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Expression of Fas Antigen and Bcl-2 Protein in Liver Tissues of Patients with Chronic Hepatitis B

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Abstract: The aim of this study was to evaluate the role of apoptosis in chronic hepatitis B, and correlate it with disease severity. We studied the expression of the Fas antigen (FasAg) and Bcl-2 protein in a group of patients with chronic hepatitis B at various stages of the disease. Four liver biopsy specimens taken from the patients with chronic hepatitis B infection and five control liver tissue specimens with normal histology were studied. Liver specimens were scored for disease severity according to Knodell's hepatic activity index (HAI). The percentage of hepatic expression was evaluated semi-quantitively. Our immunohistochemical study showed that FasAg was expressed in 92.5% of liver tissues, and detected mainly in the hepatocytes in the periportal region, especially at the advancing edges of areas of piecemeal necrosis. There was a significant correlation between hepatic expression of FasAg and the scores of the hepatic activity index and intensity portal inflammation ($p=0.04$, $p=0.02$). Bcl-2 protein expression was detected in 30% of liver tissues. Bcl-2 protein expression was found in only a few hepatocytes in the periportal region. FasAg and Bcl-2 protein expressions were not detected in liver tissues with normal histology.

The present results demonstrate that Bcl-2 protein expression partially protects hepatocytes against Fas-mediated apoptosis. Cell cycle regulation during chronic hepatitis B infection could be controlled by other genes, but further studies are required.

Key Words: Fas antigen, Bcl-2 protein, chronic hepatitis B

Introduction

Apoptosis plays a significant role in the course of hepatocyte death in acute and chronic hepatitis. Apoptosis is involved in the removal of injured or infected hepatocytes by the immune system as an adaptive and beneficial process (1). In contrast to necrosis, apoptosis is tightly controlled and regulated via several mechanisms, including Fas/Fas ligand interaction, the effects of cytokines, transforming growth factor beta and the influence of the antiapoptotic protein family. FasAg is a type I membrane protein in the tumour necrosis factor/nerve growth factor receptor super family. The primary function of Fas is to trigger apoptosis in various cell types (2-4). Antiapoptotic protein Bcl-2 is located in the mitochondrial inner envelope and prevents apoptosis induced by various treatments. Although the functional mechanism of Bcl-2 is still unclear, members of the Bcl-2 family exert an inhibitor effect on the Fas signalling pathway. However, the inhibitor effect on the Fas-mediated apoptotic signal remains controversial (1,2,4,5). Hepatocytes are very sensitive to Fas-mediated apoptosis. Acidophilic bodies, detected especially at the advancing edges of areas of piecemeal necrosis, are evidence of apoptosis. In vivo treatment with Fas-containing agents leads rapidly to fulminant hepatitis (6,7). Severals have report demonstrated that Bcl-2 is expressed in bile ductules and in small interlobular bile ducts but not in normal hepatocytes (8).

In the present study, we evaluated the role of apoptosis in chronic hepatitis B. We immunohistochemically studied the expression of FasAg and Bcl-2 protein in HBV infected liver tissues.

Materials and methods

Patients

We studied 40 patients with chronic hepatitis B infection, ranging in age from 12 to 75, and five liver samples with normal histology were used as the control group. The samples were selected at Department of Pathology at Mersin University, Faculty of Medicine, and Başkent University, Faculty of Medicine, Turgut Noyan

Research Hospital. All patients were seropositive for HBs antigen, and 12 patients were seropositive for HBeAg. Hepatitis B virus DNA polyerase activity was measured in 29 patients, and it was 812.4±142.6cpm. All patients were seronegative for anti-HCV antibody. All control patients were negative for HBsAg and Anti-HCV. All samples were fixed in 10% buffered formaline, embedded in paraffin wax and routinely processed. Histological and immunohistochemical evaluation was performed on all biopsy samples. Haematoxylin-eosin, Masson's trichrome and Gomori's reticulum were used for histochemical evaluation, and FasAg and Bcl-2 protein for immunohistochemical evaluation. Two pathologists who were unaware of the clinical findings examined the sections independently. After the independent reviews, each case was evaluated jointly and disagreements were resolved by consensus. The histological activities of liver samples were analysed according to Knodell's Hepatic Activity Index (HAI) scoring system (9,10).

