J. Infect. Dis. 177, 1382–1385.

Norder, H., Courouce, A.M., Magnius, L.O., 1992. Molecular basis of hepatitis B virus serotype variations within the four major subtypes. J. Gen. Virol. 73, 3141–3145.

Norder, H., Courouce, A.M., Magnius, L.O., 1994. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 198, 489–503.

Ogata, N., Östberg, L., Ehrlich, P.H., Wong, D.C., Miller, R.H., Purcell, R.H., 1993. Markedly prolonged incubation period of hepatitis B in a chimpanzee passively immunized with a human monoclonal antibody to the *a* determinant of hepatitis B surface antigen. Proc. Natl. Acad. Sci. USA 90, 3014–3018.

Ogata, N., Cote, P.J., Zanetti, A.R., Miller, R.H., Shapiro, M., Gerin, J., Purcell, R.H., 1999. Licensed recombinant hepatitis B vaccines protect chimpanzees against infection with the prototype surface gene mutant of hepatitis B virus. Hepatology 30, 779–786.

Okada, K., Kamiyama, I., Inomata, M., Imai, M., Miyakawa, Y., 1976. e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. New Engl. J. Med. 294, 746–749.

Okamoto, H., Imai, M., Tsuda, F., Tanaka, T., Miyakawa, Y., Mayumi, M., 1987. Point mutation in the S gene of hepatitis B virus for a d/y or w/r subtypic change in two blood donors carrying a surface antigen of compound subtype adyr or adwr. J. Virol. 61, 3030–3034.

Okamoto, H., Tsuda, F., Sakugawa, H., Sastrosoewignjo, R.I., Imai, M., Miyakawa, Y., Mayumi, M., 1988. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J. Gen. Virol. 69, 2575–2583.

Okamoto, H., Yano, K., Nozaki, Y., Matsui, A., Miyazaki, H., Yamamoto, K., Tsuda, F., Machida, A., Mishiro, S., 1992. Mutations within the S gene of hepatitis B virus transmitted from mothers to babies immunized with hepatitis B immune globulin and vaccine. Pediatr. Res. 32, 264–268.

Okamoto, H., Wang, Y., Tanaka, T., Machida, A., Miyakawa, Y., Mayumi, M., 1993. 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. J. Gen. Virol. 74, 407–414.

Ona-Nita, S.K., Kato, N., Shiratori, Y., Masaki, T., Lan, K.H., Carrilho, F.J., Omata, M., 1999. YMDD motif in hepatitis B virus DNA polymerase influences on replication and lamivudine resistance: a study by in vitro full-length viral DNA transfection, 1999. Hepatology 29, 939–945.

Oon, C.J., Lim, G.K., Ye, Z., Goh, K.T., Tan, K.L., Yo, S.L., Hopes, E., Harrison, T.J., Zuckerman, A.J., 1995. Molecular epidemiology of hepatitis B virus vaccine variants in Singapore. Vaccine 13, 699–702.

Orito, E., Mizokami, M., Ina, Y., Moriyama, E.N., Kameshima, N., Yamamoto, M., Gojobori, T., 1989. Host-independent evolution and a genetic classification of the hepadnavirus family based on nucleotide sequences. Proc. Natl. Acad. Sci. USA 86, 7059–7062.

Penna, A., Chisari, F.V., Bertoletti, A., Missale, G., Fowler, P., Giuberti, T., Fiaccadori, F., Ferrari, C., 1991. Cytotoxic T lymphocytes recognize an HLA-A2-restricted epitope within the hepatitis B virus nucleocapsid antigen. J. Exp. Med. 174, 1565–1570.

Pichoud, C., Seigneres, B., Wang, Z., Trepo, C., Zoulim, F., 1999. Transient selection of a hepatitis B virus polymerase gene mutant associated with a decreased replication capacity and famciclovir resistance. Hepatology 29, 230–237.

Pollack, J.R., Ganem, D., 1993. An RNA stem-loop structure directs hepatitis B virus genomic RNA encapsidation. J. Virol. 67, 3254–3263.

Protzer-Knolle, U., Naumann, U., Bartenschlager, R., Berg, T., Hopf, U., Meyer zum Büschenfelde, K.H., Neuhaus, P., Gerken, G., 1998. Hepatitis B virus with antigenically altered hepatitis B surface antigen is selected by high-dose hepatitis B immune globulin after liver transplantation. Hepatology 27, 254–263.

Pult, I., Chouard, T., Wieland, S., Klemenz, R., Yaniv, M., Blum, H.E., 1997. A hepatitis B virus mutant with a new hepatocyte nuclear factor 1 binding site emerging in transplant-transmitted fulminant hepatitis B. Hepatology 25, 1507–1515.

