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Immunopathogenesis of hepatitis B e antigen negative chronic hepatitis B infection

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Abstract

Hepatitis B e antigen (HBeAg) negative chronic hepatitis B represents currently the predominant form of chronic hepatitis due to the hepatitis B virus (HBV) in several parts of the world. In this review, recent data regarding the process of HBeAg negative HBV strain selection during the course of chronic HBV infection are presented and the potential virus and/or host-mediated mechanisms that lead to chronic liver necroinflammation, i.e. chronic hepatitis are outlined.

1. Introduction

Chronic hepatitis B (CHB) infection due to mutated forms of hepatitis B virus (HBV) that are unable to produce and secrete the hepatitis B e antigen (HBeAg negative strains), represents the predominant form of CHB in several parts of the world including the Mediterranean area and East Asia (Hadziyannis, 1995). In these areas, the most commonly observed mutation is located at the pre-core region (nucleotide 1896) with a G–A substitution that creates a novel translational stop codon, which prevents the synthesis of HBeAg (Carman et al., 1989; Hadziyannis et al., 1991). This form of infection termed HBeAg negative (HBeAg −) CHB, terminates to cirrhosis and hepatocellular carcinoma (HCC) in a significant proportion of patients and therefore constitutes a major public health problem in several countries (Hadziyannis, 1995; Hunt et al., 2000).

The exact factors that lead to the emergence and predominance of the HBeAg − strains during the course of CHB infection, as well as the pathogenetic mechanisms that mediate liver damage, have been the focus of an intense research over the last 15 years. In this article, new information and current concepts on the immunopathogenesis and course of HBeAg − CHB infection are presented and discussed.

2. The role of HBeAg − strains in chronic hepatitis B virus infection

A discussion regarding the immunopathogenesis of HBeAg − CHB infection must first address the critical question of whether the mutated virus can cause a de novo chronic infection or it actually emerges during the course of CHB infection

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due to the wild type (wt) alone or mixed (mutated and wt) HBV populations. Up to date, most clinical, epidemiological, animal and experimental data support the latter view.

Animal models using woodchucks infected with pre-core mutated strains of the woodchuck hepatitis virus (WHV), develop predominantly an acute self-limited infection that rarely progresses to chronicity. In a recent study by Cote et al. (2000), only 17% of the animals infected during the vulnerable neonatal period developed chronic infection compared to 70% of woodchucks infected with the wt virus. Children born to HBeAg − /anti-HBe + mothers rarely if ever develop chronic infection and similar findings have been reported in HBV infections of adults (Hadziyannis, 1995; The Incident Investigation Teams, 1997). These observations emphasize the prerequisite for the presence of the HBeAg for the establishment of chronic HBV infection and contradict the view that pre-core mutated HBV alone can cause a de novo chronic infection.

In order to better elucidate the mechanisms of liver injury in HBeAg − CHB, a discussion of the natural history of CHB is of order.

3. The four phases of chronic hepatitis B virus infection

It is now widely accepted that CHB infection consists of three well defined phases: an initial replicative or HbeAg + phase (*immune tolerance*), followed by an *immune clearance* phase with gradual disappearance of HBeAg (HBeAg loss and development of anti-HBe or HBeAg seroconversion) and a third *non-replicative phase* also referred as asymptomatic 'healthy' carrier state characterized by normal or near normal serum aminotransferase levels, little if any residual HBV and circulating anti-HBe antibodies (anti-HBe positive phase) (Fig. 1; Hadziyannis, 1995; Lee, 1997). However, in a number of patients, a fourth phase characterized by active HBV replication and substantial liver injury is observed (*replicative/reactivation phase*) (Hadziyannis, 1995). During this phase, mutated HBV strains unable to produce HBeAg predominate. The absence of HBeAg with its immunomodulatory properties and the development of anti-HBe immunity represent critical factors responsible for HBV-induced liver necroinflammation (Hadziyannis, 1995).

