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Role of silent hepatitis B virus in chronic hepatitis B surface antigen(−) liver disease

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Abstract

Despite a number of studies documenting hepatitis B virus (HBV) infection in the absence of hepatitis B surface antigen (HBsAg) a causal relationship between silent HBV infection and liver disease remain difficult to establish. In particular, both the prevalence and clinical significance of this observation are poorly understood. Why is HBV replication apparently so low in these patients? A number of studies have tried to elucidate the mechanism of HBsAg negative infections, and considerable data documenting HBV infectivity or reinfection in the absence of detectable HBsAg support the hypothesis that in some of these cases, HBV is undergoing low-level replication in the liver and this, in several situations including: (1) chronic liver disease, alcoholic liver disease, hepatocellular carcinoma; (2) viral reactivation following cancer chemotherapy or immunosuppression and (3) transmission via transfusion or from human serum to chimpanzees. In a recent study including 50 patients with chronic liver disease of unknown etiology we could detect serum HBV DNA by nested polymerase chain reaction (PCR) in 15/50 patients (50% at the cirrhosis stage) in the absence of HBsAg; in the liver of the 15 patients both HBcAg and/or HBsAg can be detected at very low-level. Viral host factors allowing HBV persistence in the absence of HBsAg can depend on several mechanisms. Coinfections with HCV can explain only a proportion of HBsAg(−) HBV infections. Secondly, HBV mutations in the core promotor region leading to a minimal viral replication, or mutations in the HBsAg-encoding region might explain the absence of serological recognition. Finally, it is possible that in some cases host immune mechanisms can maintain HBV infection in a latent state until transmission to another individual who subsequently develops a more active infection especially when immunosuppressive therapy is employed. Existence of HBsAg(−) HBV infections should be taken into account by the use of sensitive PCR tests for prevention of viral transmission in the settings of blood donations and organ transplants.

Keywords: HBV infection; Lack of HBsAg detection

1. Introduction

The existence of hepatitis B surface antigen negative (HBsAg−), hepatitis B virus (HBV) infection has been a matter of debate, but a number of studies from several laboratories have stressed

Abbreviations: anti-HBc, antibody to hepatitis core antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; PCR, polymerase chain reaction.

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the existence and the clinical relevance of 'silent' HBV infection. Indeed, HBV DNA in the absence HBsAg has been detected in several situations including: (1) chronic liver disease, alcoholic liver disease, hepatocellular carcinoma (HCC); (2) viral reactivation following cancer chemotherapy or immunosuppression and (3) transmission via transfusion or from human serum to chimpanzees. This phenomenon is not restricted to areas of high-HBV endemicity since such cases have been described in a number of Western countries including France.

2. Serologically silent HBV as reported in the literature

The clearance of HBsAg from serum usually indicates a resolution of biochemical and histologic hepatitis in patients with chronic hepatitis B (Perrillo and Brunt, 1991). However, there are several documented cases describing reactivation of latent viral infection following chemotherapy and immunosuppressive treatment (Wands et al., 1975). It was also shown that patients receiving organs from HBsAg − donors may develop HBV infection (Degos et al., 1988; Wachs et al., 1995). All these reports of reactivated infection in HBsAg − patients occurred in the setting of chemotherapy or immunosuppression which reduces immune surveillance and stimulates viral transcription via the glucocorticoid-response element in the HBV genome (Tur-Kaspa et al., 1986). It is possible that the low-levels of replication in HBsAg − patients may be a factor for the eventual development of cirrhosis and HCC.

Another serologic pattern is that of 'anti-HBc' alone, corresponding to the presence of antibodies against hepatitis B core antigen (anti-HBc) as the only marker of HBV infection. The absence of any serologic markers related to HBV also has been described in a number of clinical situations. The major antigenic region of HBsAg is located in the 'a' determinant (aa 124–147) where altered antigenicities have been described. These are often vaccine escape mutants, in most cases Gly145Arg (Carman et al., 1990). In this region of the surface gene, substitution of one or several amino acids may influence the structure of the 'a' determinant in such a way that epitope changes result in false negative results in several HBsAg screening assays, independently of the presence or absence of anti-HBc (Carman et al., 1990, 1995; Ogata et al., 1997; Oon and Chen, 1998).

