World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i12.1543

World J Gastroenterol 2011 March 28; 17(12): 1543-1548
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Juan-Ramón Larrubia, PhD, *Series Editor*

Pathogenesis of occult chronic hepatitis B virus infection

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Received: August 6, 2010 Revised: January 22, 2011
Accepted: January 29, 2011
Published online: March 28, 2011

Abstract

Occult hepatitis B infection (OBI) is characterized by hepatitis B virus (HBV) DNA in serum in the absence of hepatitis B surface antigen (HBsAg) presenting HBsAg-negative and anti-HBc positive serological patterns. Occult HBV status is associated in some cases with mutant viruses undetectable by HBsAg assays; but more frequently it is due to a strong suppression of viral replication and gene expression. OBI is an entity with world-wide diffusion. The failure to detect HBsAg, despite the persistence of the viral DNA, is due in most cases to the strong suppression of viral replication and gene expression that characterizes this "occult" HBV infection; although the mechanisms responsible for suppression of HBV are not well understood. The majority of OBI cases are secondary to overt HBV infection and represent a residual low viremia level suppressed by a strong immune response together with histological derangements which occurred during acute or chronic HBV infection. Much evidence suggests that it can favour the progression of liver fibrosis and the development of hepatocellular carcinoma.

Key words: Occult hepatitis B virus infection; Hepatitis B virus-DNA; Anti-HBc alone; Hepatitis B virus; Hepadnaviral hepatitis; Occult viral persistence; Primary occult infection; Secondary occult infection; Virus reactivation

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Aller de la Fuente R, Gutiérrez ML, Garcia-Samaniego J, Fernández-Rodriguez C, Lledó JL, Castellano G. Pathogenesis of occult chronic hepatitis B virus infection. *World J Gastroenterol* 2011; 17(12): 1543-1548 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i12/1543.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i12.1543

INTRODUCTION

Hepatitis B virus (HBV) infection is a major global health problem. It is estimated that about 350 000 000 individuals are infected by HBV; infection can induce a wide spectrum of clinical forms, ranging from a healthy carrier state to cirrhosis and hepatocellular carcinoma[1]. HBV infection is usually diagnosed when circulating hepatitis B sur-

face antigen (HBsAg) is detected. However, the availability of highly sensitive molecular biology techniques has also allowed the identification of HBV infection in HBsAg-negative individuals, with or without circulating antibodies to HBsAg (anti-HBs) and/or hepatitis B core antigen (anti-HBc)[2-5]. Furthermore, it is estimated that as much as one third of the world population have been exposed to HBV[6]. Many of these individuals may unknowingly carry the virus. The natural history of chronic HBV infection is highly heterogeneous as many host, virus and environmental factors play an integrated role in affecting rates of disease progression and long term clinical outcomes.

CHRONIC HBV INFECTION AND ITS VIROLOGICAL PHASES

The natural course of chronic infection includes four phases based on the virus-host interaction: immune tolerance, immune clearance, low or non-replication, reactivation and HBsAg negative (occult) HBV[6-8].

Inmune tolerance phase

This phase is characterized by presence of hepatitis B e antigen (HBeAg), high serum levels of HBV DNA, normal or minimally elevated serum alanine aminotransferase (ALT) and normal liver or only minimal histological activity. The immunotolerant phase may persist for 10-30 years in perinatally infected subjects, whereas it is generally short-lived or absent in childhood or adult-acquired HBV infection.

Inmune clearance phase

This initiates when immune tolerance to the virus is lost and the immune response starts to kill infected hepatocytes. This phase is characterized by fluctuating, but progressively decreasing, HBV DNA levels, high levels of ALT and hepatic necroinflammation. Serum HBV DNA levels are > 20 000 IU/mL (10^5 copies/mL) in the phase of HBeAg positive chronic hepatitis and may remain elevated or drop rapidly at the time of anti-HBe seroconversion.

Inactive carrier state

An important outcome of the active immune response is seroconversion from HBeAg to anti-HBe, with transition to the third phase of inactive HBsAg carrier state, characterized by HBeAg negativity and anti-HBe positivity, low levels of HBV DNA < 2000 IU/mL (10^4 copies/mL), normal ALT and inactive liver histology.

Reactivation phase

Some inactive HBsAg carriers may develop HBV reactivation while remaining anti-HBe positive. This phase is characterized by HBeAg negativity with anti-HBe positivity, HBV DNA levels > 2000-20 000 IU/mL (10^4-10^5 copies/mL), high ALT and moderate or severe necroinflammation with variable amounts of fibrosis on liver biopsy (HbeAg negative chronic hepatitis).

HBs negative (occult) HBV

A final phase of chronic HBV infection may eventually follow in some long term carriers, with loss of HBsAg but persistence of HBV DNA in liver. These cases usually do not have active liver disease but show the histological consequences of previous damage with variable amounts of residual fibrosis. Immunosuppression, however, may cause HBV reactivation and sometime severe exacerbation of liver disease with HBsAg, HBV DNA, and even HBeAg rebound.

