Serum tumor growth factor-β1 levels in patients with cirrhosis, chronic hepatitis B and chronic hepatitis C

Cengiz Kirmaz[1], Ender Terzioğlu[2], Omer Topalak[3], Papatya Bayrak[1], Ozge Yilmaz[4], Galip Ersoz[5], Filiz Sebik[6]

[1] Celal Bayar University, Medical Faculty, Department of Internal Medicine, Division of Immunology, Manisa, Turkey
[2] Akdeniz University, Medical Faculty, Department of Internal Medicine, Division of Immunology and Rheumatology, Antalya, Turkey
[3] Dokuz Eylul University, Medical Faculty, Department of Internal Medicine, Division of Gastroenterology, Izmir, Turkey
[4] Celal Bayar University, Medical Faculty, Department of Pediatrics, Manisa, Turkey
[5] Ege University, Medical Faculty, Department of Internal Medicine, Division of Gastroenterology, Izmir, Turkey
[6] Ege University, Medical Faculty, Department of Internal Medicine, Division of Immunology, Izmir, Turkey

Correspondence: Cengiz Kirmaz, Assist of Proff., Celal Bayar Univesity Medical Faculty, Department of Internal Medicine, Division of Immunolgy.
GSM: + 90-532-4150831 - Fax: + 90-236-2370213
E-mail: ckirmaz@mynet.com

Accepted for publication January 29, 2004

ABSTRACT. Chronic liver disease and cirrhosis are two of the most important health problems according to current gastroenterology literature. Based on the recent developments in the field of immunology, advanced follow-up and treatment modalities have been introduced for these disorders. Immune defence against viral infections depends on effective cellular immune responses derived mainly from Th1-related cytokines. Th2 type immune responses can inhibit efficient immune function by secretion of several cytokines such as IL-10, TGF-β1. In this particular study, we determined the serum levels of TGF-β1, which plays a role in immune suppression and induction of tissue fibrosis. We evaluated the role of TGF-β1 in the pathogenesis of chronic liver disease and cirrhosis. Fourteen chronic hepatitis B (CHB), 12 chronic hepatitis C (CHC) patients and 21 cirrhotic patients were enrolled in the study. The control group consisted of ten healthy people. Serum TGF-β1 levels were higher in both cirrhosis and CHC group when compared to those in CHB and control groups ($P < 0.05$). Although serum TGF-β1 levels in the cirrhosis group were higher than that in the CHC group, the difference was not statistically significant. In conclusion, elevated TGF-β1 levels in patients with CHC and cirrhosis may have a role in the pathogenesis and chronicity of these diseases.

Keywords: TGF-β1, cirrhosis, chronic hepatitis B, chronic hepatitis C, Knodell histology activity index

INTRODUCTION

Viral hepatitis is an infectious disease characterized by cellular necrosis and inflammation of the liver. Immune mechanisms play a role in its etiology and pathogenesis [1-5]. When an acute hepatitis progresses to the chronic form, it results in chronic hepatitis, cirrhosis and/or hepatocellular carcinoma [6, 7]. Recently, information about the etiology of acute and chronic hepatic diseases has broadened enormously due to the advancement of immunological techniques and knowledge. New methods of immunomodulatory treatment and immunological screening have been introduced based on the information gained during the different stages of viral (Hepatitis B, Hepatitis C) liver diseases [8, 9]. The most serious complication of acute viral hepatitis is progression to chronicity. Chronic hepatitis is defined as persistence of clinical symptoms, abnormal serum transaminases and inflammatory reaction in liver biopsy specimens for more than six months [1, 10].