Immunohistochemical staining procedures

Five-mm-thick sections were placed onto poly-L-lysine-coated slides and air-dried overnight at room temperature. Sections were deparaffinized in xylene and dehydrated through graded concentrations of ethanol. After blocking endogenous peroxidase with 0.3% solutions of H2O2 in pH 7.5 PBS, sections were heated in 0.01 mol/l citrate buffers in a microwave pressure cooker for 20 minutes. Then sections were incubated for 2 hours with anti-Bcl-2 mouse monoclonal antibody (1:50 dilution, Neomarkers, Fremont, USA), and 18 hours with anti-Fas mouse monoclonal antibody (1:10 dilution, DAKO, Hamburg, Germany). After incubation, sections were incubated for 30 minutes with an avidin-biotin-complex peroxidase kit for Bcl-2 protein and an LSAB kit for FasAg (DAKO, Hamburg, Germany). The sections counterstained with Mayer's haematoxylin. The degree of immunopositivity was evaluated semi-quantitatively. FasAg and Bcl-2 protein expressions in liver tissues were graded as follows: (-) no expression, (+) lower than 10% of all liver tissue, (++) 10% to 50% positive cells of all liver tissue and (+++) more than 50% positive cells of all liver tissue.

Statistical analysis

Statistical analyses were performed with the SPSS 9.00 computer package. The relation between FasAg and Bcl-2 protein expression with the scores of HAI, lobular degeneration, piecemeal necrosis and portal inflammation were analysed with the chi-square test. Differences with p values <0.05 were considered to be statistically significant.

Results

The mean age of the patients was 40.3±14.9 years (range 12-75 years) with a male-to-female ratio of 1.35:1. Bcl-2 protein and FasAg expression were not detected in liver tissues with normal histology, except in a few hepatocytes with FasAg. Expression of FasAg and Bcl-2 protein was detected in 92.5% (37 of the 40 liver samples) and 30.0% (12 of the 40 liver samples) of liver tissues respectively. FasAg was expressed mainly in the cytoplasm and partially in the membranes of hepatocytes. Expression was stronger in hepatocytes found in the periportal region especially at the advancing edges of areas of piecemeal necrosis and in those accompanied by many infiltrating lymphocytes around them (Fig. 1). Strong expression with FasAg was also found in ground-glass hepatocytes (Fig. 2). Bcl-2 protein expression was found in only a few hepatocytes at the periportal region of all liver samples; however, most of the regenerated bile ducts and the majority of lymphocytes present in the portal area showed strong Bcl-2 protein expression (Fig. 3).

Tables 1-4 show the relation between the expression of FasAg and Bcl-2 protein and the scores of HAI, lobular degeneration, piecemeal necrosis and portal inflammation. Liver specimens with higher scores of HAI, lobular degeneration, piecemeal necrosis, and portal inflammation showed stronger FasAg expression ($p=0.04$, $p=0.22$, $p=0.31$, $p=0.02$ respectively). However, only the correlation between the scores of HAI and portal inflammation and FasAg expression was statistically significant. No significant correlation was found between Bcl-2 protein expression and the scores of HAI lobular degeneration, piecemeal necrosis, and portal inflammation ($p=0.19$, $p=0.12$, $p=0.08$, $p=0.29$ respectively).

No significant difference was found between the expression of FasAg and Bcl-2 protein and HBV-DNA polymerase activity ($p=0.095$, $p=0.615$). FasAg and Bcl-2 protein expression did not differ between HBeAg positive and HBeAg negative patients ($p=0.122$, $p=0.208$ respectively).

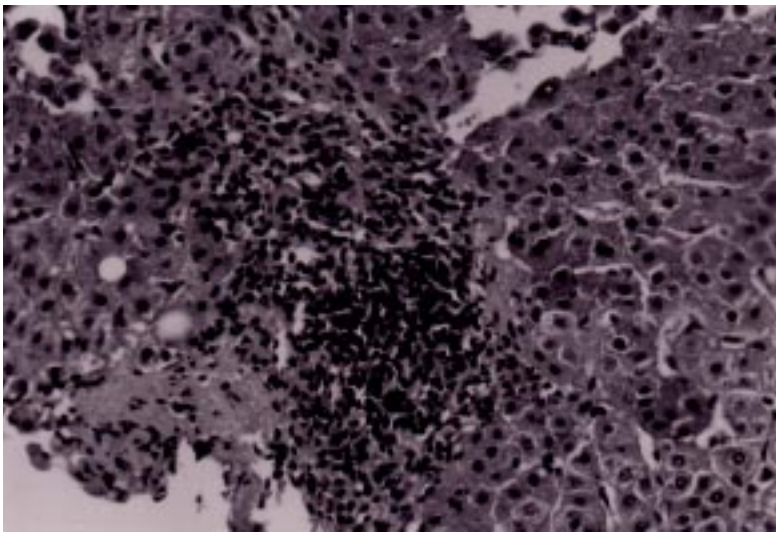

Figure 1. FasAg expression in hepatocytes in the periportal region (X200 original magnification).

