REVIEW ARTICLE

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Relevance of Hepatitis B Virus Genome Variability in Organ Transplantation

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Hepatitis B virus (HBV) can cause acute and chronic hepatitis in humans, with the latter possibly leading to liver cirrhosis and hepatocellular carcinoma. The clinical course of HBV infection is critically dependent on genetic and immune features of the host as well as on virological factors. In situations of immune suppression, e.g. in patients after organ transplantation with chronic HBV infection, severe progression of liver disease can occur, due to direct effects of immunosuppressive regimens on HBV's hepatotoxicity or replication and due to the selection of HBV mutants. HBV variants are commonly found in chronically infected patients because of the lack of proofreading capacity of the HBV reverse transcriptase. Examples of relevant HBV mutations include precore or basal core promoter mutants with increased replication capacity, escape mutants with alterations in the 'a-determinant' immune epitope, core gene deletions after renal transplantation, antiviral drug resistant strains and accumulation of complex HBV variants after long-term immune suppression. In this review, we present the virological background of HBV genetic variability, discuss frequent mutations observed in transplanted patients and address the effects of HBV genetic variability on the clinical outcome in solid organ transplant recipients.

Keywords: **Hepatitis B Virus, Host Genetic and Immune Factors, Virus Genome Variability, Organ Transplantation, Prognosis**

Introduction

Hepatitis B Virus (HBV) is a human blood-borne, usually noncytopathic and strictly hepatotropic virus that belongs to the genus Orthohepadnavirus of the Hapadnaviridae family [(1)]. HBV infection is considered as one of the major health care challenges in the world, as it is estimated that more than 350 million individuals are currently chronically infected with the virus worldwide [(2, 3)]. HBV infection shows wide clinical manifestations such as asymptomatic course, acute and chronic hepatitis as well as the life-threatening complications cirrhosis and hepatocellular carcinoma (HCC) [(4)]. The outcome of the HBV infection is not only affected by host genetic factors and the host immune system, but is also largely influenced by the genetic variability of the virus [(5, 6)]. Both host- and virus-related factors are closely linked to the outcome of HBV infection, in particular among immunocompromised patients. In this review, we present the virological background of HBV genetic variability, discuss frequent mutations observed in transplanted patients and address the effects of HBV genetic variability on the clinical outcome among immunocompromised individuals particularly in solid organ transplant recipients.

HBV molecular virology

The complete HBV particle (Dane particle) is a small, spheric, enveloped virion, around 42 nm in diameter, which contains a 3.2 kb, partially double-stranded circular DNA consisting four over-lapping open reading frames (ORFs) called P, S, C and X [(7)]. A schematic of HBV genome organization is illustrated in figure 1. The P gene covers about 80% of the whole HBV genome and overlaps the entire S

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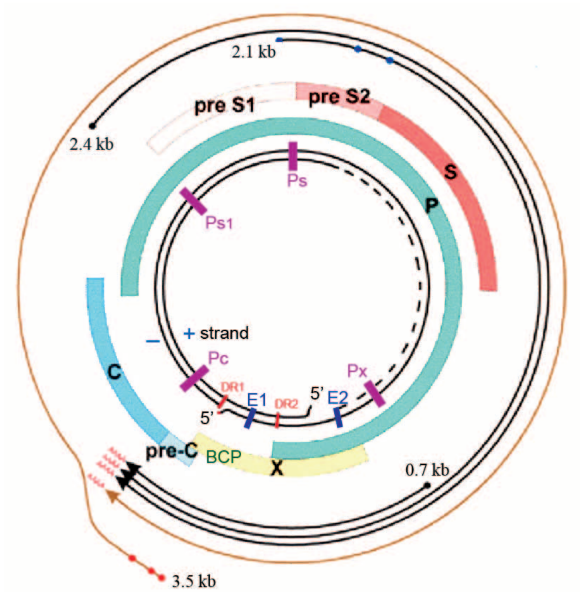