Recently, several studies on the role of cytokines in the etiology and pathogenesis of acute as well as chronic liver diseases have been published [3-5, 8, 9]. An effective cellular immune response can eliminate the viral load completely during the progress of viral hepatitis disease [4], whereas an ineffective and/or hyperactive immune response may result in chronic liver disease, cirrhosis and hepatocellular carcinoma [11-13]. Tumor growth factor (TGF)-β1 is a multifunctional cytokine in the immune system [14]. It is a homodimeric peptide with a molecular weight of 25-kDa. A better understanding of the molecular mechanism of immune response inhibition by TGF-β1 is required. TGF-β1 cannot be classified as a classical Th1 or Th2 cytokine, however, a TGF-beta-producing Th3 subset that has been recently described might play an important regulatory role in immune response [14]. Additonally, TGF-β1 that is also produced by non-parenchymal liver cells, enhances the activity of perisinusoidal cells and lipocytes while inhibiting the growth and proliferation of hepatocytes. One of its major functions is to stimulate

extracellular matrix and fibrous tissue production [15, 16]. Although TGF-β1 plays an active role in normal cell growth, it has a negative effect on hepatocyte proliferation via induction of apoptotic mechanisms. In particular, TGF-β1 is thought to be responsible for apoptosis that leads to the tissue loss and decrease in liver size seen in chronic liver diseases [17-19]. Activated liver fat storage cells (ITO) or lipocytes that express platelet derived growth factor (PDGF) receptors on their surface are the key cells in liver fibrosis. The number of these PDGF receptors increases in liver cirrhosis. It is suggested that TGF-β1 causes liver fibrosis by inducing the mitogenic effect of PDGF [20]. It is shown in the literature that liver fibrosis can be prevented by anti-TGF-β1 treatment [16].

In our study, we planned to measure serum TGF-β1 levels and to determine the role of this cytokine in disease etiology and pathogenesis, as well as in liver fibrosis, and whether it has a value as a prognostic factor or not.

PATIENTS AND METHODS

Twenty one patients with cirrhosis due to viral hepatitis, and 26 patients with chronic hepatitis [14 due to chronic hepatitis B (CHB), 12 due to chronic hepatitis C (CHC)], who were admitted to the out-patient department of Gastroenterology in Ege University Hospital were included in our study, along with ten healthy individuals as a control group. Diagnosis was based on clinical, biochemical, serological, ultrasonographic and biopsy results. At the time of a liver biopsy, the blood sample was obtained from each patient to determine serum TGF-β1 levels using ELISA technology with Amersham International Plc-Amersham UK-Biotrak commercially available kits.

Although biochemical and virological analyses were part of routine follow-up, all patients gave informed consent for liver biopsy. Approval for the study was given by the ethics committee of our hospital. HBsAg, anti-HBs, and anti-HCV antibodies were determined by automated analysis using the AXSYM MEIA technique (Abbott Laboratories, North Chicago, IL, USA). Percutaneous liver biopsy was performed successfully using the Menghini technique under sonographic control in all patients. All biopsy results were reviewed by a blinded pathologist, to obtain a consensus diagnosis and histological score. The Histological Activity Index (HAI) and fibrosis index were evaluated according to the Knodell histology activity index scoring system [21]. Briefly, HAI represents the sum of the scores attributed to the necroinflammatory lesions. Knodell HAI represents the sum of the scores for the periportal ± bridging necrosis (0 to 10), intralobular degeneration and focal necrosis (0 to 4), portal inflammation (0 to 4) with a maximum of 18 points. The Knodell HAI score for fibrosis ranges from 0 to 4 (that is, 4 for only cirrhotic fibrosis).

The Child-Pugh scoring system which was performed for all patients with cirrhosis, grades the parameters of bilirubin, albumin, prothrombin time, hepatic encepalopathy and ascites from one to three to a maximum score of 15 [22]. Exclusion criteria that all selected patients met were:

1) history of alcohol usage and evidence of alcoholic cirrhosis for patient with cirrhosis;

2) evidence of autoimmune hepatitis according to revised autoimmune hepatitis scoring system [23];

3) possibility of hemorrhage (low platelet count, prolonged prothrombin time, anemia with Hct lower than 30%) prior to liver biopy;

4) history of oral and/or parenteral corticosteroids, or other treatment that may affect serum cytokine levels, similar to interferon, anti-viral agents for a period of six months or less prior to blood sampling for TGF-β1.

Statistical analyses were done using the SPSS version 9.0 computer software program with unpaired Student's test. Correlation of HAI and serum TGF-β1 levels was investigated with the Pearson's correlation test. Statistical significance was defined as a *P* value less than 0.05 (2-tailed).