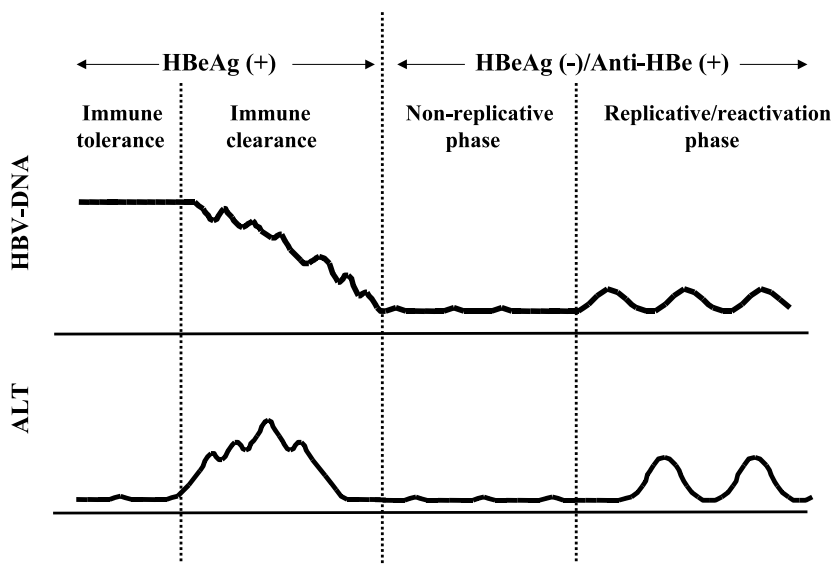

Fig. 1. The natural history of CHB infection following four sequential phases is depicted. Abbreviations: HbeAg, Hepatitis B e antigen; Anti-HBe, Antibodies against HBeAg. Modified from Hadziyannis (1995).

3.1. HBeAg + /immune tolerance phase

After initial exposure to the HBV, progression to chronic infection is related to a variety of viral and host immune factors (Hadziyannis, 1995; Lok, 2000a; Chisari and Ferrari, 1995). One of the most important determinants is the age of the host. Ninety five per cent of humans infected during their neonatal period progress to chronicity compared to 30% of children between the ages of 1–5 years and $< 5\%$ of adults (Hyams, 1995). Similarly, the duration of the initial HBeAg + phase is determined by the age at acquisition of the initial infection (Lok, 1992). Thus, for infections acquired around the neonatal period this immune tolerance phase can last for decades (10–30 years), whereas in adults infected with HBV this period is short lasting only a few weeks (Hadziyannis, 1995; Lee, 1997; Lok, 2000b).

Despite the high replicative state of HBV infection with most of the hepatocytes expressing the hepatitis B core antigen (HBcAg) and serum HBV DNA levels reaching extremely high levels, the host immune response against the virus-infected hepatocytes is minimal. Cellular and humoral immune responses against viral epitopes are barely detected while at the same time there is no evidence of immune-mediated liver damage, which explains the normal serum alanine aminotransferase (ALT) levels (Lok and Lai, 1988; Chang et al., 1988). HBcAg, one of the most potent immunogens is located, during this phase, primarily in the nucleus of infected hepatocytes, being inaccessible to the immune surveillance mechanisms of the host (Hadziyannis, 1995). The rate of spontaneous HBeAg clearance is very low during this phase, estimated at $\sim 2\%$ for the first 3 years of infection and $< 15\%$ after 20 years of infection (Lok, 2000b).

Although the precise mechanisms that induce tolerance to the various HBV components during this phase are unclear, the role of circulating HBeAg appears to be of paramount importance (Milich et al., 1998). Extensive work in experimental models and clinico-epidemiological data from humans have indicated a tolerogenic function for the secreted HBeAg (Hadziyannis, 1995; Milich et al., 1998). This phenomenon of HBeAg-mediated immune tolerance is best illustrated in infants born to mothers that are either HBeAg + or HBeAg − (Hadziyannis, 1995; Milich et al., 1990). Infants born to HBeAg + mothers usually progress to a chronic HBV infection, whereas infants that are born to HBeAg − mothers develop an acute, sometimes fulminant, infection that rarely if ever progresses to chronicity (Beath et al., 1992; Kosaka et al., 1991). Crossing of the maternal HBeAg through the placenta to the fetal circulation is the presumed mechanism of immune tolerance (Milich et al., 1990; Wang and Zhu, 2000). HBeAg has been proposed to downregulate the host antiviral immune defenses by eliciting a predominant Th2-like immune response (Milich et al., 1997), whereas at the same time downregulates Th1 cells by inducing apoptosis (Milich et al., 1998). The inability of the host to mount an efficient Th1 cellular immune response may lead to chronicity. On the other hand, when HBeAg is absent or circulates in decreased amounts a vigorous Th1 response is raised and acute-resolving or fulminant hepatitis develops.

