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The humoral immune response in acute and chronic hepatitis B virus infection

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Introduction

Infection with the hepatitis B virus (HBV) can lead to a variety of outcomes ranging from acute self-limited infection to various forms of chronic infection [i.e., asymptomatic carrier (ASC), chronic persistent (CPH) and chronic active hepatitis (CAH)]. Asymptomatic HBV carriers have normal liver morphology and function despite the presence of high levels of viral replication within their hepatocytes. This and a number of other observations have led investigators to the conclusion that liver injury and the subsequent clearance of virus during HBV infection is mediated by the immune response, and that the HBV is not directly cytopathic [1, 2]. An implication of this conclusion is that an inadequate immune response leads to chronicity. Although the success of HBV immunization programs attests to the role of antibody in protective immunity, evidence supports the role of the cellular rather than the humoral immune response in the pathogenesis of HBV-associated liver disease. For example, severe acute and chronic hepatitis can occur in the absence of humoral immunity as in the case of HBV infection in patients with agammaglobulinemia [3]. This and other clinical observations do not suggest that neutralizing antibody plays no role in eliminating virus (i.e., prevention of extracellular spread of virus), but rather that an HBV-specific humoral response is not an important mediator of liver injury and is probably not sufficient to resolve an acute or chronic infection. Therefore, a clue to the nature of the immune "defect" in chronically infected HBV patients is most likely to be found at the level of T cell responsiveness to HBV antigens. Beyond diagnostic purposes, why then is the examination of the humoral immune response to HBV antigens in acute and chronic HBV patients necessary or important? The humoral immune response during HBV infection is an important "indicator" of underlying T cell function or dysfunction. Sensitive serological assays are, therefore, a valuable asset in the diagnosis, prognosis and treatment of chronic HBV infections. For example, the presence or absence of an antibody response to an HBV antigen or the diversity of

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antibody specificities produced may predict the degree of T cell sensitization to that particular antigen. Moreover, the immunoglobulin class (IgM/IgG) and IgG subclass distribution of anti-HBV antibodies may reflect the time of onset of infection and possibly the predominance of a particular T helper (Th) cell phenotype (Th_1 vs Th_2), respectively. The kinetics of a specific antibody response may provide information relevant to antigen production and/or release from infected hepatocytes. In this report, previous and more recent studies of the humoral immune response during HBV infection are reviewed in an attempt to better understand the mechanisms responsible for long-term, persistent HBV infection.

Structural proteins of the HBV

During HBV infection, four structural antigen/antibody systems are observed: hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs); the pre-S antigens associated with HBsAg particles and their antibodies; the particulate nucleocapsid antigen (HBcAg) and anti-HBc; and an antigen structurally related to HBcAg, namely HBeAg, and its antibody (anti-HBe). The specific serological marker of HBV infection is HBsAg, which is present both in the intact virion and as free circulating filamentous and spherical 22-nm subviral particles. HBsAg is composed of a major polypeptide, P25, and its glycosylated form, GP28. HBsAg is a complex, T cell-dependent antigen possessing a common group-specific determinant, designated *a*, and two sets of subtype-specific determinants, *d/y* and *w/r*. Therefore, the four subtypes of HBsAg (*adw, ayw, adr*, and *ayr*) represent the major viral phenotypes. Additional envelope polypeptides of higher molecular weight (P42, large and GP33, middle) have recently been identified [4–6]. The larger polypeptides share the 226 amino acids of the S region at the carboxy terminus. The pre-S(2) region consists of 55 residues amino-terminal to the S region, and the pre-S(1) region consists of 119 or 108 (*ay*) residues amino-terminal to the pre-S(2) region.

The nucleocapsid of HBV is a 27-nm particle composed of multiple copies of a single polypeptide (P21), and the intact structure exhibits hepatitis B core antigenicity (HBcAg). A nonparticulate form of HBcAg, designated HBeAg, is secreted into the serum during HBV infection. Although HBcAg and HBeAg are serologically distinct, the primary amino acid sequences show significant identity (i.e., serum HBeAg lacks the C-terminal 34 residues of HBcAg and possesses an additional 10 N-terminal residues [7, 8]). This sequence homology explains the high level of cross-reactivity between HBcAg and HBeAg at the level of T cell recognition [9].

Historical serology of acute and chronic HBV infection

The serology of acute HBV infection has been extensively studied. The HBsAg, including the pre-S region, is an early marker of HBV infection. Although the anti-HBs response is often delayed in appearance, recent data suggest that anti-pre-S responses may occur quite early in infection [10]. Nevertheless, the humoral responses to the envelope antigens can show great variation from patient to patient during infection and after vaccination, ranging from high to nonresponder phenotypes. With respect to the HBcAg, high-titered antibodies are regularly produced by virtually 100% of HBV-infected patients. The high frequency of anti-HBc production is due, at least

in part, to the fact that HBcAg can function as a T cell-independent antigen [11]. IgM anti-HBc appears early in acute hepatitis B, and IgM and IgG anti-HBc can persist with slowly decreasing titers for many years. In contrast, seroconversion to anti-HBe status occurs later than anti-HBc, can be quite variable in terms of onset, and correlates with viral clearance [12]. Figure 1A depicts a typical course of an acute HBV infection.

The serology of chronic hepatitis B (CH-B) infection has been reported to be remarkably homogeneous considering the diversity of clinical classifications (i.e., ASC, CPH, CAH) and degrees of liver injury. For example, the sera of CH-B patients contain HBV virions, subviral particles composed of excess envelope proteins (HBsAg and pre-SAg), the HBeAg, and antibody to the nucleocapsid (anti-HBc), but remain seronegative for anti-envelope and anti-HBe antibodies for many years until HBV DNA clearance and loss of serum HBeAg [13]. Serum HBsAg remains positive in many resolved as well as in unresolved CH-B infections, and correspondingly anti-HBs often remains undetectable even after HBV clearance (Fig. 1B).