DEFINITION OF HBsAg LOSS

In "inactive carriers" the HBsAg may become undetectable which is defined as HBsAg loss. A loss of HBsAg is a sign for deep and usually durable suppression of HBV DNA replication and usually announces a stable status of recovery, even though other complications caused by pre-existing liver cirrhosis or fibrosis or an elevated risk for development of hepatocellular carcinoma can still lead to severe complications[9,10]. However, HBsAg may reappear and replication may reincrease, especially if the HBsAg loss was observed during a period of antiviral therapy. The spontaneous HBsAg loss in anti-HBe-positive asymptomatic HBsAg carriers has long been recognized as a very rare event with increasing probability during long term follow up. Accordingly, a cumulative rate of HBsAg seroclearance of 8.1% was described after the first 10 years of infection. Chu *et al*[11] observed that the cumulative probability of HBsAg seroclearance increased to 24.9% after 20 years and to 44.7% after 25 years of HBV infection. The authors also pointed out that age at infection, sustained remission of liver disease and male sex were factors independently associated with HBsAg seroclearance.

ETIOPATHOGENESIS OF OCCULT HEPATITIS B INFECTION

Occult HBV is found more frequently in patients with serologic evidence of HBV infection (anti-hepatitis B core antibody positive) than in core antibody-negative individuals[12,13]. Occult HBV is found in a significant proportion of patients with chronic hepatitis due to hepatitis C virus, with HBV DNA detectable in up to 30% of serum samples and 50% of liver biopsies[12]. Occult HBV infection is defined as the existence of HBV DNA in the serum, cells of the lymphatic (immune) system, and/or hepatic tissue in the absence of serum HBsAg. Most frequently, occult HBV infection follows resolution of acute hepatitis and continues indefinitely after clearance of HBsAg and biochemical improvement in liver function[13-17]. Recent estimates suggest that up to 20% of individuals with occult HBV carriage evidenced by HBV DNA detection could be nonreactive for anti-HBc or any other serological indicator of exposure to HBV[15]. Although viruses with replication deficits could theoretically explain occult HBV, the finding of cccDNA, RNA transcripts, and pregenomic replicative RNA intermediates in a large proportion of pa-

tients suggests that most occult infections are due to low-level replication of wild-type virus[19,20]. In addition, the transmissibility of acute HBV *via* liver transplant or blood product transfusion from donors with occult infection[21-23] provides evidence against the presence of defective viruses. Occult HBV may also result from mutations in HBsAg coding or transcription control regions that alter antigenicity or expression levels[24-26]. Such mutant viruses have been reported as the sole circulating strain in up to 40% of patients with occult HBV[27-30]. Occult hepatitis B infection (OBI) has been detected in the following clinical situations: (1) chronic hepatitis unrelated to HVC, atypical alcoholic hepatitis and hepatocellular carcinoma (HCC); (2) viral reactivation following immunosuppression; and (3) transmission through transplantation, transfusion or experimental transmission to chimpanzees[31]. Low level HBV DNA has been detected in liver tissue of patients with HCC[32] and in serum of blood donors and their recipients. Occult silent or serologically negative HBV infection was reported for the first time more than 20 years

that the occurrence of isolated anti-HBc could also be of value in identifying occult HBV persistence. The virus recovered from woodchucks with OBI remains infectious. It was observed that WHV harvested from PBMC isolated during OBI and *ex vivo* stimulated with lipopolysaccharide (LPS) induced classical acute WHV hepatitis in virus-naive animals[45]. In the case of hepadnaviruses, studies on occult WHV infection point to a direct link between virus persistence and its lymphotropism. They also reveal that infection of the lymphatic system could be a natural and unavoidable consequence of hepadnavirus invasion and that, under certain circumstances, such as a low virus dose, lymphoid cells can be the only temporary or permanent site of virus propagation. Another important fact established through analysis of the WHV model is that a state of occult virus persistence is a normal and ultimate aftermath of WHV infection, which is symptomatic, followed by recovery, or serologically silent from the start. A generalized concept is proposed for the possible progression pathways and outcomes of hepadnaviral infection in relation to variable virus and/or variation of virus in the host. Because of significant virological and pathobiological similarities between HBV and WHV, and close analogies in the patterns of progression and outcomes of the induced liver disease, this concept might also be applicable to human HBV infection[14].

