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The concept of hepatitis B virus mutant escape

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Abstract

Hepatitis B virus (HBV) reverse transcriptase is an error-prone enzyme, and this results in a large number of nucleotide substitutions during replication. As a result, HBV has a "quasispecies" distribution in infected individuals, meaning that HBV circulates as a complex mixture of genetically distinct but closely related variants that are in equilibrium at a given time point of infection in a given replicative environment. The quasispecies distribution of HBV implies that any newly generated mutation conferring a selective advantage to the virus in a given replicative environment will allow the corresponding viral population to overtake the other variants. Such selection processes occur at any step of infection to allow the emergence of variant viruses, such as precore and core promoter mutants during the natural course of infection, HBs antigen mutants under the pressure of active or passive anti-HBs immunization, or HBV mutants that are resistant to the antiviral action of specific HBV inhibitors.

Keywords: HBV lifecycle; HBV variability; Escape mutant

1. Introduction

Hepatitis B virus (HBV) infection is a major public health problem, with approximately 350 million individuals chronically infected worldwide (Lee, 1997). HBV is highly endemic in sub-Saharan Africa, China and South-East Asia. It is also highly endemic in the Mediterranean basin and it is present at significant levels in most industrialized countries (Lee, 1997). Two forms of chronic HBV infection can be individualized according to the presence or the absence of HBe antigen, but transitional forms exist (Ganem and Prince, 2004; Lee, 1997). Chronic HBV carriers are exposed to a risk of complications such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma, of which HBV is currently the most frequent cause (Ganem and Prince, 2004). Up to one million people die every year from the complications of HBV infection (Lee, 1997). HBV is a variable virus, due to the intrinsic properties of the HBV DNA polymerase, the enzyme that ensures viral replication. The quasispecies distribution of HBV is characterized by the coexistence of different viral populations in various proportions. Variant populations are continuously selected by the changing environment in which the virus replicates during human infection.

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2. HBV lifecycle and variability

2.1. HBV lifecycle

HBV is a member of the *Hepadnaviridae* family. Its partially double-stranded, circular DNA genome is contained in an icosahedric capsid, itself enveloped by a lipid bilayer bearing three different surface proteins. The HBV lifecycle starts with virion attachment to an unknown specific receptor complex (Ganem and Prince, 2004). The viral envelope then fuses with the cell membrane, releasing the nucleocapsid into the cytoplasm. The virus is decapsidated, and the genomic HBV DNA and HBV DNA polymerase are transferred to the nucleus. Viral DNA is repaired in the nucleus to yield a fully double-stranded DNA molecule, which is subsequently supercoiled to form covalently closed circular proviral DNA (cccDNA). cccDNA is the form in which HBV persists in host cells (Tuttleman et al., 1986). cccDNA has a very long half-life, despite the fact that it is not integrated into the cellular genome, and can probably be transmitted to progeny cells. It serves as a reservoir for viral reactivation when antiviral therapy is interrupted. cccDNA is transcribed by cellular RNA polymerases into messenger RNAs for viral protein synthesis, and into a pregenomic RNA which is subsequently encapsidated in the cell cytoplasm together with a molecule of HBV DNA polymerase. The latter has a reverse transcriptase function that catalyzes synthesis of the negatively stranded genomic DNA, while the pregenomic RNA is gradually degraded by the RNAse H activity of the polymerase in the

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nucleocapsid. A positive DNA strand is then synthesized by the polymerase, using the negative strand as template. Newly generated nucleocapsids can be recycled to yield additional cccDNA molecules in the nucleus, but most of them bud into the endoplasmic reticulum to form mature virions that are subsequently released into the pericellular space by exocytosis (Ganem and Prince, 2004).