Figure 1. The schematic diagram of the HBV genome organization illustrates four major HBV ORFs (P, S [preS1+preS2+S], C [pre-C+C] and X), circular and partially double stranded DNA (complete negative and incomplete positive DNA strands), four main transcripts (3.5 kb RNA encodes pre-genomic RNA, core, HBeAg and polymerase proteins; 2.4 kb RNA encodes large HBsAg; 2.1 kb RNA encodes middle and small HBsAg, and 0.7 kb RNA encodes X regulatory protein), four different promoters (P_x, P_c, P_{s1} and P_s), two regulatory elements (EI and EII), basal core promoter (BCP) region at the X gene sequence, and two direct repeats (DR1 and DR2).

gene. The P encodes a polymerase enzyme with distinct features in replication (reverse transcriptase, DNA polymerase and RNase H). The S gene encodes three types of envelope proteins called large (L), middle (M) and small (S) hepatitis B surface antigen (HBsAg). The C gene encodes two different proteins named hepatitis B core antigen (HBcAg), which is part of the nucleocapsid, and hepatitis B "e" antigen (HBeAg), which is secreted from the infected cell. The small X regulatory protein is transcribed from the X gene, which overlaps with the P and C genes. Because of different start codons within one gene, HBV is able to encode more than one protein from an ORF; in fact, it transcribes seven different proteins. Four promoters (preS1, S, C and X) and two enhancer elements control the expression of these proteins [(8)].

HBV genome variability

HBV (a retro-transcribing virus) is distantly linked to the retroviruses and replicates via an RNA intermediate that is converted into DNA by a reverse transcriptase (RT). The HBV RT enzyme lacks proofreading capacity; this results in a mutation rate of 1.4×10^{-5} to 3.2×10^{-5} per site each year in the virus genome [(9)]. HBV variants have been described with mutations in the different ORFs of the virus genome. The most frequent or clinically relevant mutations in the HBV genome will be briefly presented.

Mutations in the C gene

The most frequent mutations in the C gene that are found in chronically infected patients affect the transcription of the secreted HBeAg, resulting in so-called 'HBeAg-negative chronic HBV infection'. These mutations can be either located in the precore gene or in the basal core promoter (BCP) upstream the C gene. The most frequent mutation in the precore gene occurs at the codon 28 of the HBeAg. The G1896A mutation in this site creates a stop codon (TGG changed to a stop codon, TAG) which prevents production of HBeAg [(10)]. The BCP and enhancer II regions control the transcription of the precore and pregenomic RNAs. The Enhancer II overlaps with the BCP sequences and possesses transcriptional regulatory function. The BCP double mutation A1762T/G1764A, commonly found in HBeAg-negative patients, diminishes the HBeAg synthesis [(11)].

The S and P genes mutations

The 'a-determinant' domain in the S gene is a common immunodominant region, because neutralizing antibodies (anti-HBs) that develop after vaccination or infection are targeted against this epitope [(12)]. The most common mutation in the 'a-determinant' domain is G145R, but there are several reports for other mutations in this area, as well [(13)]. Mutations in the 'a-determinant' domain can result in viral escape from the humoral immune response, as the virus may not be recognized by anti-HBs antibodies. Similarly, HBsAg cannot be detected by routine blood-screening test when it possesses some specific mutations in the amino acids 124-147; thus, undetected S mutants, e.g. in blood donors, could then potentially spread to others despite regular precautions [(14)].

Clinically relevant mutations in the P gene are mostly related to antiviral drug resistance. In the catalytic domain of HBV polymerase enzyme, there exists an active and highly conserved site which carries the YMDD (Tyr-Met-Asp-Asp) amino acid motif. Certain mutations in this motif create viral strains that are resistant to antiviral drugs [15]. For instance, the mutants M204I/V/S are resistant to the nucleoside analogue lamivudine. Similar P gene mutations can lead to drug resistance to adefovir or entecavir [16, 17]. Mutations at rtA181T (in the B domain of polymerase enzyme) and N236T in the D domain are shown to confer resistance to adefovir [16]. Interestingly, resistance to entecavir mostly coincides with lamivudine drug resistance mutations [15]. Moreover, multi-drug resistance HBV variants can occur, especially in chronically infected patients that have received various antiviral drugs during their clinical course [18]. The most important HBV drug resistance mutations and their locations in the Pol gene are shown in figure 2.