3.2. Immune clearance phase/HBeAg loss and seroconversion

After a short or long period of HBeAg positivity, depending on the age at acquisition of HBV infection, immune tolerance to the virus is lost and the host starts mounting an immune-response directed against replicating HBV in the virus-infected hepatocytes (Lee, 1997; Lok et al., 1987). It is currently unclear, why this shift in immune reactivity to the various viral components occurs. This phase is characterized by fluctuating, but progressively decreasing viral DNA levels associated with or followed by increased ALT levels reflecting immune-mediated hepatocyte damage (Fig. 1). HBcAg translocates from the nucleus to the cytoplasm, where it is accessible to cytotoxic T lymphocyte (CTL)-induced responses (Hadziyannis, 1995; Chu and Liaw, 1987). Humoral immune responses to the HBcAg are prevalent as evidenced by the high circulating IgM anti-HBc titers, resembling those attained during acute HBV infection. At the same time, the levels of circulating HBeAg gradually decrease and anti-

bodies against the HBeAg (anti-HBe) appear in the circulation (HBeAg seroconversion). During this period, the rates of HBeAg loss with subsequent seroconversion are high reaching an annual rate of 10–20% (Lok et al., 1987).

The duration and severity of liver damage during the phase of HBeAg positivity varies significantly (Lok, 2000b). In most cases, this enhanced immune pressure leads to a decrease in the number of infected hepatocytes and gradual suppression in viral replication. However, this suppression rarely leads to viral clearance; HBV DNA is already integrated in the host genome, small amounts of replicative HBV DNA intermediates are detectable in the liver and extrahepatic sites of HBV replication appear to exist. So, low-level viral replication persists being detectable only by very sensitive molecular and biological techniques like the polymerase chain reaction (PCR). These changes usually precede transition to the next non-replicative, anti-HBe + phase (phase 3, Fig. 1).

However, in some patients this period of fluctuating DNA levels with liver necroinflammatory activity accompanied by persistence of HBeAg can last for years, leading to early cirrhosis or HCC (Lok, 2000b). Factors that have been associated with more severe disease include male sex, co-infection with other viruses and concomitant immunosuppressive therapy.

During this phase of HBeAg clearance, replication-competent HBV mutants not producing HBeAg, which have either emerged during the earlier stages of the infection or have even been acquired together with the transmitted wt virus and were previously circulating as quasi-species, appear to become immunologically privileged (Hadziyannis, 1995). These mutants compared to the wt virus, are lacking one or more antigenic epitopes (HBeAg) against which the host immune response is directed, when anti-HBe immunity develops. Thus, they are probably immunologically selected over the wt, becoming the predominant HBV species regardless of the amount of replicating or residual B virus (Hadziyannis, 1995).

3.3. Non-replicative/anti-HBe positive phase

When the host is able to maintain the above described strong immune response against HBV, the levels of viral DNA become barely detectable or undetectable, liver necroinflammatory activity abates, HBeAg secretion is very limited or completely absent and anti-HBe antibodies circulate in the periphery. Liver biopsies show minimal or absent necroinflammatory activity and HBcAg expression is absent (Hadziyannis, 1995). Hepatocytes with HBV DNA integrated in their genome code for the envelope proteins of the virus, which fill the cytoplasm of hepatocytes giving them a characteristic 'ground-glass' appearance (Hadziyannis, 1995).

It is during this phase that most of the residual virus is unable to produce HBeAg representing the viral reservoir for the subsequent phases or periods of HBV reactivation. Carefully performed longitudinal studies have shown that pre-core mutations accumulate around the time of and after HBeAg seroconversion (Lai et al., 1994; Maruyama et al., 1998; Chang et al., 1998). Lai et al. (1994) followed 12 HBeAg + patients before and after seroconversion. They observed that prior to seroconversion all possessed the wt virus, whereas after HBeAg seroconversion pre-core mutants emerged in seven of the 12 individuals. Similar results were obtained in a recent study by Maruyama et al. (1998), where emergence of the predominant pre-core mutant strains, occurred up to 3 years after seroconversion, again implying that this was the effect of seroconversion. In a similar study with Chinese patients, Chan et al. followed prospectively 26 HBeAg + patients that seroconverted to anti-HBe (Chan et al., 1999). Among them, only 8% had a pre-core mutation prior to seroconversion, but their proportion increased to 50% after HBeAg seroconversion. In almost half of these patients, pre-core mutants emerged 1–35 months after HBeAg seroconversion.