Data have accumulated that the serologically silent HBV infections are not only observed with acute or resolved HBV infections, but also with chronic infections and very often with HCV coinfections. Depending on the population studied, the proportion of individuals without HBsAg but with detectable HBV DNA by polymerase chain reaction (PCR) varies but is often high (Table 1). In general, HBV DNA cannot be detected in blood donors who are anti-HBc alone or in those

Table 1
Role of silent HBV in chronic HBsAg(−) liver disease

Literature	Category	Number of cases	% HBV DNA positive
Thiers et al. (1988)	Chronic hepatitis	3	100
Liang et al. (1991)	Idiopathic liver disease	67	31
Baginski et al. (1992)	Transfusion	2	100
Michalak et al. (1994)	Recovery	4	100
Rehermann et al. (1996)	Non-A, non-E CH	17	59
Uchida et al. (1994)	Non-B, non-C CH	40	88
Fukuda et al. (1996)	HCV	30	70
Uchida et al. (1997)	HCV	30	87
Koike et al. (1998)	HCV	29	95
Paterlini et al. (1990)	HCC	28	61
Cabrerizo et al. (1997)	Hemodialyzed	33	58
Cacciola et al. (1999)	HCV	200	33

devoid of any HBV serologic markers (Chemin et al., 2001). When considering individuals positive for HBV DNA without HBsAg in the context of high-risk groups, such as hemodialysis or transfused patients. HBV DNA was detected, respectively, in 19 of 33 (58%) of the former (Cabrerizo et al., 1997) or in 2/2 (100%) of the latter (Baginski et al., 1992). In a study of four patients with resolved hepatitis B infection, (Michalak et al., 1994) all were HBV DNA positive after several years. In the context of chronic liver disease with unknown etiology, percentages have varied from 3/3 (100%; Thiers et al., 1988), 21/67 (31%; Liang et al., 1991), 10/17 (59%; Rehermann et al., 1996) and 35/40 (88%; Uchida et al., 1994). The presence of HBV in patients with HCV, despite the absence of HBsAg, has been reported in 70–88% of Japanese patients (Fukuda et al., 1996; Koike et al., 1998; Uchida et al., 1997), but also with high-frequency in European HCV patients (33% of 200; Cacciola et al., 1999).

When considering patients with HCC who are devoid of HBV serologic markers, the rate of detection of HBV DNA varies, but in all cases the percentages encountered have been high, up to 61% (Paterlini et al., 1990). In a recent study (Oon et al., 1999), detection of HBsAg mutants and viral integration in human HCC was described, demonstrating that 'vaccine escape' mutants are not only implicated in acute hepatitis but also are rather frequent in HCC.

3. Questions of debate

Despite a number of studies documenting HBV infection in the absence of HBsAg, the role of such HBV infections in the outcome of hepatitis remains controversial. In particular, both the prevalence and clinical significance of this observation are poorly understood, and the role of HBV mutants not proven. Whether liver lesions can be linked to these occult HBV infections, and whether the role of extrahepatic sites of replication, often cited, may be involved remain open questions, which warrant further studies. What may occur during coinfections with other hepatitis viruses also is poorly understood. A number of studies have tried to elucidate the mechanism of HBsAg − infections, and considerable data documenting HBV infectivity or reinfection in the absence of detectable HBsAg support the hypothesis that in some of these cases, HBV is undergoing low-level replication in the liver (Cabrerizo et al., 1997; Cacciola et al., 1999; Michalak et al., 1999).

4. Examples of specific studies among HBsAg − groups

4.1. HBsAg − infections in coinfection with HCV

In the context of occult HBV infections in HCV patients, several studies (Cacciola et al., 1999; Fukuda et al., 1996; Koike et al., 1998; Uchida et al., 1997) have shown that HBsAg(−) HBV infection, with or without anti-HBc antibodies, is often found in HCV chronic carriers. Despite the clinical importance of these coinfections, no large-scale studies were performed until recently (Cacciola et al., 1999). The presence of HBV DNA was assessed by PCR in the liver and sera of 200 HBsAg(−) HCV patients and detected in 33%. Among the 66 positive patients, 46 were reactive for anti-HBc alone, while 20 were negative for all HBV markers. Cirrhosis was detected in 22/66 (33%) coinfected patients as compared to 26/134 (19%) other HCV patients, indicating that these HBsAg − HBV infections may accelerate the evolution of HCV patients toward cirrhosis. Sequencing of the PCR amplified HBV genomes did not show any changes that might explain the absence of serology and low-viral expression in these patients.