2.2. HBV quasispecies variability

HBV infection is characterized by high levels of virus production and turnover (Nowak et al., 1996; Whalley et al., 2001), whereas the HBV reverse transcriptase, like the human immunodeficiency virus (HIV) reverse transcriptase, is an error-prone enzyme lacking 3′–5′ exonuclease proofreading capacity (Cane et al., 1999; Gunther et al., 1999). This results in a large number of nucleotide substitutions during replication, that principally accumulate at the reverse transcription step. The misincorporation rate has been estimated to be of the order of 10^{10} incorrect nucleotide incorporations per day in a given patient (Zoulim, 2004). As a result, HBV, like other viruses with error-prone polymerases, such as HIV, hepatitis C virus (HCV) and poliovirus, has a "quasispecies" distribution in infected individuals (Gunther et al., 1999). This means that HBV circulates as a complex mixture of genetically distinct but closely related variants that are in equilibrium at a given time point of infection in a given replicative environment. The fact that the four main HBV open reading frames overlap, i.e. that each nucleotide is part of the coding sequence of more than one functional viral protein or regulatory element, explains why HBV quasispecies variability is subject to stronger conservatory constraints than RNA viruses with non-overlapping reading frames, such as HCV and poliovirus. The quasispecies distribution of HBV implies that any newly generated mutation conferring a selective advantage to the virus in a given replicative environment will allow the corresponding viral population to overtake the other variants, following a classical Darwinian evolutionary process (Duarte et al., 1994).

3. Mutant escape in HBV infection

3.1. Precore and core promoter mutants

Variant HBV sequences have been described both in the precore region and in the core promoter region. The most frequent precore variant results of a G → A substitution at nucleotide position 1896 which insert a stop codon hampering synthesis of the HBe protein precursor (Carman et al., 1989). Other precore sequence alterations with similar consequences have been described. Mutations in the core promoter region have also been reported. The most frequent are A → T and G → A at positions 1762 and 1764, respectively (Okamoto et al., 1994). They could play a role in the downregulation of HBe antigen expression.

Chronic HBV infection evolves over several weeks to years, typically through an initial HBe antigen-positive phase, followed by the HBe seroconversion and an HBe antigen-negative phase during which lower levels of replication are observed. This evolution is characterized by a progressive switch from a viral quasispecies dominated by "wild-type" variants, i.e. without precore or core promoter mutations, to a viral quasispecies where precore and/or core promoter variants predominate (Kajiya et al., 2001; Yuen et al., 2002). At the transition phase (i.e. around seroconversion), both populations can be detected in various respective amounts. It has been suggested that these variants are selected through CTL escape at the time HBe antigen reduction is associated with a loss of immune tolerance. The selection forces responsible for precore and core promoter variant selection however remain to be clearly identified.

3.2. HBs antigen mutants

Amino acid substitutions within the "a" determinant of HBs antigen can lead to conformational alterations that can affect the binding of neutralizing antibodies. As a result, HBV mutants have been shown to be able to escape vaccine-induced or passively transferred neutralizing responses, leading to the development of a true, HBs antigen-positive HBV infection in the presence of high titers of anti-HBs antibodies. The most frequent substitutions are the sG145R mutation, which is related to a G → A mutation at nucleotide position 587, and the sD144A mutation, but many other substitutions have been described within the "a" determinant, in the HBs antigen outside of the "a" determinant sequence, and in the pre-S region (Weber, 2005).

Selection of HBs antigen mutants has been observed principally in two clinical settings, including vaccinated newborns of HBV-infected mothers, and orthotopic liver transplant recipients receiving human monoclonal anti-HBs antibodies or hyperimmune hepatitis B immunoglobulins (Carman, 1997; Ghany et al., 1998; Protzer-Knolle et al., 1998). In contrast with the initial fear, selection of HBs antigen mutants appears to be a rare event and their prevalence in the population of HBV-infected patients remains relatively low, even in highly endemic areas (Weber, 2005). Surprisingly, selection pressure associated with extensive immunization in endemic areas has not favored the emergence of HBs antigen variant viruses, probably because these variations confer some selective disadvantages in the competition with "wild-type" viruses.

3.3. HBV inhibitor-resistant mutants

Experience with highly active antiretroviral therapy in HIV-infected patients shows that resistance to HIV reverse transcriptase inhibitors is acquired gradually, through the selection of pre-existing resistant variants and gradual