Evaluation of the pre-S (pre-S(1)Ag/pre-S(2)Ab) system in hepatitis B virus infection

M I Galán, J Tomás, M C Bernal, F J Salmerón, M C Maroto

Abstract

The diagnostic and prognostic value of pre-S(1)Ag and pre-S(2)Ab was investigated in 69 HBsAg surface antigen positive patients—14 with acute hepatitis B, 30 with chronic liver disease (six chronic persistent hepatitis, 14 chronic active hepatitis, 10 with cirrhosis) and in 25 asymptomatic carriers. Pre-S(1)Ag was found in all patients with chronic hepatitis B virus (HBV) infection regardless of viral replication. In contrast, pre-S(2)Ab was not detected in any patients. Acute hepatitis was studied sequentially with periodic controls at 20 day intervals. Pre-S(1)Ag cleared before HBsAg in six of 14 (43%) patients who progressed favourably, and the two antigens cleared simultaneously in eight of 14 (57%) cases. Patients with early clearance of pre-S(1)Ag progressed favourably, thus indicating the prognostic value of this test, which, however, is still of limited practical application given the small temporal difference between the moment of clearance of the two antigens. The first markers to clear, however, were HBeAg and DNA-HBV, which showed significant differences with respect to the clearance of HBsAg. Moreover, pre-S(2)Ab appeared before HBsAb in 57.1% of our patients and was found in some patients before pre-S(1)Ag and HBsAg had cleared (42.8%), thus allowing complete viral clearance and acute HBV infection to be predicted earlier.

The complex envelope of the B virus contains proteins encoded by three different genes. The first, called S, encodes hepatitis B surface antigen (HBsAg). Subsequently, two genes located ahead of the first were found: pre-S(1) and pre-S(2). These genes gave rise to proteins of the same name.^{1,2} Pre-S(1) protein, encoded by the entire *env* gene (pre-S + S), comprises a sequence of 108–109 amino acids, and can appear in glycosylated (gp 41) or unglycosylated form (p 39). The pre-S(2) protein is composed of 55 amino acids; the molecular weights of the unglycosylated and glycosylated forms are 33 and 36 kilodaltons, respectively. The pre-S(2) protein contains an immunodominant epitope which raises anti-pre-S(2) antibodies, as well as polymerised human serum albumin (PHSA) receptor.^{3,4}

One of the most interesting aspects of these

molecules, which is currently under intensive study, is the relation between these markers and the course of disease caused by hepatitis B virus, (HBV). With this in mind we attempted to determine the usefulness of detecting pre-S(1)Ag and pre-S(2)Ab in acute hepatitis and other chronic hepatic diseases caused by HBV, to establish their diagnostic and prognostic value compared with other markers.

Methods

Sixty nine patients were studied: 14 with acute hepatitis B, 30 with HBsAg positive chronic hepatitis and 25 asymptomatic HBV carriers, who had been hepatitis B virus e antibody (HBeAb) positive during the six months preceding the study.

Acute hepatitis was defined as a 10-fold increase in serum transaminase activities above values considered normal, plus the hepatitis B core antigen presence of IgM antibody in serum. These patients were tested every 20 days. Each analysis included serum concentrations of HBsAg, HBsAb, HBcAb, HBc IgM Ab, HBeAg, HBeAb, DNA-HBV, pre-S(1)Ag and pre-S(2)Ab.

Of the chronic liver pathologies studied, six were chronic persistent hepatitis (CPH), 14 were chronic active hepatitis (CAH), and 10 were hepatic cirrhosis. All these patients were positive for HBsAg in serum and liver. The above mentioned hepatitis markers were tested every six months in this group.

The group of asymptomatic HBsAg positive carriers were referred from the Regional Blood Transfusion Centre. All were normal on physical examination, had normal titres of transaminase activity, and were negative for HBeAg. No liver biopsies were performed in this group.

None of the patients in the study had antibodies against Delta virus, and none had received immunosuppressive or antiviral treatment.