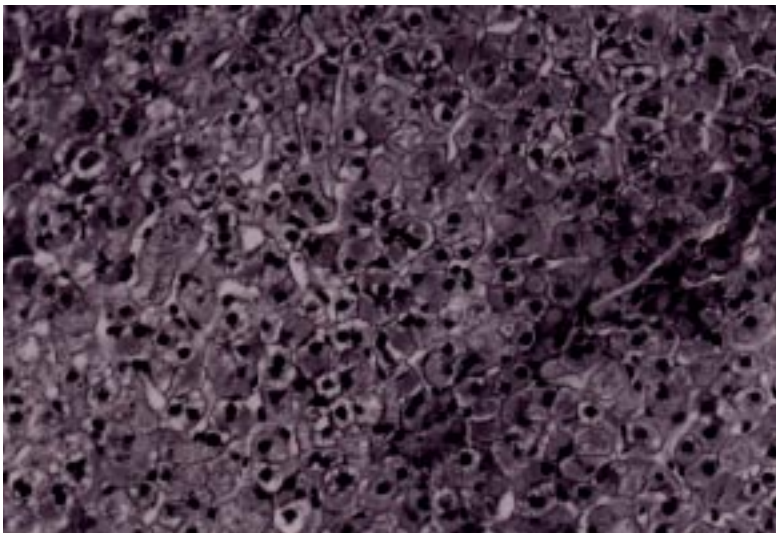

Figure 2. FasAg expression in ground-glass hepatocytes (X200 original magnification).

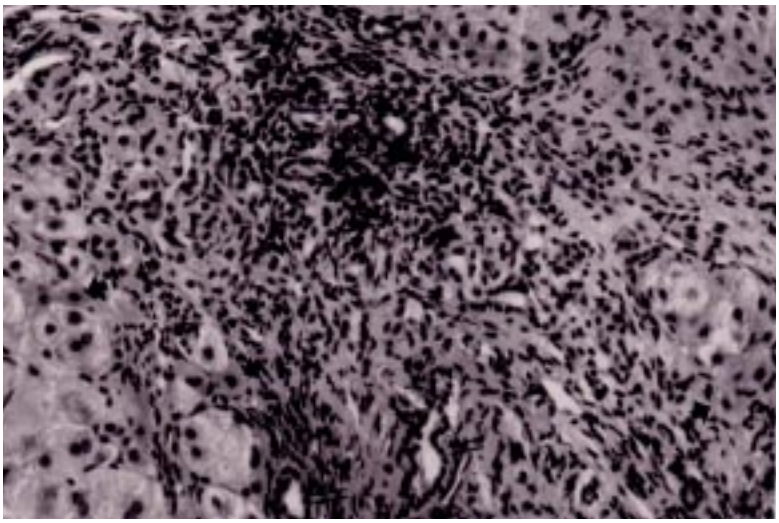

Figure 3. Bcl-2 expression in the cytoplasm of bile ducts (double arrow) and lymphocytes in the portal area. There are a few positive stained hepatocytes (arrow) (X200 original magnification).

Table 1. Relation between FasAg and Bcl-2 protein expression and the score of HAI.

Score of HAI	Fas				Bcl-2	
	(-)	(+)	(++)	(+++)	(-)	(+)
Minimal	2	6	3	0	9	2
Mild	1	8	6	0	11	4
Moderate	0	1	3	3	5	2
Severe	0	3	4	0	3	4
Total	3	18	16	3	28	12

Table 2. Relation between FasAg and Bcl-2 protein expression and the score of lobular degeneration.