HBV infection among organ transplant recipients

The natural course of chronic HBV infection is nowadays perceived as consisting of four phases: immune tolerance (high HBV-DNA, low inflammation), immune clearance (HBeAg-positive with high HBV-DNA and inflammation), inactive carrier state (low HBV-DNA and inflammation), and reactivation (HBeAg-negative, variable HBV-DNA and inflammation) [19]. Not every patient goes through every phase, and the risk for developing progressing liver disease, cirrhosis or HCC varies in the different phases. It is generally accepted that the natural course of chronic HBV infection as well as the rate and speed of clinical disease progression is critically dependent on the host immune response [20, 21]; thus, the level of liver disease and damage is exceedingly impacted by (long-term) immunosuppresion, as seen in organ transplanted patients with chronic HBV infection. For instance, rapid progression to end-stage liver disease (ESLD) is frequently observed in immunocompromised HBV infected kidney transplant recipients [22]. A number of studies demonstrated that organ transplant recipients who received long-term immunosuppressive therapy experienced more severe HBV infection and more frequently died due to ESLD, such as cirrhosis or HCC, as compared to chronically HBV infected patients without immunosuppressive medications [23-25]. In addition, there are substantial reports which illustrated that HBV genetic variability, such as mutations and deletions within different HBV ORFs, can considerably influence the hepatopathogenicity and clinical outcome, particularly among immunosuppresed patients [26-32]. However, intelligent therapeutic reactions after occurrence of mutant strains may prevent disease progression, even in immunocompromised patients with 'critical' mutations [33].

The impact of HBV C gene variability on liver disease in organ transplant patients

Within the C gene of the HBV, there exist several areas which influence HBV replication [5, 34] and outcome of disease [31], specifically (i) precore

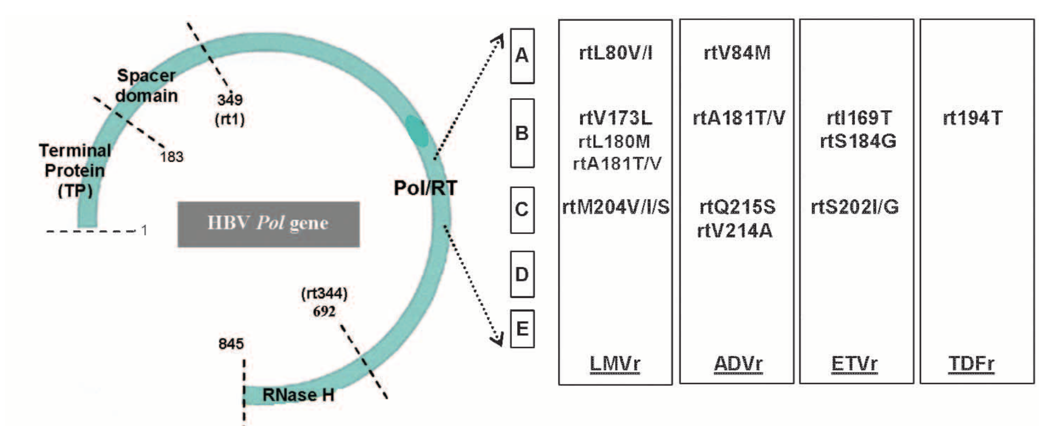

Figure 2. The most important HBV antiviral drug resistance mutations and their location in the polymerase gene of the virus [15, 16, 44, 58-60]. Numbers designate amino acid positions for different catalytic parts (terminal protein, spacer, pol/RT and RNase H) of the HBV polymerase gene. Several conserved regions within the RT domain are shown by capital letters named A to E; for instance, conserved YMDD motif is located at domain C of RT. Entecavir resistant mutations usually develop in LMV-resistant viral strains ("two-hit-model"), as LMV resistance reduces sensitivity to ETV as well. LMV: lamivudine; ADV: adefovir; ETV: entecavir; TDF: tenofovir; r: resistance.

mutant, (ii) BCP/enhancer II mutation and (iii) C gene deletion/insertion. It is currently believed that HBV precore and BCP mutant variants are associated with more severe liver disease, at least in certain HBV genotypes [11, 35]. In addition, occurrence of these mutations may also favor early graft loss after liver transplantation [36, 37].