Qiu, X.X., Schroeder, P., Bridon, D., 1996. Identification and characterization of a C(K/R)TC motif as a common epitope present in all subtypes of hepatitis B surface antigen. J. Immunol. 156, 3350–3356.

Radziwill, G., Tucker, W., Schaller, H., 1990. Mutational analysis of the hepatitis B virus P gene product: domain structure and RNase H activity. J. Virol. 64, 613–620.

Raney, A.K., Johnson, J.L., Palmer, C.N., McLachlan, A., 1997. Members of the nuclear receptor superfamily regulate transcription from the hepatitis B virus nucleocapsid promoter. J. Virol. 71, 1058–1071.

Rehermann, B., Pasquinelli, C., Mosier, S.M., Chisari, F.V., 1995. Hepatitis B virus (HBV) sequence variation of cytotoxic T lymphocyte epitopes is not common in patients with chronic HBV infection. J. Clin. Invest. 96, 1527–1534.

Salfeld, J., Pfaff, E., Noah, M., Schaller, H., 1989. Antigenic determinants and functional domains in core antigen and e antigen from hepatitis B virus. J. Virol. 63, 798–808.

Scaglioni, P.P., Melegari, M., Wands, J.R., 1997a. Posttranscriptional regulation of hepatitis B virus replication by the precore protein. J. Virol. 71, 345–353.

Scaglioni, P.P., Melegari, M., Wands, J.R., 1997b. Biologic properties of hepatitis B viral genomes with mutations in the precore promoter and precore open reading frame. Virology 233, 374–381.

Schlicht, H.J., Salfeld, J., Schaller, H., 1987. The duck hepatitis B virus pre-C region encodes a signal sequence which is essential for synthesis and secretion of processed core proteins but not for virus formation. J. Virol. 61, 3701–3709.

Schneider, R., Fernholz, D., Wildner, G., Will, H., 1991. Mechanism, kinetics, and role of duck hepatitis B virus e-antigen expression in vivo. Virology 182, 503–512.

Seeff, L.B., Beebe, G.W., Hoofnagle, J.H., Norman, J.E., Buskell, B.Z., Waggoner, J.G., Kaplowitz, N., Koff, R.S., Petrini, J.J., Schiff, E.R., Shorey, J., Stanley, M.M., 1987. A serologic follow-up of the 1942 epidemic of post-vaccination hepatitis in the United States Army. New Engl. J. Med. 316, 965–970.

Sterneck, M., Günther, S., Gerlach, J., Naoumov, N.V., Santantonio, T., Fischer, L., Rogiers, X., Greten, H., Williams, R., Will, H., 1997. Hepatitis B virus sequence changes evolving in liver transplant recipients with fulminant hepatitis. J. Hepatol. 26, 754–764.

Stevens, C.E., Beasley, R.P., Tsui, J., Lee, W.C., 1975. Vertical transmission of hepatitis B antigen in Taiwan. New Engl. J. Med. 292, 771–774.

Su, T.S., Lai, C.J., Huang, J.L., Lin, L.H., Yauk, Y.K., Chang, C.M., Lo, S.J., Han, S.H., 1989. Hepatitis B virus transcript produced by RNA splicing. J. Virol. 63, 4011–4018.

Suzuki, T., Masui, N., Kajino, K., Saito, I., Miyamura, T., 1989. Detection and mapping of spliced RNA from a human hepatoma cell line transfected with the hepatitis B virus genome. Proc. Natl. Acad. Sci. USA 86, 8422–8426.

Takayanagi, M., Kakumu, S., Ishikawa, T., Higashi, Y., Yoshioka, K., Wakita, T., 1993. Comparison of envelope and precore/core variants of hepatitis B virus (HBV) during chronic HBV infection. Virology 196, 138–145.

Tipples, G.A., Ma, M.M., Fischer, K.P., Bain, V.G., Kneteman, N.M., Tyrrell, D.L.J., 1996. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. Hepatology 24, 714–717.

Tong, S.P., Brotman, B., Li, J.S., Vitvitski, L., Pascal, D., Prince, A.M., Trepo, C., 1991a. In vitro and in vivo replication capacity of the precore region defective hepatitis B virus variants. J. Hepatol. 13 (Suppl. 4), S68–S73.

Tong, S.P., Diot, C., Gripon, P., Li, J., Vitvitski, L., Trepo, C., Guguen-Guillouzo, C., 1991b. In vitro replication competence of a cloned hepatitis B virus variant with a nonsense mutation in the distal pre-C region. Virology 181, 733–737.