Classic markers of HBV (HBsAg, HBsAb, HBcAb, HBc IgM Ab, HBeAg and HBeAb) were analysed in all serum samples with commercial immunoenzymatic methods (Abbot Laboratories Chicago, Illinois). Serum titres of DNA-HBV were measured by molecular (blot) hybridisation with a ³²P-labelled HBV DNA probe. Pre-S(1)Ag and anti-pre-S(2) peptide antibodies were studied with solid phase immunoassay analysis in microtitre plates (Microelisa System, Organon Teknika). The test is an enzyme immunoassay based on a "sandwich" principle. The wells of poly-

Microbiology Service,
University Hospital of
Granada, E-18012
Granada, Spain
M I Galán
M C Bernal
M C Maroto

Gastroenterology
Service, University
Hospital of Granada
J Tomás
F J Salmerón

Correspondence to:
Dr M I Galán.

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Pre-S(1)Ag and pre-S(2)Ab determinations in different types of HBV disease

Type of hepatitis	No	Pre-S(1)Ag	Pre-S(2)Ab
		No (%)	No (%)
Chronic HBsAg positive	30	30 (100)	
HBsAg positive carrier	25	25 (100)	
Acute HBsAg positive	14	14 (100)	12 (85.7)

Figure 1 Course of patients with acute hepatitis in whom pre-S(1)Ag and HBsAg became negative simultaneously.

Figure 2 Course of patients with acute hepatitis in whom pre-S(1)Ag became negative before HBsAg.

Figure 3 Serum markers in patients with acute HBV whose illness resolved.

styrene Microelisa strips were coated either with sheep antibody to pre-S(1), which constituted the solid phase antibody for the detection of pre-S(1)Ag, or with synthetic pre-S(2) (126–145) peptide, which constituted the solid phase antigen for the detection of antibodies against pre-S(2). The test sera were incubated in the solid phase and washed three times successively, then reincubated with either a monoclonal antibody to the “S” domain of the hepatitis B virus envelope protein (anti-S), which had been labelled with horseradish peroxidase (HRP) (to test for pre-S(1)Ag), or with synthetic pre-S(2) (126–145) peptide labelled with HRP (to test for anti-pre-S(2)). Incubation with the enzyme substrate produces a blue colour in the test well, which turns yellow when the reaction is stopped with sulphuric acid. The tests were considered positive when the spectrophotometrically measured absorbance was higher than the average of the means of the absorbance values from negative and positive controls.

Needle biopsies of liver were performed without an ultrasound scan having been taken.

The data from the analyses were statistically analysed with Friedman's test and sign tests.

Results

Pre-S(1)Ag was found in all patients with chronic liver disease and in all HBsAg positive asymptomatic carriers. In contrast, pre-S(2)Ab was absent in both these groups (table).

In the group with acute hepatitis (fig 1) serum HBsAg and pre-S(1)Ag cleared simultaneously in eight of 14 cases; in six cases the antigens could no longer be detected by the third examination (day 40); in one case they had cleared by 100 days, and in one additional patient they were absent at 20 days. In the six (42.9%) remaining patients (fig 2), pre-S(1)Ag clearance occurred before that of HBsAg. In four of these cases there was a difference of 20 days, while in all other patients HBsAg was abolished at least 40 days after pre-S(1)Ag clearance. These time differences were not significant.

Of all markers, DNA-HBV and HBeAg were the first to become negative (four and five of 14, respectively) (fig 3); serum pre-S(1)Ag, did not clear before serum HBeAg in any patient. The clearance of serum DNA-HBV and HBeAg was significant compared with HBsAg ($p \leq 0.01$); no significant difference was found compared with pre-S(1)Ag ($p \leq 0.005$).

In two of 14 (14.2%) of the patients with acute hepatitis pre-S(2)Ab could not be detected even after HBsAg and pre-S(1)Ag had become negative, although in four of 14 (28.5%) subjects, pre-S(2)Ab and HBsAb appeared simultaneously after the clearance of pre-S(1)Ag and HBsAg. In the eight (57.1%) remaining patients pre-S(2)Ab was found before HBsAb had appeared ($p \leq 0.01$); in six of these patients pre-S(2)Ab was present 20 days before HBsAb had been cleared from the serum (fig 4).

Figure 4 Course of patients with acute hepatitis in whom pre-S(2)Ab was detected before HBcAg.