RELATIONSHIP OF OCCULT HEPADNAVIRAL INFECTION AND THE HOST'S IMMUNE SYSTEM

The host's immune-surveillance probably has a critical role in the development of occult HBV infection, as suggested by at least two arguments: (1) there is evidence showing that a vigorous memory T-cell response against HBV antigens is still present many years after clinical recovery from acute B hepatitis, probably because the long-lasting persistence of the occult infection produces a minute amount of antigens able to maintain an efficient antiviral T-cell response[46,47]; and (2) all the conditions inducing immunosuppression may provoke the reactivation of the occult HBV infection with the reappearance of the typical serological profile of the productive infection[48,49]. During occult infection a balance between the virus and the host's immune system is established, and as well as the cytotoxic T lymphocytes, the cytokines synthesized in the liver might also exert a control on HBV replication[16]. On the other hand, it also is conceivable that this extrahepatic pattern of virus expression is a consequence of another property of the virus that, at low doses, displays its natural predisposition for invading lymphoid cells. In this regard, of note are our findings documenting the existence of the cell-binding site in the preS1 domain of the WHV envelope which mediates a strictly host and cell-type-specific recognition. Synthetic analogues of the site have considerably greater ability to interact with woodchuck lymphoid cells than with hepatocytes[50]. Different molecular forms of HBV DNA, including replicative intermediates and cccDNA, and viral RNA have been detected in lymphoid cells of patients with clinically evident hepatitis B[51,52], although discrimination between synthesized *de novo* viral nucleic acids and those potentially adhered to the cell surface has not always been made. Also, traces of HBV antigens have been found on peripheral blood mononuclear cells isolated from actively infected individuals[53]. The observation that cultured peripheral blood mononuclear cells from WHsAg-positive animals produced infectious virus strongly argued that lymphoid cells can support the complete replication cycle of WHV[54].

TRANSMISSIBILITY OF MATERNAL OCCULT HEPADNAVIRAL INFECTION TO NEWBORNS

Vertical transmission of viral hepatitis is considered to be a cause of many perinatal viral infections, and it is postulated that a specific variant can influence virus tropism and outcome of infection in the newborn.

Since mother-to-child transmission is one of the main routes for HBV dissemination, it was important to determine whether hepadnavirus persistently carried as an occult infection can be passed from mothers to their newborns. To investigate this possibility, Michalak *et al*[44] have examined woodchuck offspring born in captivity and they found not only that such mothers transmit WHV to newborns but also that the induced infection is asymptomatic and progresses for years after birth in the absence of serum WHsAg, anti-WHs and, in contrast to convalescent adults, anti-WHc-antibodies. Results of these experiments revealed that under certain natural conditions long-term persistence of hepadnavirus can be maintained exclusively at an extrahepatic location. Thus, there were newborns that did not have any detectable WHV DNA in sequential liver biopsies, yet the viral genomes were expressed in livers of other offspring from the same litter. There also were offspring that initially had lymphoid-restricted patterns of WHV infection, yet at 19 mo after birth became liver WHV DNA reactive[55].

Albeit maternal transmission of hepadnavirus from mother to offspring might differ in some aspects in humans and woodchucks, the essential pathobiological similarity between HBV and WHV suggests that HBV could also be passed from apparently healthy mothers convalescent from hepatitis B to their babies. Children born to these mothers may persistently carry traces of infectious virus and, possibly, have an increased long-term risk for the development of disorders of the lymphatic system and, in some cases, the liver.

CONCLUSION

Occult HBV infection is defined as HBV DNA detection in serum or in the liver of HBsAg-negative patients with or without serologic markers of previous viral

exposure. With the introduction of highly sensitive diagnostic tests for viral proteins the paradigms of HBV infection were challenged. Accumulated evidence indicates that occult HBV can be both a source of virus contamination in blood and organ donations, as well as the reservoir from which full blown hepatitis can arise. The oncogenic potency of occult HBV persistence becomes progressively evident. Certain co-morbidities support occult HBV infection as co-infection with hepatitis C virus or human immunodeficiency virus. Persistence of OBI could involve different mechanisms: mutation or integration of the viral sequence which may alter HBsAg expression, decrease of HBV replication, or hindrance of HBsAg through circulating immune complex. OBI has important clinical implications: any case reports indicate that immunosuppression caused by chemotherapy[56], immunomodulatory agents[57], or immune deficiencies, such as HIV infection[58] or hematological malignancies[59], can reactivate occult infection. However most patients with hepatitis B who recover from the infection do not experience any problems during their lives.

In the case of hepadnavirus, studies on occult WHV infection point to a direct link between virus persistence and its lymphotrophism. Infection of the lymphatic system could be a natural and unavoidable consequence of hepadnavirus invasion and that, under certain circumstances, such as low virus dose, lymphoid cells can be the only temporary or permanent site of virus propagation. Occult HBV may also result from mutations in HBsAg coding or transcription control regions that alter antigenicity or expression levels[19,25,26]. In addition, the transmissibility of acute HBV *via* liver transplant or blood product transfusion from donors with occult infection[5,22,23] provides evidence against the presence of defective viruses.

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