

## Association of the Hepatic Expression of $\beta$ 2-Microglobulin with Spontaneous Hepatitis Be Antigen Seroconversion in Chronic Hepatitis B

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Hepatic expression of hepatitis B core antigen (HBcAg) and  $\beta$ 2-microglobulin ( $\beta$ 2-MG) was studied in 42 patients with liver biopsy-proven chronic hepatitis B; 31 were hepatitis Be antigen (HBeAg)-seropositive and 11 were anti-HBeAg antibody (anti-HBe)-seropositive at base line. The degrees of expression of both HBcAg and  $\beta$ 2-MG at base line were significantly higher in HBeAg-seropositive patients than in anti-HBe-seropositive patients ( $p < 0.001$  and  $p < 0.001$ , respectively). During two-year-follow-up, 14 of 31 HBeAg-seropositive patients were seroconverted to anti-HBe (HBe-seroconversion). When the base line hepatic expression of HBcAg and  $\beta$ 2-MG was compared between the patients with subsequent HBe-seroconversion and the non-seroconverted patients,  $\beta$ 2-MG expression on hepatocytes in the patients with subsequent HBe-seroconversion was significantly more intense than that in the non-seroconverted patients ( $p < 0.025$ ). In contrast, the degrees of HBcAg expression at base line were not different between the two groups. These results suggest that the hepatic expression of  $\beta$ 2-MG as well as that of HBcAg correlates with the serum level of HBeAg and that the increased expression of  $\beta$ 2-MG is followed by HBe-seroconversion in patients with chronic hepatitis B.

**Key Words:**  $\beta$ 2-microglobulin, HBcAg, chronic hepatitis B

### Introduction

The hepatocyte damage induced by hepatitis B virus (HBV) infection is not due to a direct viral cytopathic effect, but mainly due to T cell-mediated cytotoxicity against the HBV-associated antigen expressed on the infected hepatocytes.<sup>1,2,3</sup> Adherence of lymphocytes to the infected hepatocytes is thus necessary for the immune response. The process of cell to cell adhesion is mediated by antigenic determinants displayed in conjunction with the major histocompatibility complex (MHC) products on the infected hepatocyte.<sup>4</sup>

The hepatic expression of HBV nucleocapsid antigens such as hepatitis B core antigen (HBcAg) and hepatitis Be antigen (HBeAg) was reported to be not only a marker of HBV replication but also a major target antigen for T cell-mediated cytotoxicity in chronic hepatitis B.<sup>5,6,7</sup> The serum level of HBeAg is also clinically used as a marker of HBV replication.<sup>8</sup> Previous studies demonstrated that the seroconversion from HBeAg to anti-HBe (HBe-serocon-

version) occurs spontaneously in the patients with chronic hepatitis B, resulting in the decrease in liver inflammation activity.<sup>9,10</sup> Several groups showed that bridging hepatic necrosis during acute exacerbation was associated with HBe-seroconversion.<sup>8,10</sup> However, little is known about the relation of HBe-seroconversion with the hepatic expression of the HBV-related antigens or the MHC class I products.

In the present study, the hepatic expression of HBcAg and  $\beta$ 2-microglobulin ( $\beta$ 2-MG), a subunit of the HLA class I products,<sup>12</sup> was studied in 42 patients with a biopsy-proven chronic hepatitis B in order to clarify the association of expression of HBcAg and  $\beta$ 2-MG with HBe-seroconversion.

### Materials and Methods

Fifty-five liver biopsy specimens from 42 patients with chronic hepatitis B were used in this study. All the patients were hepatitis B surface antigen-seropositive for more than 2 years and had biopsy-proven chronic hepatitis. The patients who had the antibody to hepatitis delta virus or hepatitis C virus were excluded from this study, as were the patients with drug or alcohol abuse. These 42 patients were followed for, at least, 2 years after receiving the first liver biopsies. The histological degrees of chronic hepatitis in the patients were based on the international classification<sup>13</sup> (Table 1). HBe-seroconversion occurred spontaneously in

**Table 1.** Summary of the patients.

	HBeAg (+)	Anti-HBe (+)
male:female	27:4	9:2
age (mean $\pm$ SD)	31.7 $\pm$ 6.5 y. o	29.0 $\pm$ 4.0 y. o
Histological diagnosis		
CPH	3	6
CAH2A	13	5
CAH2B	15	0