3.4. Reactivation phase of HBeAg − chronic hepatitis B

The anti-HBe + phase can run for decades or even lifelong without evidence of significant viral replication and liver inflammation, referred by many as the asymptomatic or 'healthy' hepatitis B surface antigen (HBsAg) carrier state (Hadziy-

annis, 1995; Lee, 1997; Lok, 2000b). Although considered an inactive period, close monitoring of patients with sensitive methods of measurement of viral DNA levels, ALT levels and IgM anti-HBc titers, have shown that in a significant proportion of patients, HBV reactivation can occur over time (Hadziyannis et al., 1991). Viral reactivation episodes followed by immune-mediated liver injury and increased ALT levels characterize and define HBeAg− CHB, which represents 10 to more than 30% of all cases of chronic HBV infections, while its frequency is progressively increasing in recent years (Hadziyannis, 1995; Chan et al., 2000; Knoll et al., 1999).

Enhanced viral replication leads initially to a resurgence of the host immune responses causing immune-mediated liver necroinflammation and decrease in viral DNA levels (Hadziyannis, 1995). It has been hypothesized that during these cycles of viral reactivation → liver injury → decrease in viral DNA levels, immune pressure is exerted more vigorously on the wt strain, which expresses the additional circulating epitopes of the HBeAg compared to the mutant strains (Hadziyannis, 1995; Maruyama et al., 1998). Continuous immune pressure from the host may then lead to the selection and predominance of the pre-core mutants.

Recent findings have suggested a potential mechanism of HBeAg mutant selection during chronic HBV infection. Diepolder et al. showed that the presentation of HBcAg or HBeAg to CD4+ T cells is different (Diepolder et al., 1999). So, cells containing only HBcAg (such as hepatocytes infected with mutant strains) do not elicit a response from specific CD4+ T cells, whereas CD4+ T cells recognize and lyse cells harboring HBeAg (e.g. hepatocytes infected with wt virus; Diepolder et al., 1999). CD4+ T cells are known effector cells that can induce liver necroinflammation in vivo (Franco et al., 1997). A protracted immune pressure on hepatocytes containing the wt virus by these effector T cells can lead to the selection of hepatocytes infected with mutant strains and gradual decrease in the levels of circulating HBeAg (Milich, 1999).

Data supporting the concept that the selection of pre-core mutants follows HBeAg seroconversion and long-lasting periods of viral replication are derived from several epidemiological observations. In anti-HBe+ patients with persistently normal liver enzymes and low or undetectable HBV DNA levels, pre-core HBV mutants are detectable in mixtures together with the wt virus, while the age of these patients is older compared to that of HBeAg+ patients from the same country (Carman et al., 1989). On the other hand, longitudinal follow-up of chronically infected HBV patients, has shown a gradual accumulation of pre-core HBV mutants (Hadziyannis, 1995; Lai et al., 1994; Alexopoulou et al., 1997; Hamasaki et al., 1994; Brunetto et al., 1991a; Okamoto et al., 1990). Furthermore, patients with pre-core HBV mutants and chronic hepatitis tend to be significantly older compared to HBeAg− patients with normal liver enzymes or HBeAg+ patients with chronic hepatitis (Hadziyannis et al., 1991).

More rapid accumulation of pre-core mutants has been observed in infants developing fulminant hepatitis after transmission of a mixture of wt and mutant strains from their mothers (Raimondo et al., 1993; von Weizsacker et al., 1995), in patients developing HBeAg seroconversion after withdrawal of immunosuppressive therapy (Daikoku et al., 1995) and also in recurrent hepatitis infection after liver transplantation (Torre et al., 1999).

