



Review Article

Hepatitis B e Antigen–Negative Chronic Hepatitis B

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Introduction

An estimated 350 million individuals in the world have chronic HBV infection.¹ Although positive for HBsAg, most of them are HBeAg negative. In recent community-based studies from different parts of the world, the prevalence of HBeAg negativity in chronic HBV infection has been found to range between 70% and 100%.² The term HBeAg-negative CHB defines the condition as disease caused by strains of HBV that are not producing HBeAg.³ It does not specify the mutations responsible for the lack of HBeAg, *i.e.*, a precore stop-codon mutation,⁴ a double basic core promoter (BCP) mutation, or other,⁵ and it says nothing about the level of HBV replication. Few years ago, the diagnosis of chronic hepatitis B (CHB) was thought to require the presence of hepatitis B e antigen (HBeAg), as a reliable and sensitive marker of hepatitis B virus (HBV) replication.⁶ Individuals positive for hepatitis B surface antigen (HBsAg) but negative for HBeAg were considered to have non replicative HBV infection, and if their liver enzymes were normal or nearly normal they were referred to as asymptomatic or healthy HBsAg or HBV carriers²

If, on the other hand, they displayed elevated serum aminotransferases and liver histology indicative of chronic hepatitis, they were generally thought to be suffering from other superimposed or complicating conditions.⁸ In the early 1980s it became apparent that HBV could replicate in the absence of HBeAg. Patients from the Mediterranean area, although negative for HBeAg and positive for antibodies to HBeAg (anti-HBe), were reported to have CHB with replicating HBV.¹⁰ The term anti-HBe-positive or HBeAg negative CHB was then proposed and subsequently became widely accepted. In 1989 the molecular basis of this form of CHB was discovered^{11,12} with the identification of HBV mutations preventing HBeAg formation from an otherwise normally replicating HBV.^{13,14} With time, it became apparent that HBeAg-negative CHB, initially viewed as an atypical and rare form of CHB mainly restricted in the Mediterranean area, had a much wider geographical distribution and that its frequency was increasing.³ Its molecular virology and immunology were found to be more complex than initially thought¹⁶ whereas the selection of precore HBV mutants was shown to be largely

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determined by the HBV genotype.⁵ Mutations abolishing or diminishing HBeAg formation were identified along with changes in other parts of the HBV genome.⁵

Natural Course and Prognosis

Information from prospective studies on the natural history of HBeAg-negative CHB is limited. As mentioned, some patients will have advanced liver fibrosis at the time of their presentation. Approximately 40% of the patients identified in the early studies had histologically confirmed cirrhosis.¹⁵ During follow-up, less than 15% of patients achieve long-lasting remission.¹⁵ In addition to persistent disease activity with different ALT patterns, severe and even fatal episodes of HBV reactivation may occur.¹⁶ In general, the long-term prognosis is poor. In Italy, one third of patients have been found to develop cirrhosis over a mean period of 6 years.¹⁷ In a large Greek series of 322 patients, mortality and development of HCC within 4 years from presentation were found to be 29% and 14%, respectively, being much higher compared with a group of patients with HBeAg-positive CHB.¹⁸ Compatible with the role of precore mutants in liver carcinogenesis is the detection of integrated precore HBV mutants in liver tumor tissue.¹⁹ Long term follow-up studies are needed to better understand the natural history and prognosis of HBeAg-negative CHB.

Emergence and Selection of Hbeag-Negative Hbv Mutants

When the precore HBV variant with the novel stop codon was identified as a major cause of HBeAg-negative CHB, 2 possibilities were considered: (1) that it represents a stable HBV strain that causes hepatitis with transition to chronicity or (2) that it emerges during the course of typical HBV infection with the wild-type virus and subsequently becomes selected during the phase of immune clearance (HBeAg loss and seroconversion to anti-HBe).³ The first possibility has been quickly ruled out on the basis of clinical³ and experimental data showing that in contrast to acute HBV infection with