(i) Precore mutant: In a study on HBV-infected liver transplantation patients, results revealed more variations in the HBV precore and core sequences, and these mutations were significantly associated with recurrence of HBV infection and post-transplantation and graft survival [37]. Moreover, the genotype D of HBV had a strong association with precore mutant variants. Similar results were observed among bone marrow/stem cell, liver, heart and renal transplant patients [38-43].

(ii) BCP/enhancer II mutation: Particular point mutations at the BCP (A1762T/G1764A) and enhancer II region were found in patients with fulminant hepatitis [35, 44]. As illustrated (Fig. 1), the BCP is located within the HBV X gene, so that BCP mutations can affect the X gene, as well. For instance, certain BCP mutations may create a binding site for the transcription factor hepatocyte nuclear factor 1 (HNF1), resulting in higher viral replication and a more severe outcome especially in immunocompromised patients [26, 45]. BCP mutations in immunocompromised hematopoietic stem cells transplanted patients appear to be associated with a higher incidence of liver dysfunction [43]. Likewise, in kidney transplant recipients, BCP/enhancer II mutations were recognized as the most prevalent HBV population in the long-term immunosuppressed patients with severe liver disease, whilst these variants were not detectable in those with mild disease [26]. In contrary, this association was not observed in the heart transplant recipients [46].

(iii) The C gene deletion/insertion: Major deletions/insertions in the HBV C gene were specifically discovered in HBV-infected renal transplant recipients and associated with a more progressive and severe liver disease. In a study by Günther and co-workers, nine out of 24 HBV-infected renal transplant patients were infected by HBV variants with core gene deletions [27]. Interestingly, the majority of the HBV C gene deletions occurred in the middle of the C gene, and all were in-frame. Patients with core gene deletions showed liver cirrhosis manifestation, and five of them also died due to ESLD. These results were statistically significant comparing control HBV-infected patients who received no immunosuppressive treatment. Their survey demonstrated that immunosuppressive regimen together with C gene deletion HBV variants may lead to severe deterioration of the liver in renal transplant patients [26, 47]. Other investigations confirmed this observation, as the development of liver cirrhosis was associated with persistence of specific insertions/deletions in the HBV BCP and core genes in immunocompromised patients after renal transplantation [48, 49].

Surprisingly, core gene deletions particularly occur in renal transplant recipients. In a large series, we investigated 60 liver, 50 heart and 30 kidney transplanted patients chronically infected with HBV. Core gene deletions (large and small deletions) were only found after kidney, but not after heart or liver transplantation [31]. In this large series of cases, large and short C gene deletions were always in-frame and located in the middle of the C gene. The replication capacity of mutated HBV can be tested in vitro by cloning mutated HBV constructs and by transfecting these vectors in human hepatoma cells. The in-vitro analysis of the replication capacity revealed that mutants with large core deletions were replication incompetent, meaning they required coinfection with wild type virus for replication (Fig. 3). However, mutants with large core gene deletions showed nuclear accumulation of core protein. These large core gene deletions were particularly associated with progressive liver disease in renal transplant recipients [31].

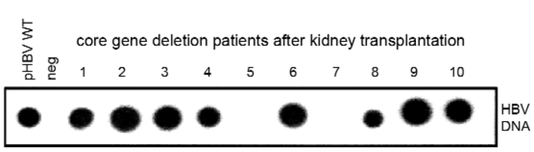

Figure 3. Core gene deletions in patients after kidney transplantation. One-third of patients (10/30) after kidney transplantation had core gene deletions. HuH7 cell line was transfected with HBV replication-competent constructs carrying the different deletions. Vector controls pBS (empty-vector), wt (pHBV 2.0) and core gene deletion mutants (patients 1-10) were utilized. The amount of newly synthesized progeny DNA was determined by dot blot analysis using a ^{32}P-labeled HBV probe. As illustrated, samples 5 and 7 were derived from two patients infected by large core deletion HBVs which could not synthesize progeny HBV DNA (modified from Bock *et al.* [31]).