CPH: chronic persistent hepatitis

CAH2A: chronic active hepatitis with moderate inflammatory activity

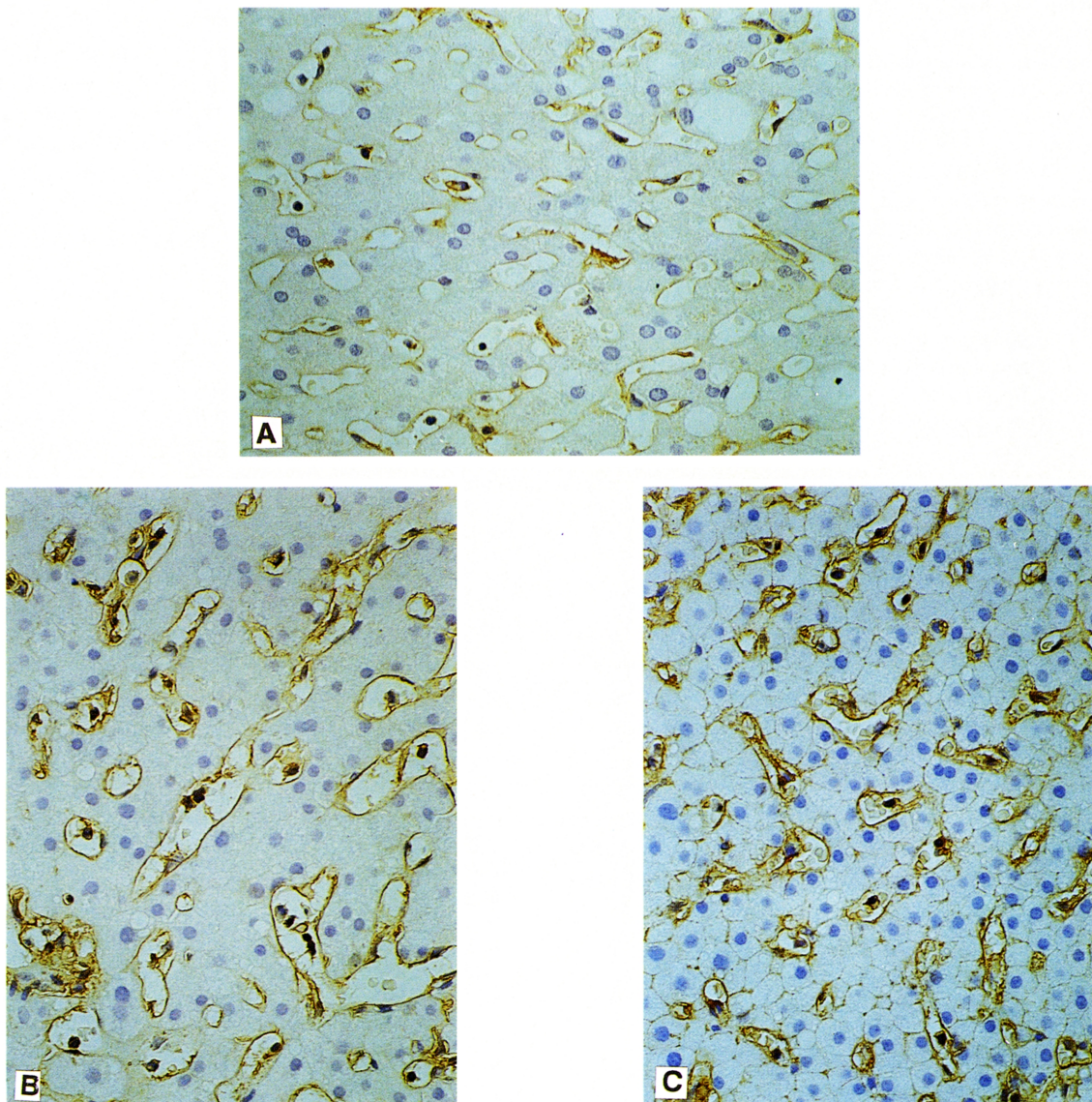
CAH2B: chronic active hepatitis with severe inflammatory activity

14 of 31 HBeAg-seropositive patients during follow-up, while 17 patients remained HBeAg-seropositive. The second liver biopsy specimens were obtained from 10 of the 14 HBe-seroconverted patients after HBe-seroconversion. Three of 17 non-seroconverted patients also allowed to receive the second liver biopsies at intervals of more than one year.