Table 1
Patients and control group characteristics, serologic situation, HAI (Histological Activity Index), Fibrosis index, Child Pugh score, Biochemical parameters and serum TGF-β1 levels.
See text for *P* values

Parameter	Patients with CHB (*n* = 14)	Patients with CHC (*n* = 12)	Cirrhotic Patients (*n* = 23)	Control (*n* = 10)
Age (year ± SD)	30.64 ± 5.3	30.75 ± 4.9	38.16 ± 7.6	31.0 ± 4.78
Female/Male	6/8	5/7	9/14	4/6
Duration of seropositivity (year ± SD)	8.76 ± 2.09	7.42 ± 2.21	12.34 ± 5.04	–
Serology (HbsAg/anti-HCV Ab)	14/0	0/12	11/10	0/0
HAI (Mean ± SD)	9.21 ± 1.81	8.25 ± 2.7	–	–
Fibrosis index (Mean ± SD)	1.78 ± 1.48	1.75 ± 1.36	4	–
Child-Pugh Score (Mean ± SD)	–	–	7.22 ± 1.65	–
Serum bilurubin mg/dL (Mean ± SD)	1.18 ± 0.44	1.11 ± 0.31	1.99 ± 0.58	0.9 ± 0.4
Serum albumin g/dL (Mean ± SD)	4.18 ± 0.66	3.93 ± 0.47	3.01 ± 0.84	4.08 ± 0.72
Serum TGF-β1 (ng/ml) (Mean ± SEM)	62.93 ± 13.96	91.92 ± 5.68	117.86 ± 12.77	76.7 ± 3.84

RESULTS

The study group consisted of 21 non-alcoholic, cirrhotic patient (with both 11 HBsAg and 10 anti-HCV antibody positive), 14 HBsAg and 12 anti-HCV antibody-positive chronic active hepatitis patients. Chronic active hepatitis patients with HBV and HCV infection did not differ significantly according to their baseline clinical characteristics as shown in *Table 1*. Demographic characteristics of patient and control groups were similar. Other characteristics of these patients and control group are shown in *Table 1*.

Biochemical parameters were similar in all groups except for serum bilurubin and albumin. Serum bilurubin levels in patients with CHB, CHC and cirhosis were higher than those in the control group ($P < 0.05$). The highest level of serum bilirubin was shown in patients with cirrhosis. Additionally, serum bilirubin levels did not vary among different patient groups. Serum albumin levels in patients with CHB and CHC were not significantly different from the control group. Patients with cirrhosis had lower serum albumin levels when compared to patients with CHB, CHC and control group ($P < 0.05$).

When hepatic biopsies of patients with chronic hepatitis were compared, the mean HAI values in CHB and CHC patients were 9.21 ± 1.81 and 8.25 ± 2.7 respectively. The difference between these two groups was not statistically significant. The mean score of fibrosis which is a criterion of HAI was evaluated as 1.78 ± 1.48 in CHB; 1.75 ± 1.36 in CHC patients. The difference in fibrosis indices between the groups was not significant either.

The mean (± SEM) serum level of TGF-β1 was 76.7 ± 3.84 ng/ml and 62.93 ± 13.96 ng/ml in the control group and in CHB patients, respectively. The difference between these groups was not statistically significant. In patients with CHC, the mean serum level of TGF-β1 was 91.92 ± 5.68 ng/ml, and it was significantly higher than that in the CHB and control groups ($P < 0.05$). Mean serum levels of TGF-β1 in cirrhotic patients with a positive HbsAg and a positive anti-HCV antibody were 114.75 ± 17.56 ng/ml and 119.92 ± 10.43 ng/ml, respectively and this difference was not statistically significant. Similarly, clinical and biochemical findings, as well as Child-Pugh scores for these two groups were similar so, the results of the patients in both groups were evaluated as one single group designated the cirrhotic patients. Thus, the highest TGF-β1 level that was detected in patients with cirrhosis (117.86 ± 12.77 ng/ml) was not significantly higher than that of patients with CHC.

In patients with cirrhosis, the TGF-β1 level was positively correlated with the Child-Pugh score ($r = 0.625$; $P = 0.002$) *(Figure 1a)* as well as serum bilirubin levels ($r = 0.726$; $P = 0.0001$), whereas it was negatively correlated with serum albumin levels ($r = -0.502$; $P = 0.04$).