Tong, S.P., Li, J.S., Vitvitski, L., Trepo, C., 1992. Replication capacities of natural and artificial precore stop codon mutants of hepatitis B virus: relevance of pregenome encapsidation signal. Virology 191, 237–245.

Tong, S.P., Li, J.S., Vitvitski, L., Kay, A., Trepo, C., 1993. Evidence for a base-paired region of hepatitis B virus pregenome encapsidation signal which influences the patterns of precore mutations abolishing HBe protein expression. J. Virol. 67, 5651–5655.

Trautwein, C., Schrem, H., Tillman, H.L., Kubicka, S., Walker, D., Böker, K.H.W., Maschek, H.J., Pichlmayr, R., Manns, M.P., 1996. Hepatitis B virus mutations in the pre-S genome before and after liver transplantation. Hepatology 24, 482–488.

Uchida, T., Aye, T.T., Shikata, T., Yano, M., Yatsuhashi, H., Koga, M., Mima, S., 1994. Evolution of the hepatitis B virus gene during chronic infection in seven patients. J. Med. Virol. 43, 148–154.

Ulrich, P.P., Bhat, R.A., Kelly, I., Brunetto, M.R., Bonino, F., Vyas, G.N., 1990. A precore-defective mutant of hepatitis B virus associated with e antigen-negative chronic liver disease. J. Med. Virol. 32, 109–118.

Villari, D., Pollicino, T., Spinella, S., Russo, F., Campo, S., Rodino, G., Squadrito, G., Longo, G., Raimondo, G.,

1995. Hepatitis BE antigen detection in formalin-fixed liver biopsy specimens. A tool to investigate wild-type and E-minus variant HBV infection. Am. J. Clin. Pathol. 103, 136–140.

Wallace, L.A., Echevarria, J.E., Echevarria, J.M., Carman, W.F., 1994. Molecular characterization of envelope antigenic variants of hepatitis B virus from Spain. J. Infect. Dis. 170, 1300–1303.

Wang, H.P., Rogler, C.E., 1991. Topoisomerase I-mediated integration of hepadnavirus DNA in vitro. J. Virol. 65, 2381–2392.

Wang, J., Lee, A.S., Ou, J.H., 1991. Proteolytic conversion of hepatitis B virus e antigen precursor to end product occurs in a postendoplasmic reticulum compartment. J. Virol. 65, 5080–5083.

Waters, J.A., Kennedy, M., Voet, P., Hauser, P., Petre, J., Carman, W., Thomas, H.C., 1992. Loss of the common 'A' determinant of hepatitis B surface antigen by a vaccine-induced escape mutant. J. Clin. Invest. 90, 2543–2547.

Weinberger, K.M., Bauer, T., Böhm, S., Jilg, W., 2000. High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. J. Gen. Virol. 81, 1165–1174.

Wynne, S.A., Crowther, R.A., Leslie, A.G.W., 1999. The crystal structure of the human hepatitis B virus capsid. Cell 3, 771–780.

Xu, Z.C., Yen, T.S.B., 1996. Intracellular retention of surface protein by a hepatitis B virus mutant that releases virion particles. J. Virol. 70, 133–140.

Yang, W., Summers, J., 1995. Illegitimate replication of linear hepadnavirus DNA through nonhomologous recombination. J. Virol. 69, 4029–4036.

Yu, X., Mertz, J.E., 1997. Differential regulation of the pre-C and pregenomic promoters of human hepatitis B virus by members of the nuclear receptor superfamily. J. Virol. 71, 9366–9374.

Yuan, T.T., Faruqi, A., Shih, J.W., Shih, C., 1995. The mechanism of natural occurrence of two closely linked HBV precore predominant mutations. Virology 211, 144–156.

Yuan, T.T., Lin, M.H., Qiu, S.M., Shih, C., 1998a. Functional characterization of naturally occurring variants of human hepatitis B virus containing the core internal deletion mutation. J. Virol. 72, 2168–2176.

Yuan, T.T., Lin, M.H., Chen, D.S., Shih, C., 1998b. A defective interference-like phenomenon of human hepatitis B virus in chronic carriers. J. Virol. 72, 578–584.

Yuh, C.H., Chang, Y.L., Ting, L.P., 1992. Transcriptional regulation of precore and pregenomic RNAs of hepatitis B virus. J. Virol. 66, 4073–4084.

Zhang, Y.Y., Summers, J., 1999. Enrichment of a precore-minus mutant of duck hepatitis B virus in experimental mixed infections. J. Virol. 73, 3616–3622.