Discussion

Our results show that pre-S(1)Ag was present in all chronic asymptomatic carriers, in agreement with other studies (Abstract presented at the International Symposium on Viral Hepatitis and Liver Disease, London, May 1987), thus confirming that pre-S(1)Ag behaves like HBsAg. The presence of pre-S(1)Ag in chronic carriers has been relatively poorly studied, and the results to date are contradictory. Some workers have found variable percentages, ranging from 52% to 80%^{5,16} (Abstract presented at the International Symposium on Viral Hepatitis and Liver Disease, London, May 1987), although these differences may have been due to the variations in sensitivity of the techniques used.

The pre-S(1) antigen was also found in all our patients with chronic HBV disease, regardless of whether the virus was replicated or unreplicated. Hadziyannis *et al.*⁸ in their radioimmunoassay studies, found different percentages of the pre-S(1)Ag system, depending on the stage of viral replication. They suggested that changes in the expression of viral proteins occur or that DNA-HBV might be incorporated with isolated expression of HBsAg. The production of pre-S(1)Ag is likely to persist, although at very low concentrations only detectable with enzyme linked immunosorbent assay (ELISA). Machida *et al* support this view,⁹ and Klinkert *et al* have shown that pre-S(1)Ag and pre-S(1)Ab co-exist in chimpanzee serum,¹⁰ a finding explained by the formation of immuno-complexes.

The production of pre-S(1)Ag is probably related to that of HBsAg, but not to viral replication, although if the rate of replication is high, the concentration of HBsAg and pre-S(1)Ag will also be raised. Treatment with α -interferon, which inhibits DNA-HBV formation and hence viral replication, has no effect on the synthesis of pre-S genes.¹¹

In agreement with earlier findings,¹² pre-S(2) antibody was negative in chronic HBV

carriers, supporting the hypothesis that its neutralising effect contributes to viral clearance. Other authors have found these antibodies in advanced chronic liver disease,¹³ although these findings are probably exceptional. In view of recent evidence that pre-S(1) antigen contains a binding site to hepatocytes, however, it may be of interest to determine antibody to pre-S(1) in these patients once a reliable analysis becomes available for anti-pre-S(1) in serum.

One of the most interesting aspects of these findings is the behaviour of pre-S(1)Ag in relation to HBsAg, particularly when this pre-S antigen is cleared before HBsAg, a phenomenon which seems to indicate a more favourable prognosis in acute hepatitis. In none of our patients did the pre-S(1) antigen become negative after the HBsAg had done so, hence clearance of this marker seems to occur when B virus infection resolves, with complete viral clearance. The clearance of pre-S(1)Ag from the serum indicates a favourable prognosis, but should not replace HBsAg as an indicator of the course of acute infection, as the number of analyses that this would require does not justify the limited practical benefit obtained.

Serum concentrations of HBeAg and DNA-HBV are significant markers of a favourable outcome when these antigens clear quickly in patients with acute HBV infections;¹⁴ DNA-HBV is likely to clear more rapidly—about 10 weeks after the appearance of the symptoms.^{15,16}

In acute infections pre-S(2) antibody is present more or less consistently, appearing in connection with viral clearance and full clinical recovery. This antibody is present transiently during the recovery and early convalescent phases.^{6,17-19} Our findings concur with earlier data: pre-S(2)Ab was found quickly, before HBsAg and pre-S(1)Ag clearance in some patients, and significantly earlier than the appearance of HBsAb. The simultaneous presence of pre-S(2)Ab with HBsAg and pre-S(1)Ag does not rule out pre-S(2)Ab positivity several days before HBsAb are detected, as analyses were performed 20 days apart in our study. Alberti *et al* found pre-S(2)Ab in up to 30% of their patients five to seven years after recovery from acute hepatitis.¹² These findings suggest that the immune response to pre-S(2)Ag is a marker for HBV clearance, and is not involved in the pathogenesis of liver damage caused by HBV.

The detection of pre-S(2) antibody provides no advantage despite its rapid clearance, as this always occurs after HBsAb has appeared in the serum, thus giving it some degree of prognostic but limited practical value. Serum determinations of pre-S(2)Ab would probably be more useful in patients with acute prolonged hepatitis B.

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