In patients with CHC, the serum TGF-β1 level was positively correlated with HAI ($r = 0.523$; $P = 0.04$) *(Figure 1b)*. However, the serum TGF-β1 level was not correlated with HAI in patients with CHB ($r = 0.261$; $P = 0.25$). The fibrosis index did not correlate with TGF-β1 levels significantly in CHB patients ($r = -0.2455$; $P = 0.4$). However, there was a sigificant correlation between this index and serum TGF-β1 levels in CHC patients ($r = 0.723$; $P = 0.008$) *(Figure 1c)*.

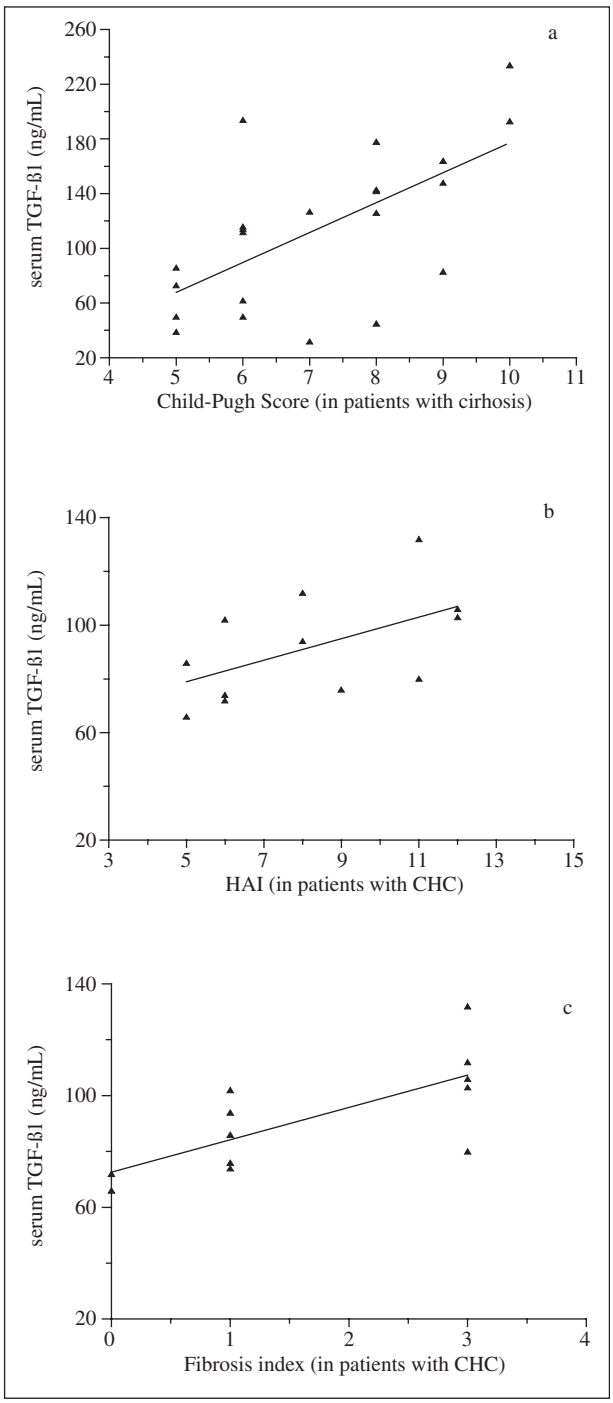

Figure 1
a: Positive correlation between serum TGF-β1 and Child-Pugh staging in a patient with cirrhosis; **b:** Positive correlation between serum TGF-β1 and HAI (Knodell histology activity index) in a patient with CHC; **c:** Positive correlation between serum TGF-β1 and fibrosis index in a patient with CHC.

DISCUSSION

It is known that TGF-β1 is a stimulator of extracellular matrix proliferation and tissue fibrosis and is also a strong immunosuppressive cytokine. It also induces apoptosis of liver cells [14, 15, 17-19, 24]. BALB/c mice deficient in the immunoregulatory cytokine TGF-β1 spontaneously develop necroinflammatory liver disease. Runder *et al.* demonstrated in these mice that TGF-β1 has a critical role in homeostatic regulation of hepatic immune system by

inhibiting the development or expansion of the hepatic cytolytic CD^{4+} T cells [25].