Humoral immune response to the pre-S envelope antigens

The immune responses to the pre-S antigens of the HBV envelope have been less well studied due to their more recent discovery. However, anti-pre-S antibodies have been shown to be virus neutralizing in chimpanzee protection studies [15–17] and several groups have examined the humoral immune response to these antigens during HBV infection. Neurath et al. [18] demonstrated that the pre-S(1) sequence 12–32 is recognized by antibodies in sera of individuals recovering from acute HBV infection [18]. It was noted that anti-p 12–32 can be detected prior to anti-HBs or anti-HBc seroconversion but declines rapidly after production of anti-HBs and anti-HBc. The fine specificity of anti-pre-S(1) production during acute HBV infection was studied using a panel of pre-S(1) synthetic peptide analogs. It was shown that the human anti-pre-S(1) response can include multiple specificities since peptide sequences represented by p1–21, p12–32, p32–53, and p94–117 were variably recognized by sera from different HBV-infected patients [19,20]. Analysis of the temporal occurrence of anti-pre-S(1) in an HBV-infected chimpanzee also illustrated the early appearance of this antibody, which was detected shortly after pre-S(1) antigen appeared during acute disease [21]. Several studies have examined pre-S(1) antibody production in acute and chronic HBV infection and found that antibodies to pre-S(1) appear at an early stage of acute resolving HBV infection, but the antibodies are absent [22] or of differing specificity [20] in the sera of patients with an HBV infection entering a chronic course and in the sera of chronic carriers. In another study of chronically infected patients, an interesting observation was that among asymptomatic carriers anti-pre-S(1) was detected in those seropositive for anti-HBe but not in those positive for HBeAg [23]. The authors suggested that anti-pre-S(1) may have a role in clearing HBV from the circulation because pre-S(1)-containing polypeptides are preferentially expressed on virions [4].

Several groups have examined in vivo antibody production to the pre-S(2) region of HBsAg during HBV infection. Alberti et. al. [24] examined acut-phase sera (negative for anti-S) for antibody that would precipitate radiolabeled intact virions and termed this antibody anti-Dane particle (DP). Patients who subsequently seroconverted to anti-HBe status rapidly exhibited high anti-DP activity, whereas patients with low

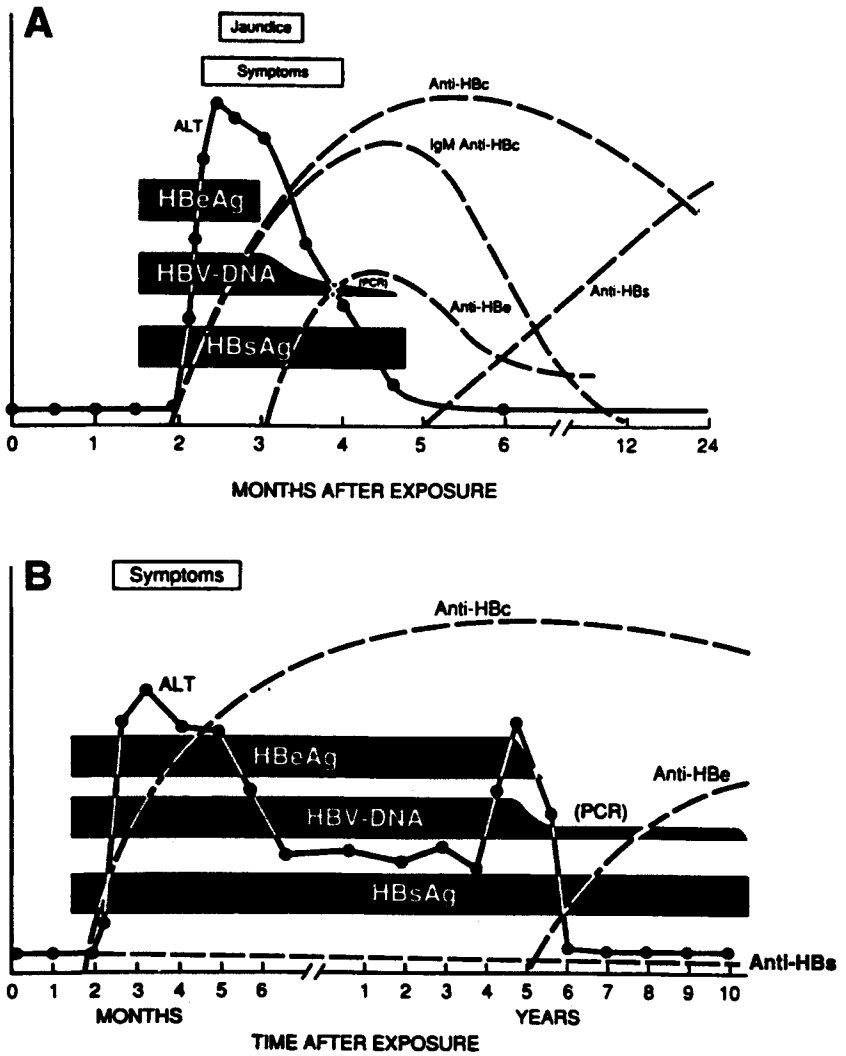

Fig. 1. **A** Typical course of a case of acute hepatitis B. Initially, hepatitis B virus (HBV) DNA can be detected by blot hybridization, but as the disease resolves only low levels detectable by polymerase chain reaction can be detected. *ALT*: alanine aminotransferase: *HBeAg, HBcAg, HBsAg*: HBV e, core and surface antigens, respectively; anti-HBe, anti-HBc, anti-HBs: antibodies to HBeAg, HBcAg and HBsAg, respectively. **B** Representative course of a case of chronic hepatitis B (CH-B) in which acute infection is followed by chronic infection. Ultimately, there is a remission in disease when seroconversion from HBeAg to anti-HBe occurs. Adapted from [14]