4.2. HBsAg − infections in hemodialyzed patients

HBV DNA in serum and blood cells also from HBsAg(−) hemodialysis patients and staff also was investigated (Cabrerizo et al., 1997). This report described the detection of HBV DNA by PCR in both groups. It was present in serum for 19 (58%) and in the peripheral blood mononuclear cells (PBMCs) of 54% of patients. HBV DNA was present in six staff members, all with previous recorded acute hepatitis B. Serum treat-

ment with DNAse prior to PCR showed that the HBV DNA was protected, indicating that viral particles are produced. Detection of HBV RNA by RT-PCR demonstrated that active transcription occurred in PBMCs as suggested by several other studies (Chemin et al., 1992; Korba et al., 1987; Pardoe and Michalak, 1995).

4.3. HBsAg− infections in the context of transfusion

Blood donors infected with HBsAg mutants and those circulating low-levels of viral protein may escape detection by HBsAg screening assays and therefore, may affect the safety of the blood supply. PCR was used to investigate the presence of HBV in serum from three blood donors and two transfusion recipients who developed non-A, non-B hepatitis (Baginski et al., 1992). Both donors and recipients had serologic markers for HCV, but not for HBV, infection. PCR revealed the presence of both HCV RNA and HBV DNA in the patients' sera. Cloning and sequencing of both donor–recipient pairs demonstrated that HBV had been transmitted through transfusion, but no changes in the viral sequences were able to explain the absence of HBV serology in these patients. In these cases, the most probable hypothesis would be viral interference between HBV and HCV. A recent study (Jongerius et al., 1998) described a new HBV mutant in a blood donor negative for HBsAg by several HBsAg screening assays. In this case, genomic sequencing revealed changes from Gln to Arg at position 129 and from Met to Thr at position 133. This combination of mutations has not been previously described, and conformational changes in the 'a' determinant may be responsible for the lack of detection of HBsAg by the different commercial tests.

4.4. HBsAg− infections after recovery from hepatitis

A study performed in woodchucks (Michalak et al., 1999) convalescent from acute infection with HBV-related woodchuck hepatitis virus (WHV) showed that WHV DNA persists for life in the liver and lymphoid cells of these animals after recovery. All anti-WHc reactive animals displayed WHV DNA by PCR, and, in some cases WHV DNA was detected in the absence of anti-WHc. Convalescent animals were not susceptible to WHV reinfection. Remarkably, there was persistence of low-grade necroinflammatory injury and development of HCC after recovery from acute hepatitis, suggesting a role of these silent HBV infections in the pathology of liver disease. A recent study by the same group (Coffin and Michalak, 1999) demonstrated that female woodchucks with occult hepadnaviral carriage may transmit the virus to their offspring, with persistent infection within lymphoid cells but not always in the liver.

4.5. HBsAg mutants in hepatocellular carcinoma

It has been shown for some vaccine escape mutants that (i) they are capable of independent replication, (ii) they are infectious in chimpanzees (Ogata et al., 1997) and (iii) they can be involved in acute human hepatitis (Carman et al., 1995; Hsu et al., 1995). The report of Oon and Chen described the presence of HBV mutants in human HCC patients with changes within the 'a determinant' of HBsAg that are similar to those described in 'vaccine escape' cases. In this study, 20 cases of HBsAg mutants were detected in a cohort of 62 Singapore HCC patients. HBsAg mutations at positions 145 (Gly to Arg) and 133 (Met to Thr) were found. Interestingly, this latter mutation was mostly found in aggressive HCC. Further characterization of those mutants may provide more information about their potential role in the development of HCC.

5. Recent results on HBV DNA detection obtained in the context of non A–E hepatitis

5.1. Ultrasensitive PCR test for HBV

This assay consisted of two-step amplifications (nested or semi-nested PCR) by means of selected primers located in well-conserved regions of the HBV genome (selected by alignment of more than

Table 2
Clinical and histopathological status of non-A, non-E patients

	Patients no.	Risk factor (%)	Anti-HBc[a] positive (%)	Severe fibrosis or cirrhosis (%)	Steatosis (%)
HBV DNA[b] positive	15	20	73	53	0
HBV DNA[c] negative	35	28	6	16	25

[a] These are additive results of anti-HBc testing obtained by a competition assay (CORAb, Abbott) and a sandwich test (Ortho Diagnostics).
[b] 4/15 had past anti-HBS antibodies at very low-titer.
[c] Three patients had no available biopsies.

81 full-length genomes in Genbank). PCR was performed from serum or paraffin-embedded formalin-fixed liver biopsies.