wild-type HBV, *de novo* precore mutant HBV infection rarely, if ever, goes into chronicity. Infants born to HBeAg-negative mothers³ and adults acquiring HBV from HBeAg-negative infected individuals²⁰ do not develop chronic HBV infection. Acute or fulminant hepatitis caused by transmission of precore variants can occur.²⁰ Precore HBV mutants either emerging in the course of HBV infection or cotransmitted, coexist as minor species within a predominant population of wild-type precore HBV during the phases of HBeAg positivity.²¹ Wild-type HBV has a clear advantage over the precore mutant or other HBeAg-negative strains and therefore predominates during that period. This is probably not related to the replicative capacity of wild type HBV but to the diminished host immune response against the virus during that phase (immune tolerant phase).³ The presence of circulating HBeAg is likely one of the most significant factors determining the immune tolerance of the host to the replicating virus. It is well established that this protein possesses unique tolerogenic properties.²² HBeAg has been also shown to down-regulate the host antiviral immune defenses by eliciting a predominant Th2-like immune response²³ while down-regulating Th1 cells by inducing apoptosis.²² Currently unclear, however, are the critical factors that, at a certain point during the course of chronic infection, initiate the loss of immune tolerance against the replicating wild-type virus and clearance of HBeAg (immune clearance phase).²⁴ Once immune pressure to the wild-type virus starts to mount, selection of HBeAg-negative mutants and their predominance over the wild-type HBV is hastened.³ But it remains to be determined why HBV mutants that are not producing HBeAg would be privileged to become selected over the wild-type virus during or after HBeAg loss and seroconversion. It is becoming clear that in the absence of immune-mediated hepatocyte damage, HBeAg-negative mutants are not selected.^{3,25,26} This is best illustrated by HBV genome analyses in patients with persistently high viral replication and normal aminotransferases (indicating absence of liver injury) during the HBeAg positive phase, where only few, if any, mutations are detectable.²⁷

Therefore, their selection is not a primary event implicated in the loss of tolerance to HBV but most likely secondary to the already-mounted immune response against HBV. HBeAg-negative mutants most likely exhibit certain biological properties that render them less vulnerable to host immune reactions compared with wild-type HBV. The immunologic or other advantages of the HBeAg-negative mutants compared with the wild-type HBV can be summarized as follows:

1. Preferential immune pressure on the HBeAg-positive/ wild-type virus may be exerted by humoral-mediated mechanisms to specific epitopes of the HBe protein. Antibodies against conformational and linear HBe epitopes are detectable years before and during the clearance of HBeAg.²⁸ They exert humoral immune pressure against the HBeAg-positive virus (wild-type) and could also participate in liver injury through antibody-dependent cell cytotoxic mechanisms.³ However, a direct cytotoxic action of these specific antibodies against HBeAg expressed in infected hepatocytes is not supported by recent animal data.²⁹
2. CD4-mediated mechanisms may also be implicated in this selection process. Diepolder et al. recently showed a more efficient presentation of HBeAg to CD4 (-) T cells compared with the hepatitis B core antigen (HBcAg).³⁰ So, cells expressing both HBcAg and HBeAg such as hepatocytes infected with wild-type virus could elicit a stronger immune response from specific CD4 (-) T cells compared with cells infected with mutant strains expressing only HBcAg.³⁰ CD4 (-) T cells recognizing various HBV proteins are known effector cells that can induce liver necroinflammation *in vivo*.³¹ A sustained immune pressure on hepatocytes infected with the wild-type virus could gradually lead to the selection of the mutant strains.³²
3. The role of cytotoxic T lymphocytes (CTL, mainly CD8-) is less clear. Because the precore/core (HBe) and core (HBc) proteins

share CTL epitopes, CTL responses cannot really distinguish between them. Although this may be the case during periods of high viral replication (HBeAg-positive phases), sustained low-level viral replication after HBeAg seroconversion, may favor the selection of HBeAg-negative mutants, because hepatocytes expressing concomitantly HBcAg and HBeAg CTL epitopes (*i.e.*, those infected with the wildtype strain) could become a preferential immune target for CTLs compared with cells expressing only HBcAg epitopes (*i.e.*, those infected with mutant strains).²⁵ Some recent studies also provide evidence that coincident mutations, deletions, or other changes in the core region may also be important in this context.³³