The rate and timing of the emergence/dominance of the pre-core mutated HBV strains and the subsequent development of HBeAg− CHB, is most likely a highly complex process influenced by a combination of factors including:

1. rate of viral replication, with frequent accidental emergence of mutant strains as it occurs during the HBeAg seroconversion period (Chan et al., 1999),
2. severity of liver necroinflammation, which causes continuous cell destruction and generation of new uninfected hepatocytes ('replication space') that favors the emergence of mutant strains (Zhang and Summers, 2000),
3. host immune factors, such as the presence of the tolerogenic HBeAg, MHC haplotypes (Ahn et al., 2000; Thio et al., 1999), genetic

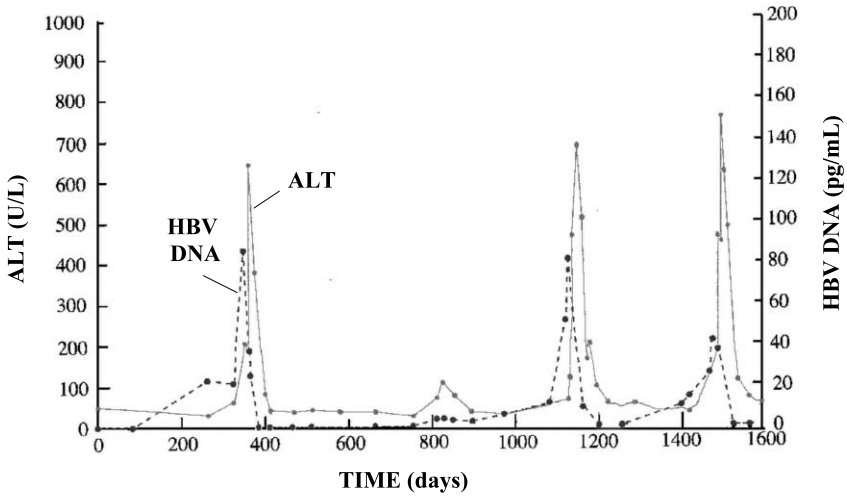

Fig. 2. A typical case of a patient with HBeAg− CHB infection is illustrated. The levels of ALT and HBV DNA are plotted over a 5-years period. From Hadziyannis (1995).

polymorphisms in cytokine production (Hohler et al., 1998) and

4. viral factors, such as the HBV genotype or the presence of mutations in other areas, such as the basal core promoter or core regions (Hunt et al., 2000).

Recent data have suggested that the frequency of the pre-core mutations may be related to specific HBV genotypes (Lok et al., 1994). It has been observed that the pre-core G–A mutation in nucleotide 1896, which creates the novel stop codon, occurs almost exclusively in patients with non-A genotype infections (Hunt et al., 2000; Lok et al., 1994). These genotypes are harboring a thymine (T) at position 1858, which binds with A at position 1896 creating an additional Crick–Watson bond, strengthening the stem and the epsilon (ε) encapsidation signal of the stem loop structure (Lok et al., 1994). The T1858 has been detected in 60–90% of patients from Asia and Middle East (genotypes B–E), but it is rare in North America and Europe (genotype A; Hunt et al., 2000). These findings may explain the geographical distribution of the 1896 pre-core mutation, although additional studies including larger patient populations are needed.

Regardless of the mechanism(s) responsible for the emergence of the mutant strains, in patients with HBeAg− CHB a vigorous immune response against the virally infected hepatocytes is raised. Cytoplasmic localization of HBcAg is a prominent feature accompanied by enhanced expression of major histocompatibility complexes (MHC) in hepatocytes (Hadziyannis, 1995; Lau et al., 1993). Immunoglobulins being able to fix complement in vitro deposit at the same sites implying a role for antibody/complement dependent cytotoxicity (ADCC; Hadziyannis, 1995). Cellular (CD4 and CD8-mediated) and humoral immune responses against various HBcAg epitopes are augmented in the periphery, whereas IgM anti-HBc levels are elevated resembling those of acute hepatitis B (Hadziyannis, 1995).

These replication/reactivation episodes occur either in an intermittent or a persistent pattern that is reflected clinically by monitoring HBV DNA levels and ALT activity (Hadziyannis et al., 1991; Brunetto et al., 1991b). A typical example of a patient with HBeAg− CHB and intermittent pattern of ALT activity is shown in Fig. 2. A good correlation between viral DNA levels and severity of liver inflammation has been clearly shown in a recent study of HBeAg− CHB (Lindh et al., 2000). This enhanced immune reactivity can be

directed either towards the wt and/or the pre-core mutant strains, leading to indistinguishable liver injury and finally cirrhosis and HCC.

4. Conclusions

Chronic hepatits B in patients who are chronically HBeAg − is a major health issue in several parts of the world. Better understanding of the viral and host factors that regulate the appearance and dominance of mutated strains, as well as the mechanisms of liver injury, will certainly aid in the development of more efficacious treatment strategies for these patients.

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