In this study, etiologic factors of cirrhosis in patients were HBV and HCV infections. However, we did not detect any clinical or biochemical difference between these two groups. Child-Pugh scores and serum TGF-β1 levels were similar in these groups, too. Therefore we interpreted that; in patients who develop cirrhosis due to viral etiology, the immunopathogenetic mechanism leading to cirrhosis is similar despite the difference in viral etiology. Increased levels of TGF-β1 in patients with cirrhosis might be attributed to a chronic viral hepatitis background since TGF-β1 may be produced to suppress ongoing immune reactions on the basis of a relatively weak Th2-derived immune response. Detection of the highest level in cirrhotic patients is evidence of the role of TGF-β1 in the induction of fibrosis. Moreover, a positive correlation of TGF-β1 with Child-Pugh staging supported the role of TGF-β1 in the etiopathology of cirrhosis. Previous studies have also detected high serum TGF-β1 levels in cirrhotic patients [26, 27]. TGF-β1 levels are correlated positively with Child-Pugh staging and bilirubin, and negatively with albumin; therefore it is possible to use measurement of TGF-β1, along with this staging as a predictive or even a prognostic factor to evaluate the disease process. Furthermore, Ohara and Kusano showed that inhibiton of TGF-β1 function by anti-TGF-β1 antibody improves the survival rate in cirrhotic rats [28]. Their results may indicate that increased TGF-β1 is a poor prognostic factor in patients with cirrhosis. Additionally, Flisiak *et al.* had noted that plasma TGF-β1 level is an indicator of a liver function impairment and a possible marker of hepatic fibrosis progression in cirrhotic patients [27]. Thus, these results may support our comments on the prognosis of cirrhosis.

In another study, recombinant TGF-β1 suppressed hepatitis B-stimulated IFN-γ synthesis in a dose-dependent manner and suppressed anti-HBc antibody production [29]. High levels of TGF-β1 in our patients may be attributed to this anti-inflammatory action. In a work reported from Japan, neutralization of TGF-β1 in hepatitis C patients increased hepatitis C-specific cytotoxic T cell reaction [30]. Apparently, high levels of TGF-β1 in our patients with CHC may also suppress specific immune reactions including cytotoxic T cell function which may contribute to the development of chronic disease.

In our study, we have found higher serum TGF-β1 levels in patients with CHC than that in patients with CHB. This may explained the worse prognosis and higher tendency to chronic progression in CHC infection which was also supported by, a positive correlation between serum TGF-β1 levels and HAI in patients with CHC. HAI indices were not significantly different between CHB and CHC patients. Similarly, fibrosis indices of these patients did not differ significantly either. However, the strong correlation of TGF-β1 levels with fibrosis, along with HAI in CHC patients indicates an important role for TGF-β1 in pathogenesis for fibrosis in these patients. It has been shown before that Hepatitis C virus core antigen upregulates TGF-β1 [31]. Ray *et al.*, in a study to compare liver biopsies of patients with chronic liver disease due to HCV and due to other etiologies, detected that TGF-β1 mRNA was present in patients with HCV-related chronic liver disease but not in the other group. In the same study, TGF-β1 mRNA and protein levels were two logs and approximately 30 times higher in HCV transfected HepG2 cells than in HBV- and mock-transfected cells respectively [32]. In the light of these previous reports in literature, and of our results, it can be concluded that TGF-β1 plays a more important role in chronic liver disease due to HCV when compared to other etiology. Futhermore, Tsai *et al.* has reported that patients positive for both HBsAg and anti-HCV antibody had much higher serum TGF-β1 levels when compared to patients positive only for HBsAg [33]. As has been observed in this study too, anti-HCV positivity is related to an increase in serum levels of TGF-β1. This may explain our finding of elevated TGF-β1 levels in CHC patients. Higher risk of progression to chronicity in patients with CHC may be explained by the elevated levels of TGF-β1. Similarly, the presence of the positive correlation between HAI and TGF-β1 in CHC but not in CHB patients may be interpreted as this cytokine having a more vital role in the immunoinflammatory pathogenesis of CHC. Additionally, this supports the idea that TGF-β1 causes more extensive damage in patients with CHC than in patients with CHB, and may be used as a prognostic marker for these patients.