4. In addition to these possibilities for immunologic selection of HBeAg-negative HBV variants, certain precore or BCP mutants may have an inherent replicative advantage. In HBV genotypes B, C, D, and E the change of G to A at nucleotide 1896 creates not only a novel stop codon but also a new base pair located in the stem of the encapsidation signal of the pregenomic RNA.³⁴ This increases the stability of the signal and may also increase the replicative efficiency of these mutants compared with that of the respective wild-type HBV genotype. The same has also been claimed for the double BCP mutation, which becomes selected in the case of HBV genotype A and down-regulates HBeAg production at a transcriptional level, probably increasing at the same time the replicative efficiency of the virus.³⁵ However, existing data on this topic are controversial with some studies showing a replicative advantage of these mutants³⁶ and others not.³⁸ In recent studies, mutations in other genes coding for regulatory elements of the virus that alter the replicative efficiency of the virus have been claimed.³⁹ Further studies, including whole genome analyses of HBeAg-negative strains, may help clarify the importance of additional molecular changes in the viral genome.

Pathogenesis

HBeAg-negative CHB can develop either close to the phase of HBeAg loss or much later in the course of the HBeAg (-)/anti-HBe (-) phase. In both cases it is induced by replicating HBV regardless of the presence of HBeAg mutations. After the discovery of precore mutants, it was postulated that these HBV variants were cytopathic to the liver.³

Currently it is accepted that pathogenetic mechanisms of liver cell damage in HBeAg-negative CHB stem from immune responses against replicating HBV.⁴⁰ While in the majority of HBeAg-negative patients, efficient control of the infection is achieved through suppression of HBV replication, in some individuals it is not. Ongoing HBV replication (mutant or not) in HBeAg-negative individuals triggers strong immune responses against the virus that were first described as early as in 1991.⁴⁰ These mechanisms of immune-mediated liver damage are most likely similar to those operating in HBeAg positive CHB.⁴¹ The typically encountered pattern of liver necroinflammation reflected by increases in serum ALT preceded by elevations in serum HBV DNA levels.³ In certain patients, liver cell injury is quite severe resembling that of acute or even fulminant hepatitis B.⁴² This close relationship between viral DNA levels, ALT activity, and liver damage in patients with HBeAg-negative CHB has been clearly shown in a recent study by Lindh et al. During these episodes, T helper cell responses to HBe epitopes, levels of IgM anti-HBe and serum tumor necrosis factor and interleukin 2 were at levels almost identical to those observed in fulminant hepatitis B. Cytolytic and cytokine-mediated noncytolytic mechanisms of liver injury are probably participating in this process. CTL responses to common HBe epitopes expressed in infected liver cells are most likely responsible for the lysis of these hepatocytes leading to aminotransferase elevations. Furthermore, noncytolytic cytokine-mediated pathways may also operate, because in most cases of HBeAg-negative CHB with ALT flares, the HBV DNA levels start to decrease before ALT spikes.³ This mechanism of viral down-regulation

has been also shown in experimental models of acute hepatitis B.⁴³ Long-term follow-up studies and epidemiologic observations in HBeAg-negative patients have clearly shown that the development of CHB may occur years or decades after HBeAg seroconversion.⁴⁴ The determinants of this late resurgence of HBV replication that is followed by a vigorous host immune response leading to liver inflammation remain unknown.