Each specimen was fixed in 10% formaldehyde solution, embedded in paraffin and used for routine histology. For immunohistochemistry, deparaffinized sections were stained by the Biotin-Streptavidin complex method (Bio Genex Labo., U. S. A.). In brief, the sections were reacted for 30min at room temperature with rabbit polyclonal anti-human  $\beta 2$ -MG antibody (DAKO; working dilutions = 1:500) or rabbit polyclonal anti-HBcAg antibody (DAKO;

working dilutions = 1:200). After washing with phosphate buffered saline (PBS), pH7.4, three times, the sections were reacted for 20min with biotinylated goat anti-rabbit immunoglobulin G, followed by incubation for another 20min with peroxidase-conjugated streptavidin, and washed in PBS, pH7.4. The reaction products were visualized by incubation for 10min in 0.61 M Tris HCl buffer, pH7.4, containing 0.05% diaminobenzidine and 0.01%  $H_2O_2$ . The intensity of the hepatic expression of  $\beta 2$ -MG and HBcAg was graded independently by three observers without the prior knowledge of the clinical data.

Expression of  $\beta 2$ -MG on hepatocytes was graded as follows; (-) negative, (+) weakly positive, (++) strongly positive. The representative cases are displayed in Fig. 1. The hepatic expression of HBcAg was also scored semi-



**Fig. 1.** Expression of  $\beta 2$ -MG on hepatocytes.

The staining intensity of  $\beta 2$ -MG on hepatocyte membrane was negative (A), weakly positive (B) and strongly positive (C). (x 200)

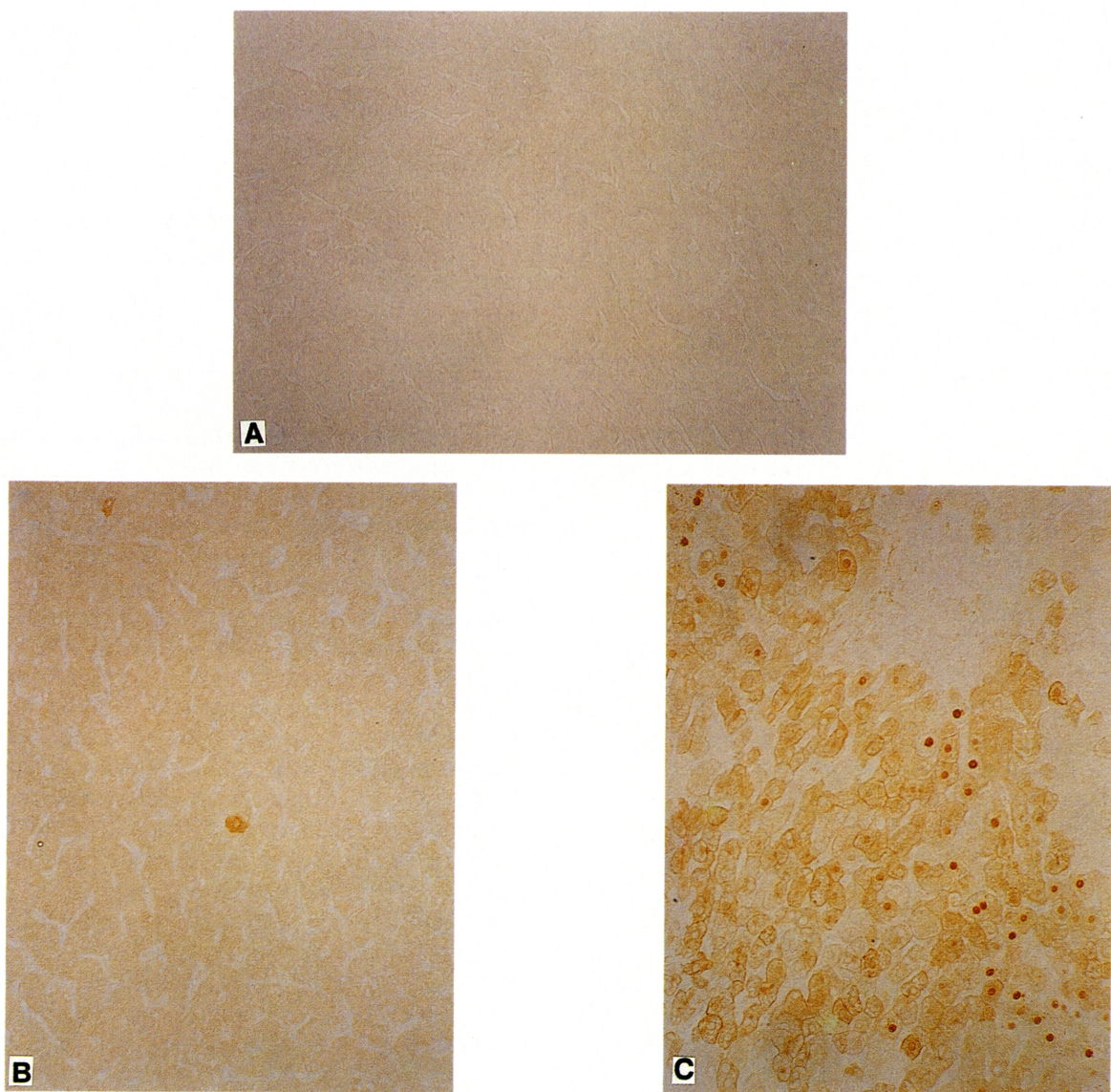
quantitatively as follows; (-) negative, (+) scattered distribution of hepatocytes containing HBcAg in a few lobules, (++) scattered or diffuse distribution of hepatocytes containing HBcAg in more than a few lobules (Fig. 2).