Score of lobular deg.	Fas				Bcl-2	
	(-)	(+)	(++)	(+++)	(-)	(+)
0	0	1	2	0	3	0
1	3	10	7	0	15	5
3	0	6	5	3	9	5
4	0	1	2	0	1	2
Total	3	18	16	3	28	12

Table 3. Relation between FasAg and Bcl-2 protein expression and the score of piecemeal necrosis.

Score of piecemeal n.	Fas				Bcl-2	
	(-)	(+)	(++)	(+++)	(-)	(+)
0	3	6	4	0	11	2
1	0	5	5	0	8	2
3	0	2	0	1	1	2
4	0	1	1	0	2	0
5	0	1	1	1	1	2
6	0	3	5	1	5	4
Total	3	18	16	3	28	12

Table 4. Relation between FasAg and Bcl-2 protein expression and the score of portal inflammation

Score of portal infl.	Fas				Bcl-2	
	(-)	(+)	(++)	(+++)	(-)	(+)
0	0	0	0	0	0	0
1	2	9	4	0	11	4
3	1	7	6	2	12	4
4	0	2	6	1	5	4
Total	3	18	16	3	28	12

Discussion

Apoptosis plays an important role in embryonic development, tissue remodelling, immune regulation and tumour regression. The Bcl-2 family and death factor family regulate apoptosis. Fas is a typical member of the death factor family. It is reported that T lymphocytes are important for the activation of Fas induced apoptosis in chronic hepatitis B. Viral antigens expressed in infected hepatocytes activate T lymphocytes, and at the end of this process Fas-induced hepatocyte apoptosis begins. This is a beneficial process for removing virus-infected cells, but overexpression of FasAg may lead to fulminant hepatitis (2, 11-14). Fas is abundantly expressed not only in the liver but also in the heart and lungs. This system may be also be involved in cytotoxic T lymphocyte mediated disease in these tissues (13).

In the present study, we detected FasAg in the hepatocytes of liver samples from the patients with chronic hepatitis B infection, but not in normal liver tissues, except for a few positive hepatocytes. FasAg expression was stronger in liver tissues with higher HAI scores. Bcl-2 protein expression was found in only a few hepatocytes in these cases, and was negative in normal liver tissues. There are other reports with FasAg in chronic hepatitis B and C that have similar results (1,15). These findings suggest that Fas-mediated apoptosis plays a significant role in liver cell death in viral hepatitis. Expression of FasAg was stronger in hepatocytes' cytoplasm, especially at the advancing edges of the region in piecemeal necrosis among the lymphocytes. There was a correlation between hepatic expression of FasAg and intensity of portal inflammation ($p=0.02$). Ando et al. demonstrated in their experimental study with HBV-transgenic mice that cytotoxic T lymphocytes bind HBsAg-positive hepatocytes and induce apoptosis in them. These results suggest that the Fas system may be a way of controlling apoptosis by cytotoxic T lymphocytes, and this

continuous attack of lymphocytes may result in advancing hepatic injury (1,16). These data support our findings of stronger expression with FasAg in ground-glass hepatocytes (Fig. 2)

In cell cycle control, Bcl-2 is an important antiapoptotic protein. But our results demonstrated that Bcl-2 cannot prevent Fas-mediated hepatocyte apoptosis. The great sensitivity of hepatocytes to Fas-mediated apoptosis might be partially due to the small amount of antiapoptotic proteins in these cells, and the expression of Bcl-2 protein in hepatocytes during chronic inflammation of liver allows their survival partially in a Fas-induced apoptotic storm. Despite the broad antiapoptotic activity of Bcl-2 in various apoptotic pathways, the ability of Bcl-2 to inhibit Fas-mediated apoptotic pathways has remained controversial. Costa et al. demonstrated that Bcl-2 gene expression in the hepatocytes of transgenic mice protects them against Fas-induced fulminant hepatitis. This protective effect is the result of the blocking of caspase-3 activity by Bcl-2 in response to Fas triggering (6). Immunohistochemical studies with Bcl-2 protein expression in hepatomas have demonstrated that Bcl-2 protein expression occurs in 6-19% of hepatomas (17).

In conclusion, in this study we have provided evidence that the high prevalence of FasAg expression shown in liver tissue with more severe necro-inflammatory activity and Bcl-2 protein expression partially protects hepatocytes against Fas-mediated apoptosis. It seems beneficial, but it is possible that Bcl-2 protein provides some transformed cells, and it may be a mechanism of developing hepatoma from chronic hepatitis B infection and cirrhosis.

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