In conclusion, the role of the immune response in both acute and chronic hepatitis and in cirrhosis patients can not be denied. The results of our study and previous other studies demonstrate that, high serum TGF-β1 levels may be related to continuing efforts to suppress the ongoing immune reaction. Moreover, high levels of serum TGF-β1 levels in cirrhotic patients may indicate that the cirrhotic process may be due to the fibrotic effects of this cytokine.

REFERENCES

1. Hsu HH, Feinstone SM, Hoofnagle JH. 1995. Acute viral hepatitis. In: Mandell GL, Dougles RG, Bennett JE, eds. *Principles and practice of infectious diseases*. New York: Churchill Livingstone Inc, 1136.
2. Di Bisceglie AM, Hoofnagle JH. 1996. Chronic viral hepatitis. In: Zakim D, Boyer TD, eds. *Hepatology: A Text Book of Liver Disease*. Philadelphia: W.B. Saunders Company, 1299.
3. Sekiyama KD, Yoshiba M, Thomson AW. 1994. Circulating proinflamatory cytokines (IL-1 beta, TNF-alfa, and IL-6) and IL-1 receptor antagonist (IL-1 Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol* 98: 71.
4. Cacopardo B, Fatuzzo F, Russo R, Celesia BM, La Rosa R, Lupo G, Cosentino S, Nunnari A. 1992. Serum Tumor Necrosis Factor in acute and fulminant hepatitis. *B J Infect Dis* 166: 448.
5. Yoshioka K, Kakumo S, Arao M, Tsutsumi Y, Inove M. 1989. Tumor Necrosis Factor alpha production by peripheral mononuclear cells of patients with chronic liver diseases. *Hepatology* 10: 769.
6. Zuckerman AJ, Harrison J. 1987. Hepatitis B virus chronic liver disease and hepatocellular carcinoma. *Postgraduate Med J* 63: 13.
7. Ghendon Y. 1987. Perinatal transmission of hepatitis B virus in high incidence countries. *J Virol Methods* 17: 69.
8. Vincent GB, Grqeme JM, Alexander Adrian LWF. 1990. Immunopathogenesis of viral hepatitis. In: Torgan RS, Shanahon F. eds. Immunology and immunopathology of the liver and gastrointestinal tract. *Iguka-Shain Medical Publishers* 225.
9. Andus T, Bayer J, Gerok W. 1991. Effects of cytokines on the liver. *Hepatology* 13: 364.
10. Krawitt EL. 1995. Chronic Hepatitis. In: Mandell GL, Dougles RG, Bennett JE, eds. *Principles and practice of infectious diseases*. New York: Churchill Livingstone Inc, 1153.

11. Ferrari C, Penna A, DeglAntoni A, Fiaccadori F. 1988. Cellular immune response to hepatitis B virus antigens: an overview. *J Hepatol* 7: 21.

12. Tsai SL, Chen PJ, Lai MY, Yang PM, Sung JL, Huang JH, Hwang LH, Chang TH, Chen DS. 1992. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for Hepatitis B antigen seroconversion. *J Clin Invest* 89: 87.

13. Tsai SL, Liaw YF, Chen MH, Huang CY, Kuo GC. 1997. Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. *Hepatology* 25: 449.

14. Cerwenka A, Swain SL. 1999. TGF-beta1: immunosuppressant and viability factor for T lymphocytes. *Microbes Infect* 1: 1291.

15. Massague J. 1999. The transforming growth factor beta family. *Annu Rev Cell Biol* 6: 597.

16. Nakamura T, Sakata R, Ueno T, Sata M, Ueno H. 2000. Inhibition of transforming growth factor beta prevents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. *Hepatology* 32: 247.

17. Thoresen GH, Refsnes M, Christoffersen T. 1992. Inhibition of hepatocyte DNA synthesis by transforming growth factor-beta1 and cyclic AMP: effect immediately before the G1/S border. *Cancer Res* 52: 3598.