Clinical Aspects

Males are predominate with the male to female ratio ranging from 4.6 to 17 depending on the population studied.³ Few patients can date the onset of their disease with acute hepatitis; intrafamilial acquisition of HBV infection in early life seems to be the most common mode of virus transmission.³ In Greece approximately 40% of the tested siblings of the patients are HBsAg (-)/anti-HBe (-).³ Drug addicts, homosexuals, and multiply transfused patients rarely have HBeAg-negative CHB.³ At presentation most patients are asymptomatic, having been discovered incidentally during HBsAg screening of blood donors or evaluation of asymptomatic ALT elevations. A small percentage present with clinically overt episodes of liver necroinflammation.⁴⁴ Other patients become aware of their underlying chronic liver disease only as it reaches an advanced stage with complications.³ In general, there are no major differences in presentation compared with HBeAg positive CHB.³ During follow-up, 2 main patterns of disease activity are observed: (1) persistently increased serum aminotransferase levels without tendency for spontaneous remission, with an average 3- to 4-fold increase in ALT values (30%-40% of cases)⁴⁴ and (2) an erratic pattern of ALT changes with frequent flare up of disease activity (45%-65%).⁴⁰ Sometimes flares are severe resembling acute hepatitis B with ALT values exceeding 1,000 IU/L and positive IgM anti-HBe.⁴⁵ Changes in anti-HBe IgM titers during the course of HBeAg-negative CHB represent a sensitive marker of HBV replication as well as liver damage and also have been used extensively in monitoring response to antiviral treatment (mainly interferon alpha [IFN]).⁴⁵ Increases in serum HBV DNA usually

precede flareups of enzyme activity, then may drop dramatically and even become undetectable. Intervening periods of inactivity with completely normal ALT levels may be long lasting, but in most cases the disease recurs. Only 6% to 15% of patients exhibit sustained spontaneous remission.⁴⁰ The diagnosis of HBeAg-negative CHB is based on: First, HBsAg positivity with HBeAg negativity for more than 6 months, preferably a year. This criterion excludes patients at the unstable stage of HBeAg seroconversion.⁴⁶ Second, increased ALT levels, either persistent or intermittent. Third, HBV replication documented by either HBV DNA in the serum and/or HBcAg in the liver. If quantitative PCR assays are used, then HBV DNA levels should exceed 10⁵ copies/mL. If serum HBV DNA and/or HBcAg in liver are not detectable, which is particularly true during severe exacerbations of liver disease, then sequential testing is necessary to document that liver damage is indeed HBV induced. Prior to spikes of ALT activity, serum HBV DNA levels increase significantly⁴⁰, whereas in approximately 80% of patients high levels of anti-HBc IgM or total anti-HBc titers are also found during or after the ALT elevations.³ If these diagnostic criteria are not fulfilled, the etiology of liver cell damage in HBsAg (-)/HBeAg (-) patients may not of isoleucine by valine at the same position (M552V) as well as a combined change of leucine to methionine at position 528 (L528M) have also been observed. Similar results were subsequently reported in French patients, and the assumption has been made that the M552I mutant may be more frequently selected among genotype D strains. However, other factors could also account for these associations such as the concomitant presence of BCP mutations and of additional changes in other parts of the HBV genome.⁴⁷ Transient reversion to wild-type HBV has also been reported in 2 studies,⁴⁷ but this was not seen in a long-term study of 70 Greek patients treated with lamivudine.⁴⁸

Summary

HBeAg-negative CHB has now a worldwide distribution, developing in the course of HBeAg-positive chronic HBV infection during or after the

phase of HBeAg loss and its seroconversion to anti-HBe. It is caused by replicating noncytopathic HBV mutants either unable to produce HBeAg (precore mutants) or with down-regulated transcription of the precore/core messenger RNA (BCP mutants). The most frequently encountered and stable HBeAg-negative mutants in the world are those with a novel translational precore stop codon. They are HBV genotype determined and become selected in genotypes B, C, D, and E (non-A genotypes). They prevail in South Europe, the Mediterranean basin, and Asia, whereas they are rather infrequent in the United States. The incidence of HBeAg-negative CHB is increasing in the world. The selection of HBeAg-negative HBV mutants is determined both by viral and host factors, the same being true for their ability to replicate in the presence of anti-HBe immunity. Clinical, virologic, and biochemical features as well as the natural course of HBe negative CHB have been reviewed, and the efficacy of IFN and antiviral therapy as well as the problem of viral resistance have been critically presented.

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