Statistical analyses were carried out using the chi-square test. P values < 0.05 were considered statistically significant.

## Results

### *Difference in the hepatic expression of HBcAg and $\beta$ 2-MG between HBeAg-seropositive patients and anti-HBe-seropositive patients*

The degree of HBcAg expression in 29 of 31 HBeAg-seropositive patients was more than 1+, whereas expression of HBcAg was negative in 10 of 11 anti-HBe-seropositive patients. The difference was statistically significant ( $p < 0.0001$ ). The intensity of expression of  $\beta$ 2-MG in HBeAg-seropositive patients was also significantly higher than that in anti-HBe-seropositive patients ( $p < 0.01$ ) (Table 2).



**Fig. 2.** The hepatic expression of HBcAg.

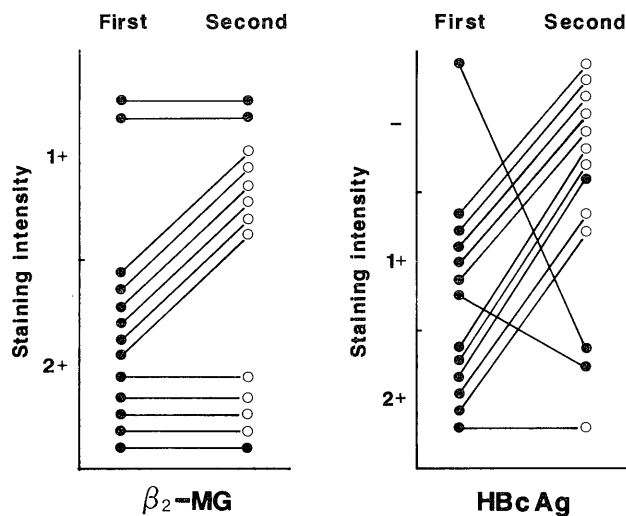
Score of the staining intensity was described in Materials and Methods. Negative (A), scattered distribution of hepatocytes containing HBcAg in a few of the lobules (B), Scattered or diffuse distribution of hepatocytes containing HBcAg in more than a few of the lobules (C). (x 200)

**Table 2.** Difference of the hepatic expression of  $\beta$ 2-MG and HBcAg between HBeAg-seropositive patients and anti-HBe-seropositive patients.

	$\beta$ 2-MG expression			HBcAg expression		
	-	+	++	-	+	++
Serum HBeAg (+) n = 31	1	12	18	2	10	19
Serum HBeAg (-) n = 11	0	11	0	10	1	0
p-value	p < 0.01			p < 0.001		

Statistical analysis was carried out using chi-square test.

When the changes in the hepatic expression of HBcAg and  $\beta$ 2-MG before and after HBe-seroconversion were analyzed in the 10 HBe-seroconverted patients, expression of HBcAg and  $\beta$ 2-MG became less intense after HBe-seroconversion in 9 and 6, respectively, of the 10 seroconverted patients; in contrast, expression of  $\beta$ 2-MG showed no interval changes in all of the three non-seroconverted patients and expression of HBcAg increased in two of the three patients (Fig. 3).