or absent anti-DP activity seroconverted slowly or not at all. Using a synthetic peptide representing residues 120–145 within the pre-S(2) region (N-terminal 26 residues) in a solid-phase radioimmunoassay (RIA), it was demonstrated that HBV-infected acute-phase sera contain antibodies that recognize this sequence [6]. Examination of the fine specificity of antibodies reacting with the p120–145 sequence revealed that multiple

specificities exist that primarily recognize the pl33–143 sequence [25]. The pre-S(2)-specific antibodies appear early during infection and decline in titer rather rapidly [18]. Several studies have suggested a transient nature for antibodies to pre-S(2) during HBV infection [26,27]. An examination of antibodies capable of inhibiting the interaction between HBsAg and polymerized human serum albumin (pHSA) revealed the appearance of antibody in the early recovery phase of acute hepatitis co-occurring with HBsAg and elevated aminotransferase levels and preceding the anti-HBs response. However, in chronic HBV infection, antibodies were detected in only 1% of 358 asymptomatic carriers seropositive for anti-HBe and in none of 67 sera that were seropositive for HBeAg [28]. Assuming this assay was specific for anti-pre-S(2), these results indicate that pre-S(2) antibody is not produced by asymptomatic carriers regardless of HBeAg/anti-HBe status, and in this respect anti-pre-S(2) parallels anti-S region antibody production. A caveat to this interpretation is that anti-pre-S(2) may not be detected in a single serum sample due to its apparent transient nature. However, temporal analysis of anti-pre-S production during acute and chronic HBV infections also indicated a correlation between HBV clearance and anti-pre-S production. No anti-pre-S(2) was detected in acute patients who progressed to chronicity or in patients with chronic hepatitis with persistence of HBsAg and pre-S antigens regardless of HBeAg/anti-HBe status [29,30].

In summary, the pre-S antigens represent new serological markers of HBV infection that may provide information relevant to viral replication and infectivity. The corollary is that immune responses may be important to viral neutralization mechanisms, and analysis of anti-pre-S antibody production may have diagnostic and prognostic value. Whether this new information will be beneficial beyond that currently derived from the standard serology and HBV DNA levels remains to be determined.

Revised view of the serology of chronic HBV infection

If cellular immune responses are responsible for liver cell injury and HBV clearance, there should be serological evidence of these underlying cellular processes. However, the presence of HBsAg and HBeAg in the serum may affect the ability to detect circulating antibodies and may obscure the onset of seroconversion. The available commercial assays usually detect anti-HBs and anti-HBe antibodies only after the respective antigens have been cleared from the serum (see Fig. 1). To address the possibility that antibodies to the structural HBV antigens are produced but not detected in CH-B infection, several groups have developed sensitive immunoassays for the detection of anti-HBe and anti-envelope (anti-HBenv) antibodies, regardless of the presence of the antigens in serum [31–33]. We and others [32–34] have utilized a direct binding enzyme immunoassay (EIA) to detect IgG anti-HBe in the sera of HBeAg-positive CH-B patients. This EIA method is more sensitive than the commercial anti-HBe assays, which are based on antigen neutralization [32]. To detect anti-envelope antibodies (i.e., anti-S and anti-pre-S) in the presence of a vast excess of the particulate HBsAg, a method of detecting IgG anti-HBenv that is immune-complexed to HBsAg was developed [31]. Using these more sensitive immunoassays, a series of serum samples from 200 HBsAg and HBeAg-positive CH-B patients with various degrees of liver disease were recently analyzed [32]. All patients were seronegative for antibodies specific for the envelope antigens or the HBeAg when the current commercial assays were utilized. In contrast, virtually all chronically infected patients with liver disease