5.2. Results on incidence, histopathology and immunohistochemistry

PCR amplification of HBV DNA demonstrated the presence of circulating HBV sequences in 30% of 50 non-A, non-E hepatitis patients as shown in Table 2. PCR was carried out by amplification of two different regions of the HBV genome (S and X genes) which reinforces the finding that these patients harbour HBV DNA sequences. In all cases, HBV DNA detection in serum was further confirmed using paraffin-embedded, formalin-fixed liver biopsies (PCR amplification of the S gene) and/or on serial serum samples (at least 2) from the same patient, with consistent positive results.

Fifty patients with evidence of chronic hepatitis without identifiable etiology were evaluated (Table 2). From 20 to 28% of these patients had evidence of documented risk factors for hepatitis viruses, and 50% had moderately elevated transaminases (1.5–2 times upon normal limit). Of the fifty patients, 13 were positive for anti-HBc using two tests of different format (Table 2); 11 of these (85%) were HBV DNA positive. Among the 15 patients found to be HBV DNA positive, seven had cirrhosis and one severe fibrosis. Steatosis was not observed in any of the HBV positive patients, but was found in 25% of the HBV negative cohort. In almost all cases, patients were classified in the Metavir score as A_0 or A_1, suggesting that the liver injury process was of low-intensity. Identification of cirrhosis or severe fibrosis in 8/15 HBV positive patients emphasises that low-grade HBV infection was associated with significant liver disease sequelae in these non-A, non-E hepatitis patients. Lobular lesions were a frequent finding on liver biopsies from all 50 patients, whatever their HBV DNA status (data not shown).

To investigate whether HBV genomes detected in the liver were competent for gene and protein expression, immunostaining for HBsAg and HBcAg was performed on liver biopsies. Nine out of 15 HBV carriers were positive either for HBsAg or HBcAg in the liver at low-levels (data not shown).

5.3. Example of mutations in the HBsAg

Amplified HBV genomic PCR fragments were sequenced to examine for the presence of viral gene mutations. As illustrated in Fig. 1 for one patient in whom the entire HBV genome could be amplified and sequenced, amino acid substitutions were observed that could explain the absence of serologic detection of HBV. In this case, mutations were identified in the HBsAg region which are different from the normally described 'vaccine escape' mutants. Eleven substitutions in the HBsAg gene were primarily concentrated within the 'a determinant'. Six rare mutations were already known, while five had never been described before. These mutations, often involving the loss or

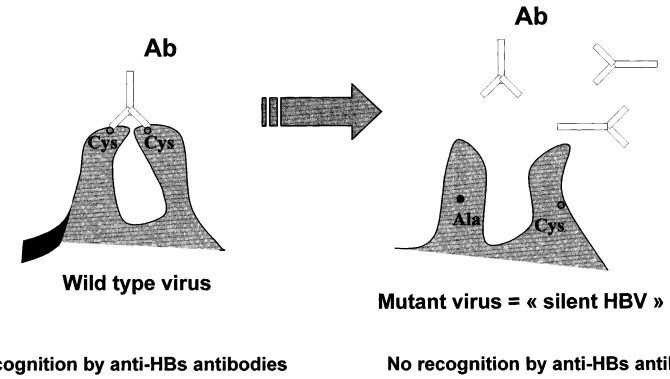

Fig. 1. Changes in the secondary structure of HbsAg leading to the failure of recognition by anti-HBs.

the introduction of Pro or Gly residues, could lead to changes in the secondary structure of HBsAg, explaining why the mutant virus is not recognized by anti-HBs antibodies (Fig. 1).

6. Conclusion

The proportion of chronic hepatitis cases of unknown etiology in which HBV DNA is detectable depends on different parameters such as: (1) the method used for HBV DNA detection – even by PCR, the choice of primers and the experimental conditions are critical; (2) the origin of the samples, prevalence being potentially higher in clinical settings with liver disease patients and/or in regions of high-HBV endemicity and (3) whether serum, PBMCs or liver tissue are tested.

Viral or host factors allowing HBV persistence in the absence of HBsAg can depend on several mechanisms. First, viral interference in coinfection with HCV is now well documented, but can explain only a proportion of HBsAg(−) HBV infections. Whether a new viral agent could interfere with HBV infection remains a speculation to be documented. Secondly, HBV mutations in the core promotor region leading to minimal HBV replication, or mutations in the HBsAg-encoding region might explain the absence of serological recognition. Nevertheless, these latter mechanisms seem to explain only a subset of the HBsAg(−) patients found to be positive for HBV DNA by nested PCR. Finally, it is possible that in some cases host immune mechanisms can maintain HBV infection in a latent state until transmission to another individual who subsequently develops a more active infection especially when immunosuppressive therapy is employed.

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