18. Oberhammer Fa, Pavelka M, Sharma S, Tiefenbacher R, Purchio A, Bursch W, Shulte-Hermann R. 1992. Induction of apoptosis in cultured hepatocytes and in recessing liver by transforming growth factor-beta1. *Proc Natl Acad Sci USA* 89: 5408.

19. Lin JK, Chou CK. 1992. In vitro apoptosis in the human hepatoma cell line induced by transforming growth factor-beta1. *Cancer Res* 52: 385.

20. Carr BI, Hayashi I, Branum EL, Moses HL. 1986. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type B transforming growth factor. *Cancer Res* 46: 2330.

21. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. 1981. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1: 431.

22. Bissell DM, Maher JJ. 1996. Hepatic fibrosis and cirrhosis. In: Zakim D, Boyer TD, eds. *Hepatology: A Text Book of Liver Disease*. Philadelphia: W.B. Saunders Company, 506.

23. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WGE, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston ALWF, Fainboim L, Heathcote J, Homberg J-C, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RNM, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Mieli-Vergani G, Nakanuma Y, Nishioka M, Penner E, Porta G, Portmann BC, Reed WD, Rodes J, Schalm SW, Scheuer PJ, Schrumpf E, Seki T, Toda G, Tsuji T, Tygstrup N, Vergani D, Zeniya M. 1999. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatology* 31: 929.

24. Abou-Shady M, Friess H, Zimmermann A, di Mola FF, Guo XZ, Baer HU, Buchler MW. 2000. Connective tissue growth factor in human liver cirrhosis. *Liver* 20: 296.

25. Rudner LA, Lin JT, Park IK, Cates JM, Dyer DA, Franz DM, French MA, Duncan EM, White HD, Gorham JD. 2003. Necroinflammatory liver disease in BALB/c background, TGF-beta 1-deficient mice requires CD4 + T cells. *J Immunol* 170: 4785.

26. Calabrese F, Valente M, Giacometti C, Pettenazzo E, Benvegnu L, Alberti A, Gatta A, Pontisso P. 2003. Parenchymal transforming growth factor beta-1: its type II receptor and Smad signaling pathway correlate with inflammation and fibrosis in chronic liver disease of viral etiology. *J Gastroenterol Hepatol* 18: 1302.

27. Flisiak R, Pytel-Krolczuk B, Prokopowicz D. 2000. Circulating transforming growth factor beta(1) as an indicator of hepatic function impairment in liver cirrhosis. *Cytokine* 12: 677.

28. Ohara K, Kusano M. 2002. Anti-transforming growth factor-beta1 antibody improves survival rate following partial hepatectomy in cirrhotic rats. *Hepatol Res* 24: 174.

29. Kakumu S, Ito Y, Takayanagi M, Yoshioka K, Wakita T, Ishikawa T, Higashi Y, Yang ZQ. 1993. Effect of recombinant human transforming growth factor beta-1 on immune responses in patients with chronic hepatitis *B Liver* 13: 62.

30. Kanto T, Takehara T, Katayama K, Ito A, Mochizuki K, Kuzushita N, Tatsumi T, Sasaki Y, Kasahara A, Hayashi N, Hori M. 1997. Neutralization of transforming growth factor beta 1 augments hepatitis C virus-specific cytotoxic T lymphocyte induction in vitro. *J Clin Immunol* 17: 462.

31. Taniguchi H, Kato N, Otsuka M, Goto T, Yoshida H, Shiratori Y, Omata M. 2004. Hepatitis C virus core protein upregulates transforming growth factor-beta 1 transcription. *J Med Virol* 72: 52.

32. Ray S, Broor SL, Vaishnav Y, Sarkar C, Girish R, Dar L, Seth P, Broor S. 2003. Transforming growth factor beta in hepatitis C virus infection: in vivo and in vitro findings. *J Gastroenterol Hepatol* 18: 393.

33. Tsai JF, Jeng JE, Chuang LY, Chang WY, Tsai JH. 1997. Urinary transforming growth factor beta1 levels in hepatitis C virus-related chronic liver disease: correlation between high levels and severity of disease. *Hepatology* 25: 1141.