(i.e., CPH and CAH) and approximately 50% of chronic patients without liver disease (ASC) demonstrated anti-HBe and anti-envelope antibodies when sera were tested in the more sensitive immunoassays (Figs. 2, 3). Furthermore, the asymptomatic patients could be serologically distinguished from the symptomatic patients based on antibody fine specificity, titer, and IgG subclass. For example, the production of anti-HBc and anti-HBe antibodies of the IgG_3 isotype correlated with symptomatic infection. This and other studies reporting the presence of circulating HBsAg- and HBeAg-containing immune complexes (IC) [32, 34–39] and free anti-HBe antibodies [32, 33] in the sera of CH-B patients demonstrate that chronically infected HBV patients are not immunologically unresponsive as suggested by the current commercial assays. In fact, only 44.4% of ASC patients are characterized by the absence of anti-HBe and anti-envelope antibody production (Table 1). In light of these new serological findings, the concept of a relatively non-overlapping seroconversion from HBeAg-positive to anti-HBe-positive status and the total absence of anti-HBs antibodies during CH-B infection (Fig. 1B) is no longer tenable. Anti-HBe conversion can occur many years prior to the loss of HBeAg or liver injury. Similarly, anti-envelope antibodies may co-exist with virions and subviral HBsAg particles for many years before viral clearance and loss of HBsAg. A more accurate view of the serology of chronic HBV infection is demonstrated by the patient represented in Fig. 4. This patient, diagnosed with CAH demonstrated three episodes of liver disease during the 3 years of observation, and is representative of many symptomatic CAH patients. Note that humoral immune responses specific for HBcAg, HBeAg, and HBsAg were present and that antibody levels increased in parallel with alanine aminotransferase (ALT) elevations. A recent study revealed significant correlations between increasing levels of serum HBV DNA, HBeAg, HBeAg-containing IC, and liver injury [40]. These results suggest that increases in viral replication and accumulation of viral proteins in the serum (HBeAg) and intracellularly (HBcAg) and the subsequent immune response play an important role in initiating the repeated bouts of liver injury often seen in CH-B infection. The anti-HBc and anti-HBe antibody responses were characterized by IgG_1 and IgG_3 subclass antibody production. Note that the anti-HBe response was positive before the first ALT elevation and the anti-envelope response became detectable during the first ALT elevation using the more sensitive assays. In contrast, an anti-HBe antibody seroconversion was not detected by the commercial anti-HBe assay until after the third ALT elevation and loss of HBeAg from serum, and anti-HBs antibodies were never detected by the commercial assay (Fig. 4). Serological studies and evidence from a murine model suggest that anti-envelope antibody production in CH-B patients may be mediated, at least to some extent, by HBc/HBeAg-specific Th cells [32,41]. This is consistent with several recent reports that HBc/HBeAg-specific but not HBsAg-specific T cell proliferative responses are demonstrable in CH-B patients [42–45].

The frequency and kinetics of IgM anti-HBc production have been well characterized and have been found to be useful for diagnosing acute HBV infections. However, the current assays for anti-HBc and anti-HBe detection do not discriminate between IgG isotypes since they are based upon a competitive format. Several groups have examined the IgG isotype distribution of anti-HBc and anti-HBe antibodies in acute and chronic HBV infection using experimental immunoassays. Similar to other viral antigens, IgG_1 is the major isotype of anti-HBc elicited in both acute and chronic HBV infections [32, 33, 47, 48]. However, the IgG_3 and IgG_4 isotypes of anti-HBc are also detected in acute HBV (AH-B) infected patients [48]. An unexpected find-

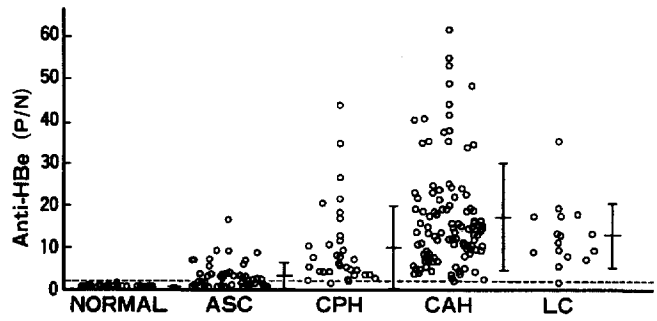

Fig. 2. Prevalence of anti-HBe antibody production in CH-B carriers determined by an experimental direct enzyme immunoassay (EIA). *ASC*: asymptomatic carriers; CPH, CAH: chronic persistent, chronic active hepatitis, respectively; *LC*: liver cirrhosis; P/N: positive/negative ratio. Adapted from [32]

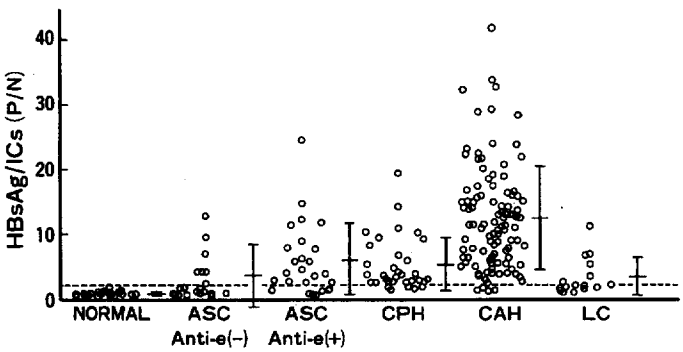

Fig. 3. Prevalence of anti-envelope antibody production in CH-B carriers. Anti-envelope antibody production was determined by measuring HBsAg/anti-HBs immune complexes (*ICs*). Adapted from [32]

ing is the high detection rate of IgA_1 anti-HBc in acute HBV infections [48,49]. The kinetics of IgA_1 anti-HBc production appear similar to that of IgM anti-HBc. In CH-B infection anti-HBc and anti-HBe of the $IgG_{1,3}$, and $_4$ isotypes can also be detected [33,47,48]., In a panel of well-characterized CH-B patients, IgG_3 anti-HBc and anti-HBe antibodies correlated with symptomatic chronic infection, whereas, IgG_1 anti-HBc was produced by all chronic carriers and IgG_1 anti-HBe was produced by a high percentage of asymptomatic as well as symptomatic CH-B patients [32].