The impact of the HBV S variability on liver disease in organ transplant patients

Mutations affecting the S gene have been frequently described in patients after organ transplantation. The obvious reason is the established therapy of HBV-infected organ transplantation recipients with anti-HBs antibodies, the hepatitis B immune globulin (HBIG). Treatment with HBIG creates a selection pressure for S-mutants that escape the humoral immune response or the neutralizing antibody treatment, respectively [28, 29, 50]. As shown above, the 'a-determinant' domain within the HBV S gene is the major epitope targeted by the humoral immune response [13]. Mutations in the 'a-determinant' domain may alter the antigenicity and binding to HBs-Ab, which then allows escape from neutralization by anti-HBs [51]. S and pre-S mutations in (liver and kidney) transplanted patients are associated with reinfection, severe and rapidly progressive liver disease, and also reduced graft and patient survival, especially after HBIG treatment [28, 50]. In one study done among liver transplanted patients, the major changes during two mutations were observed in the CCAAT-box of the preS region after transplantation; these mutations were associated with severe fibrosing cholestatic hepatitis after liver transplantation [29]. The most frequent mutations described in the literature for transplanted patients were detected at residues which were located within 'a'-determinant domain such as M133T, F134Y, T143S, D144A and G145R [28, 42, 50].

Complex HBV variants with multiple mutations in organ transplant patients

Not only can one mutation affect two ORFs in the HBV genome [52] (especially in the overlapping pol and S genes), but also several mutations are able to occur in one infected patient, in particular among (long-term) immunosuppresed transplanted recipients. In Preikschat *et al.*'s survey, 38 renal transplanted patients infected with HBV were monitored via full-length genome analysis [49]. Results demonstrated that more than one-third of patients who experienced liver cirrhosis and ESLD were infected with HBV variants with amalgamation of mutations/deletions/insertions in the BCP, C and preS regions, in contrast with other renal transplanted patients who had no liver cirrhosis and ESLD [49]. Recently, in vitro phenotypic assessments have been employed to determine the phenotype of such complex HBV variants. It has been revealed that development of cirrhosis in renal transplant recipients with chronic HBV infection is linked to the accumulation of complex HBV variants carrying deletions in the core gene and/or preS region and deletions in the core promoter [30, 53]. The in vitro investigation results have disclosed that complex HBV variants had reduced levels of pre-C and surface mRNAs and increased pregenomic RNA and consequently HBV replication comparing wild type virus, which may cause cytotoxicity or disturb the host cell physiology [53].

Conclusion

The variability of the HBV genome can influence the outcome of HBV infection in organ transplant recipients. Particularly, HBV core gene deletions, which are mostly observed after renal transplantation, can have a strong impact on liver disease progression. There exist some potential mechanisms which may explain augmented HBV replication and consequently rapid liver damage in transplanted recipients. First, it has been characterized that HBV possesses a glucocorticoid receptor binding sequence in its genome resulting increased activity of the HBV enhancers [54, 55], while steroids are often an inherent component of immunosuppressive regimens after organ transplantation. Second, the immunosuppressive medicines suppress the virus-specific immune response [56, 57], which favors a higher viral load [24]; besides, following liver transplantation, additional organ mass is accessible for the virus to replicate [32]. Moreover, higher viral replication and a weak endogenous antiviral immune response facilitate the development of mutant strains. Recent in vitro experiments revealed that mutations/deletions in different parts of the HBV genome (especially in the BCP and C regions) further enhance the replication mediated at the level of encapsidation and reverse transcription [26, 30, 31]. Finally, complex HBV variants can emerge in immunosuppressed patients under antiviral and HBIG therapy that considerably change the phenotype of the virus, often with enhanced HBV replication [53]. Therefore, patients after organ transplantation need a very close monitoring with respect to virological, biochemical and histological alterations. Effective therapeutic measures must be applied if critical HBV mutant strains emerge to prevent progression of liver disease and to assure graft and patient survival.

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