**Fig. 3.** Changes in the hepatic expression of  $\beta$ 2-MG and HBcAg before and after HBe-seroconversion.

● : HBeAg seropositive state  
○ : anti-HBe seropositive state

#### Association of the hepatic expression of HBcAg and $\beta$ 2-MG with subsequent HBe-seroconversion

Expression of HBcAg and  $\beta$ 2-MG at base line was compared between 14 patients with subsequent HBe-seroconversion and 17 non-seroconverted patients (Table 3). The mean age and the sex distribution were not different between the two groups. The degree of  $\beta$ 2-MG expression at base line was significantly more intense in the patients with subsequent HBe-seroconversion than in the non-seroconverted patients ( $p < 0.025$ ), whereas the degree

**Table 3.** Association of the hepatic expression of  $\beta$ 2-MG and HBcAg with subsequent HBe-seroconversion.

	$\beta$ 2-MG expression			HBcAg expression		
	-	+	++	-	+	++
HBeAg (+) $\rightarrow$ Anti-HBe (+) male:female = 12:2 age = 33.1 $\pm$ 6.3 y. o.	0	2	12	1	6	7
HBeAg (+) $\rightarrow$ HBcAg (+) male:female = 15:2 age = 30.5 $\pm$ 6.2 y. o.	1	10	6	1	4	12
p-value	p < 0.025			p > 0.25		

Statistical analysis was carried out using chi-square test.

of HBcAg expression was not different between the two groups ( $p > 0.05$ ).

#### Discussion

The hepatic expression of HBcAg and the MHC class I products is closely associated with liver inflammation activity in chronic hepatitis B.<sup>4,14-17</sup> In this study, the hepatic expression of HBcAg and  $\beta$ 2-MG, a topographic distribution of which is similar to the MHC class I products,<sup>18</sup> was analyzed in 42 patients with chronic hepatitis B. The degree of the hepatic expression of HBcAg in HBcAg-seropositive patients was significantly higher than in anti-HBe-seropositive patients. When expression of HBcAg was studied before and after HBe-seroconversion in HBe-seroconverted patients, HBcAg expression was clearly reduced after HBe-seroconversion. Since the serum level of HBeAg is associated with HBV replication in hepatocytes, these results indicate that the hepatic expression of HBcAg closely correlates with HBV replication. The intensity of the hepatic expression of  $\beta$ 2-MG in HBcAg-seropositive patients was also significantly higher than that in anti-HBe-seropositive patients. Several studies showed that expression of the MHC class I products on hepatocytes was associated with the serum aminotransferase level, but not with the serum level of HBeAg.<sup>19</sup> The discrepancy between the previous and the present results seems to be, in part, due to the patients studied, because the activity of liver inflammation in HBeAg-seropositive patients was much higher than that in anti-HBe-seropositive patients in our study. Chu *et al.*<sup>16</sup> also found that the hepatic expression of the MHC class I products was associated with viral replication and histological activity in chronic hepatitis B, with the most intense display in the patients with HBeAg-seropositive chronic active hepatitis.

HBe-seroconversion occurs spontaneously in chronic hepatitis B, resulting in normalization of the serum aminotransferase level and the histological improvement.<sup>9,10</sup>

Fattovich *et al.* demonstrated that approximately 60% of HBeAg-seropositive patients with chronic hepatitis B were seroconverted to anti-HBe during a mean follow-up period of 5 years, and that liver inflammation was repressed in 90% of the patients after HBe-seroconversion.<sup>20</sup> Several workers reported similar results.<sup>10,11</sup> However, it remains obscure how the hepatic expression of the HBV-related antigens or the MHC class I products is associated with HBe-seroconversion. Therefore, we focused on the relationship between their expression and HBe-seroconversion. In this study, the base line intensity of  $\beta$ 2-MG expression was significantly higher in the patients with subsequent HBe-seroconversion than in the non-seroconverted patients, but the degree of HBeAg expression was not different between the two groups at base line. These results suggest that the increased expression of  $\beta$ 2-MG on hepatocytes but not the increased HBV replication is followed by HBe-seroconversion. HBe-seroconversion is thought to result from the enhanced immune response of the host to HBV. In fact, some investigators showed that acute exacerbation in chronic hepatitis B was frequently found during HBe-seroconversion with the increased expression of the MHC class I products on hepatocytes.<sup>16,19</sup> These findings agree well with our results.

Recently, interferon has been used clinically in the treatment of chronic hepatitis B. Although interferon directly suppresses the replication of HBV in hepatocytes, the clinical results of interferon therapy cannot be explained only by the anti-replicative effects of interferon on HBV.<sup>21,22</sup> Since interferon induces the hepatic expression of the MHC class I products *in vitro* and *in vivo*,<sup>23,24</sup> it is possible that clinical effects of interferon are mediated by the increased expression of the MHC class I products on hepatocytes in chronic hepatitis B.

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