In summary, the use of more sensitive serological assays has revealed that substantial immune responsiveness can occur during CH-B infection, and three representative serological profiles have been observed. Additional serological profiles may emerge as more heterogeneous populations of CH-B patients are examined. However, the serological profiles described in Table 1 are much more consistent with the concept of immune-mediated liver injury and HBV clearance in CH-B infection than is the conventional serology, which suggests that CH-B patients are immunologically nonresponsive despite ongoing liver disease. The ability to detect anti-HBe and anti-envelope antibody seroconversion events early will be useful as a means of monitoring the onset of underlying cellular immune responses (i.e., Th cell sensitization), whereas current antibody detection methods merely confirm antigen clearance after

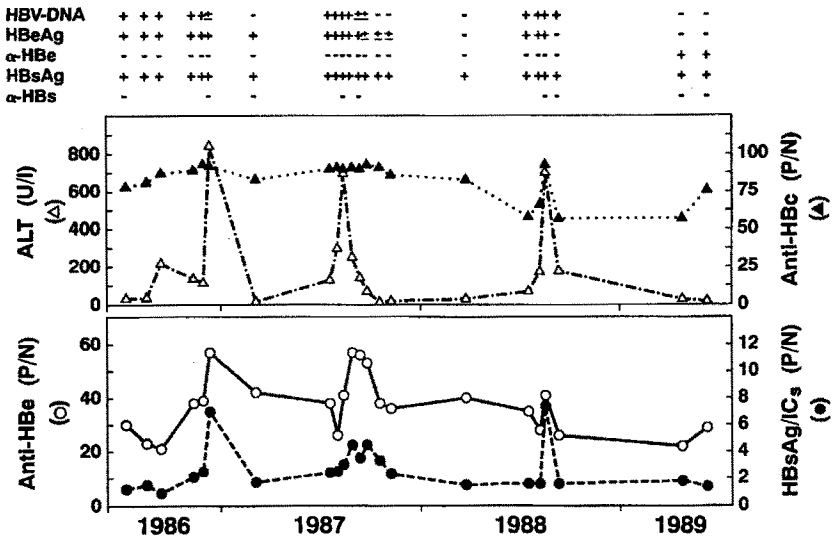

Fig. 4. Serological profile of a representative symptomatic CH-B patient determined using experimental immunoassays as compared to current commercial assays (Abbott, *top*). Adapted from [32]

Table 1. Summary of the revised serological profiles of chronic hepatitis B infection and possible roles of hepatitis B virus (HBV) e antigen (HBeAg)-specific T helper (Th) cells

Clinical diagnosis	Frequencey		Serological profile	HBeAg-Th	Functions	Liver disease
ASC	44.4%	I.	↓ α-HBc(G_1) [a]HBV DNA ++[a]	0	–	0
ASC	55.6%	II.	↑ α-HBc(G_1) α-HBe (G_1) α-HBenv HBV DNA ++	Th_2-like	↑Antibody production	0
CAH CPH	~ 100%	III.	↑↑ α-HBc (G_1 + G_3) ↑ α-HBe (G_1 + G_3) ↑ α-HBenv HBV DNA + to 0	Th_1-like + Th_2-like	↑Inflammatory response α-HBc/HBe → G_3?	+ + to ±

Ag, antigen; ASC, asymptomatic carrier; CAH, chronic active hepatitis; CPH, chronic persistent hepatitis; α-HBc, antibody to HBV core Ag; G_1/G_3, IgG_1/IgG_3; α-HBe, antibody to HBeAg; α-HBenv, antibody to HBV envelope protein; ↓, decreasing; ↑, increasing

[a] HBV-DNA: ++, > 1000 pg/ml; +, 5–1000 pg/ml; 0 < 5 pg/ml (adapted from [32])

the fact. Furthermore, early detection of anti-HBe antibody production especially of the IgG_3 subclass may predict future liver injury. Similarly, anti-HBe-positive ASC

patients may benefit from immunomodulatory therapy [i.e., interferon (IFN)], whereas, anti-HBe-negative ASC patients may not.

The specificity and source of Th cell function for anti-HBc, anti-HBe, and anti-envelope antibody production in the context of CH-B infection may also be surmised from a complete serological analysis. We propose that the production of low titer and IgG_3 subclass-deficient anti-HBc and anti-HBe responses in asymptomatic versus chronic active hepatitis patients indicates reduced Th cell function in asymptomatics and may reflect differential engagement of Th cell subsets amongst these patient groups. The serological profile and absence of liver disease in anti-HBe-positive ASC is consistent with an exclusive Th_2-like response. Alternatively, the serology and evidence of immune-mediated cytotoxic responses in CAH patients suggest a Th_1-like or combined Th_1-Th_2-like response (Table 1). Interestingly, in a murine system, we have observed that certain HBeAg-specific T cell site-MHC combinations preferentially elicit either a Th_1-like (proliferation) or a Th_2-like (antibody) response [79]. In addition to the diagnostic value of these new immunoassays for the serological characterization of CH-B carriers, these results suggest possible therapeutic approaches for terminating the chronic carrier state. For example, it may be beneficial to convert the predominantly Th_2-type HBeAg-specific Th cell response evident in ASC into a Th_1-predominant response which may more efficiently mediate viral clearance.

Distinguishing between acute and symptomatic CH-B infection

Differentiating between an AH-B infection and an acute exacerbation (AE) of a CH-B infection can present problems for the clinician. This is especially true because patients with CAH and CPH often show a cyclic pattern of hepatitis characterized by AE of liver injury alternating with normal liver function. During the symptomatic phases of infection, patients with both acute and chronic HBV are likely to present with similar serological profiles as determined by the available commercial assays. For example, during symptomatic periods, patients with acute and chronic HBV have increased liver enzyme levels, the HBsAg is present in the serum, and they produce antibodies to the HBcAg, but antibodies specific for the HBsAg or the HBeAg are not detected. Antibody production to HBcAg occurs early in the course of the acute phase of HBV infection and can persist for many years, and chronically infected patients produce high titers of anti-HBc. In contrast to most viral infections, patients with acute and chronic HBV often produce both IgM and IgG anti-HBc antibodies; therefore, the mere presence of IgM anti-HBc is not diagnostic of an acute infection. However, higher levels of IgM anti-HBc are generally produced during the acute phase as compared with chronic infection, and this quantitative difference has become the only serological means of differentiating an acute HBV infection from an AE of a chronic infection [46]. The usefulness of IgM anti-HBc assays in the differentiation of acute from chronic HBV infection has also been questioned [50, 51]. The distinction between acute and chronic HBV infection is important in terms of prognosis and possible treatment modalities.

Because the use of more sensitive assays for the detection of anti-HBe and anti-HBs antibodies has modified our view of the serology of CH-B infection, it was of interest to apply the use of these same immunoassays to AH-B infection. In a recent study, serum samples from HBeAg-positive patients with acute and chronic HBV, taken during periods of peak liver enzyme increases, were evaluated for the

presence of anti-HBe, HBeAg-containing IC, HBsAg-containing IC, IgM anti-HBc, IgG anti-HBc, and a novel anti-HBc specificity [52]. The results indicated significant differences in the serology of acute and chronic HBV infection and extended the ability to distinguish between acute and symptomatic chronic infection well beyond IgM anti-HBc production. Sera from patients with CH-B showed significantly higher levels of free anti-HBe, HBeAg/anti-HBe IC, and HBsAg/anti-HBs IC compared with AH-B patient sera. Interestingly, the most significant and possibly the most useful difference in the serology of patients with acute and chronic HBV was the presence of a novel specificity of IgG anti-HBc antibody in the sera of patients with chronic (but not acute) HBV infection. Patients with CH-B infection produce high-titer anti-HBc antibodies that cross-react with the nucleocapsid of the woodchuck hepatitis virus, which we designated as anti-HBc^{W} [52].

It may seem contradictory that CH-B patients exhibit higher levels of HBV-specific serum antibodies and IC as compared to AH-B patients who clear the infection relatively efficiently. Again, the predominance of different Th cell subsets in acute and chronic HBV infection may explain the higher antibody levels during chronic infection. For example, predominance of the Th_2 subset during chronic infection would favor antibody production over cell-mediated immunity (CMI), whereas predominance of the Th_1 subset in acute infection would favor CMI which may result in efficient viral clearance mechanisms. We are currently examining differential Th cell subset recognition of HBeAg in a transgenic murine model, and methods to shift the Th_1-Th_2 balance (see below).

An experimental model of HBe/HBcAg-specific immune responses

Because of the importance of the immune response specific for nucleocapsid antigens in HBV clearance, we have generated HBeAg- and HBcAg-expressing transgenic (Tg) mice. Studies in HBeAg-Tg mice suggested that the HBeAg may cross the placenta and function as a tolerogen in utero [53]. Babies born to HBV-infected mothers may be unable to mount an HBe/HBcAg-specific Th cell response after neonatal or perinatal infection due to T cell tolerance and, therefore, become chronically infected. Indeed, greater than 90% of such babies become chronic carriers of HBV [54]. We have also demonstrated previously that certain HBeAg-specific Th cells can evade tolerance induction in HBeAg-Tg mice (i.e., 129–140-specific and IA^b-restricted Th cells) [55]. Recently, mechanisms of escape from tolerance have been studied. It appears that the 129–140-specific Th cells that evade tolerance induction are of low avidity and are predominantly of the Th_2 subset [80]. The HBeAg-specific Th cells responsible for anti-HBe production in CH-B patients (see Table 1) may also represent low avidity, Th_2-like T cells that evaded or have emerged from the tolerant state. Because HBeAg-Tg mice on an H-2^b background possess Th cells reactive with "self" antigen (i.e., HBeAg), it is possible to elicit in vivo autoantibody production (i.e., anti-HBe) by activating HBeAg-specific Th_2 cells simply by injecting the synthetic T cell site 129–140. Such Th_2 cell activation results in the cognate interaction of the Th cells with B cells specific for HBeAg (which are not tolerant in this model) and high levels of anti-HBe antibodies (titer $> 10^5$) are produced which neutralize detection of the circulating "self" HBeAg [55].

The finding of residual and functional HBeAg-specific Th cells in the periphery of HBeAg-Tg mice suggests the possibility that in the context of HBeAg-positive

chronic infection, immunologically silent, HBeAg-specific Th cells may be present and capable of responding to activation if provided with an appropriate stimulus. In the transgenic model, quiescent HBeAg-specific Th cells can be activated by a single injection of the synthetic T cell site 129–140. Similarly, in human CH-B patients injection of selected HBeAg-derived synthetic or recombinant antigens may activate quiescent Th cells in vivo. It is also significant that the functional HBeAg-specific Th cells which evade tolerance induction in the Tg model are primarily of the Th_2-type. Using this Tg system we have screened a number of compounds for the ability to modulate anti-HBe production in vivo. For example, anti-CD4, anti-IL-2 receptor, anti-IL-4 receptor (unpublished observations), CTLA4Ig [56], IA^b-binding peptides [55], and IL-12 all suppress anti-HBe autoantibody production in vivo. Recent experiments in the HBeAg-Tg model have indicated that Th_2-mediated anti-HBe antibody production can be shifted to a Th_1-like response using IL-12 as a modifier, and reciprocally a Th_1-mediated response can be shifted to a Th_2-like response using IL-4 [81]. If Th_2-like, HBeAg-specific Th cells dominate the immune response in asymptomatic HBV carriers, as we have previously suggested [32,40,52], it may be possible to alter the Th_1-Th_2 balance in these patients to favor viral clearance rather than mere antibody production. Therefore, a vaccine/cytokine combination may serve as an effective therapeutic vaccine strategy to treat chronic HBV carriers. The HBeAg-Tg model may also be useful to screen drugs or therapies useful for the treatment of Th_2-mediated allergic or autoimmune diseases. In many ways the immune response to HBV antigens in chronically infected patients resembles an autoimmune process in light of the fact that long-term carriers have "lived" with HBV antigens for many years. However, the goals of immunotherapy for chronic HBV infection and autoimmune disease are quite different. In the treatment of autoimmunity the primary goal is to down-regulate the immune response, whereas in CH-B infection the primary goal is to redirect the immune response in a manner which is more effective in clearing the infection. In certain instances the goal of therapy may be to activate rather than down-regulate the immune response in CH-B patients.

Secondary manifestations of the humoral immune response in CH-B infection

Immune complex disease

Given the frequent co-occurrence of anti-HBe and anti-HBenv antibodies and their respective antigens in the serum of HBV-infected patients, the presence of circulating IC is not surprising. Indeed, IC diseases have been described in carriers of HBV [57]. The various manifestations include: glomerulonephritis [58–60], arthritis [61], and polyarteritis nodosa [62, 63]. Hepatitis B-associated glomerulonephritis (HBGN) has become a well-recognized complication of HBV infection. The majority of patients are male children [60]. While the exact incidence of HBGN in adults and children is not known, the percentage of HBV-infected patients demonstrating glomerular deposits containing HBV antigens has been reported to be relatively low [64, 65]. Both HBeAg and HBsAg have been implicated as antigens detected in granular deposits in the glomeruli along with immunoglobulin and complement components [59, 60]. However, at least one study emphasized the predominance of HBeAg-mediated glomerulonephritis [59]. It was suggested that because of the size difference between HBeAg and the particulate HBsAg, IC involving HBeAg would be expected to pref-

erentially deposit in glomerular capillary walls, whereas HBsAg-containing IC would deposit in vascular walls as described in cases of polyarteritis nodosa [66].

Although immunosuppressive therapies have been utilized in HBGN patients, remission of the glomerulonephritis often occurs as the patients seroconvert from HBeAg to anti-HBe. Therefore, use of medications such as steroids and azathioprine may suppress the HBV-specific immune response and actually delay seroconversion and may be contraindicated [59]. In the context of the more sensitive serological assays, it is notable that in symptomatic CH-B patients, the free HBeAg and immune-complexed HBeAg levels rise and fall in the serum in parallel with ALT values (Fig. 5). Because a relatively constant source of HBeAg is required for the maintenance of immunopathological processes in the kidney [59], the cyclic appearance of HBeAg in the serum of symptomatic CH-B patients may protect this group from severe IC disease. Alternatively, IC-positive (HBeAg/anti-HBe; HBsAg/anti-HBs) asymptomatic chronic carriers (see profile II, Table 1) may be at increased risk for IC disorders due to the steady-state presence of serum HBeAg and HBsAg. This may explain the increased incidence of HBGN in children from areas endemic for HBV infection. A majority of these children are infected neonatally and may begin to emerge from HBeAg-specific T cell tolerance mechanisms with age and produce low levels of anti-HBe and anti-HBs antibodies in the absence of liver injury.

Autoantibodies

Apart from antibody-mediated autoimmune hepatitis, infection by HBV may also elicit autoantibody production. Distinct pathways may result in the development of autoantibodies during HBV infection. First, the development of primarily virus-specific antibodies which recognize similar motifs on self structures may lead to autoreactivity. This mechanism has been termed molecular mimicry [67]. This pathway is probably not as relevant for the HBV-specific immune response as it may be for the response to the hepatitis C virus (HCV). It was recently shown that a sequence derived from a putatively host-derived protein, designated GOR, displays a 62% homology with one of the major linear antigenic regions of the HCV core protein [68–70]. However, whether these putative autoantibodies have any relation to HCV pathology is unclear and has been widely debated [70, 71].

In a second mechanism, the induction of autoantibodies results from the active lysis of virally infected cells and this mechanisms may have a more obvious relevance to HBV infection. Several studies have demonstrated the presence of autoantibodies in HBV infections which are mainly directed to subcellular components. A number of different potential antigens may be released and, thereby, be accessible to B cells by this route. A large array of autoantibodies have been described in HBV infections including autoantibodies directed against actin, tubulin, myosin, the hepatic asialoglycoprotein receptor, double-stranded DNA, laminin or keratin, mitochondria, parietal cells, polymerized human albumin, and thyroglobulin, as well as endothelial cell antibodies and sarcolemmal antibodies [72–74]. Patients with end-stage liver disease and hepatocellular carcinoma may develop autoantibodies to nuclear and nucleolar antigens at a frequency of approximately 30% [75].

Lastly, an additional iatrogenic route of induction of autoantibodies in CH-B patients appears to occur in patients treated with IFN-α or IFN-γ [76, 77]. However, only a minority of treated patients develop antinuclear antibodies, and no clinical signs

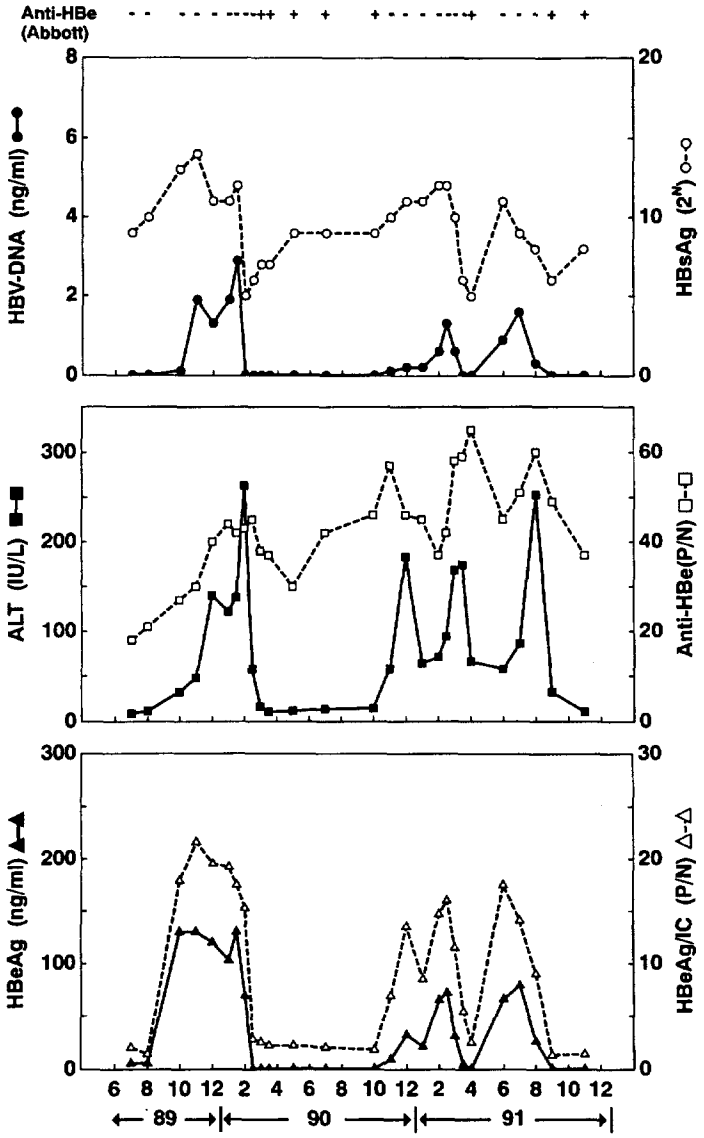

Fig. 5. Biochemical and serological analysis of a patient with CAH showing multiple acute exacerbations of liver injury. Serum samples were analyzed for HBV DNA and HBsAg (*top*); ALT and anti-HBe (direct EIA) (*middle*); and HBeAg and HBeAg/anti-HBe ICs (*bottom*). The anti-HBe status as determined by commercial assay (Abbott) is also shown. Adapted from [40]

of autoimmune disease are apparent [77]. In summary, it is clear that autoantibody production may be elicited by the lysis of HBV-infected cells, but whether these antibodies actually add to the HBV pathology remains to be determined.

Conclusions

Resolution of an AH-B infection and the associated liver injury are believed to be immune mediated. Therefore, the inability to resolve an HBV infection reflects a defect(s) in the HBV-specific immune response resulting in chronic infection. A number of studies suggest that the immunological defect may reside at the level of T cell responsiveness. Although any number of defects in the complex interactions between the host immune response and HBV may result in persistent infection, a likely candidate is an inefficient HBeAg-specific Th cell response. For example, we have proposed a model in which exposure to HBeAg in utero tolerizes the HBe/HBcAg-specific Th cell response and results in chronicity after neonatal or perinatal HBV infection [53]. Immunosuppression or genetic T cell nonresponsiveness to HBe/HBcAg may explain chronicity after adult HBV infection. However, the chronicity rate after symptomatic HBV infection in adults is relatively low (i.e., $< 10\%$). Chronic or persistent HBV infection encompasses a heterogeneous patient population ranging from severe to limited liver disease to the asymptomatic carrier state. Presumably, chronic liver disease reflects ongoing HBV-specific immune responses sufficient to cause liver cell injury but insufficient to clear the infection completely. Nevertheless, the currently available serological assays suggest that the humoral immune responses to HBeAg and HBsAg during CH-B infection are universally negative regardless of clinical status or degree of liver injury. To resolve this apparent contradiction, we and others have designed more sensitive serological assays capable of detecting anti-HBe and anti-HBs antibodies in the presence of excess circulating antigens. Using sensitive experimental immunoassays it is clear that chronically infected HBV patients produce a variety of antibodies and that the quantity and quality of antibody production correlates with the degree of liver disease. In fact, HBV-specific humoral immune responses in chronic infection appear remarkably intact considering the degree to which Th cell function can be compromised and the excess production of subviral particles and proteins during chronic HBV infection. However, at least one study did suggest a B cell defect during HBV infection [78]. It was suggested that envelope-specific cytotoxic T lymphocytes may lyse HBsAg-specific B cells that present envelope peptides in the context of the MHC class I pathway. Such a mechanism would be expected to suppress anti-HBs production.

In summary, the serological responses of CH-B patients serve as efficient "markers" of the degree and specificity of underlying T cell responsiveness. This information is relevant to the diagnosis and prognosis of CH-B patients and may be useful in determining appropriate treatment modalities. For example, the use of the experimental immunoassays can distinguish between asymptomatic and symptomatic CH-B patients and between AH-B patients and CH-B patients undergoing an AE of liver disease. Furthermore, a survey of a large number of CH-B patients using the more sensitive immunoassays suggested that the balance between HBV-specific Th_1- and Th_2-type cells may be important in preventing and/or resolving chronic HBV infection. Lastly, use of the sensitive experimental immunoassays may allow the selection of CH-B patients most likely to benefit from immunomodulatory therapy. For example, asymptomatic as well as symptomatic CH-B patients with evidence of pre-existing HBV-specific immune responses may be more responsive to immune-enhancing therapies (i.e., IFN-α).

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