EFFECT OF SERA OF CIRRHOTIC PATIENTS WITH OR WITHOUT HEPATITIS B VIRUS INFECTION ON PROTEIN SYNTHESIS IN HEPATOMA CELLS

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Summary. – The *in vitro* effects of sera of 11 patients with liver cirrhosis on protein synthesis in PLC/PRF/5 cells were studied. Hepatitis B virus (HBV) infection was documented in 7 patients. Increased random production of several cell proteins of M_r of approximately 25, 65, 90 and 130 K was shown by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). There was no correlation between HBV-positive and HBV-negative cirrhosis and the induced proteins. One of them was identified as alpha-1 foetoprotein by immunoblot analysis. C-reactive protein (CRP) was determined only in one case; production of interleukin-6 (IL-6) was not detected.

Key words: liver cirrhosis; HBV; hepatoma cells; protein synthesis; alpha-foetoprotein; C-reactive protein; SDS-PAGE; immunoblot

Introduction

Cirrhosis may develop as a result of the action of various aetiologic agents. Hepatic cirrhosis is most often caused by chronic hepatitis and is characterized by changes in liver architecture and by necrosis of liver cells (Liaw *et al.*, 1988, Bissel and Roll, 1990).

An important role in the control of gene expression in liver cells play cytokines. The effects of these regulatory protein is a part of the acute phase response resulting in the alteration of several plasma proteins. Some of them are also involved in the pathogenesis of chronic hepatitis and cirrhosis (Abb *et al.*, 1985, Castilla *et al.*, 1991).

Circulating regulatory factors are also demonstrated in plasma of patients with various diseases. Therefore we decided to study the effects of sera of patients with liver cirrhosis, HBV-positive or HBV-negative, on protein synthesis in human hepatoma PLC/PRF/5 cells *in vitro*.

Patients and Methods

Patients with liver cirrhosis were diagnosed from liver biopsy and histological results. Table 1 summarizes markers of HBV- and HCV-related cirrhosis. A sample of venous blood was taken from all the subjects and the obtained sera were stored at –20 °C until assayed.

Serological studies. HBsAg, HBeAg, anti-HBs, anti-HBe, anti-HBc (total) and anti-HCV were determined by commercially available ELISA kits (ABBOT Diagnostics, SEVAC).

Cell cultures. Human hepatoma PLC/PRF/5 cells (Alexander *et al.*, 1976), obtained from the Ivanovskii Institute of Virology, Moscow, were grown in MEM supplemented with 5 % heat-inactivated calf serum, 2 mmol/l L-glutamine, 0.01 mmol/l non-essential amino acids, penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells were grown on 24 well-plates (Sigma) in 2 ml media per well at 37 °C in 5 % CO_2 atmosphere.

SDS-PAGE was performed in 7.5–15 % linear gradient slab gels as described by Laemmli (1970). After electrophoresis gels were silver stained.

Immunoblot analysis. Proteins from gels were electroblotted to PVDF (Immobilon, Millipore), or nitrocellulose (Schleicher and Schuell) membranes (30 V, 4 °C, overnight) using a buffer described by Towbin *et al.* (1979). For identification of CRP and alpha-1 foetoprotein we used appropriate polyvalent monospecific antibodies (SEVAC) saturated with the proteins of control PLC/PRF/5 cells. Antibody to alpha-1 foetoprotein was also saturated with human albumin. For the detection of IL-6 a monoclonal antibody was used (gift of Dr. Aarden, Blood Transfusion Service, Amsterdam).

Table 1. Pathogenetical markers of patients with liver cirrhosis

Patient No.	Sex	Age	Viral hepatitis serum markers					
			HBsAg	HBeAg	a-HBe	a-HBs	a-HBc	a-HCV
1	M	60	+	+	–	–	+	ND
2	M	49	+	+	–	–	+	ND
3	M	45	+	+	–	–	+	ND
4	M	21	+	–	+	–	+	ND
5	F	57	+	–	+	–	+	ND
6	F	17	+	–	ND	–	+	ND
7	F	61	–	–	ND	+	+	–
8	F	33	–	–	–	–	–	–
9	F	46	–	–	–	–	–	–
10	F	56	–	–	–	–	–	–
11	F	30	–	–	–	–	–	–

M – male
F – female
(+) – present, (–) – absent, ND – not done

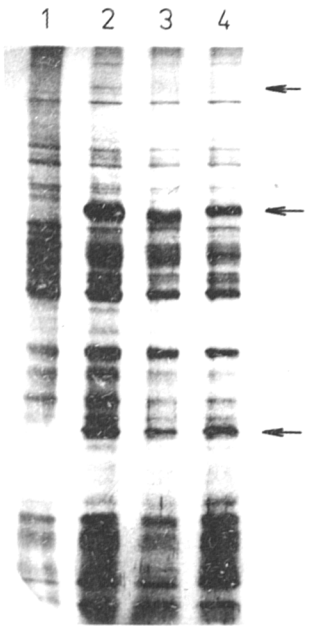

Fig. 2
SDS-PAGE of proteins induced by patients' sera in PLC/PRF/5 cells
Control cells (lane 1). Cells treated with sera of patient No. 6 (lane 2). No. 4 (lane 3) and No. 1 (lane 4). Arrows indicate increased synthesis of cell proteins.

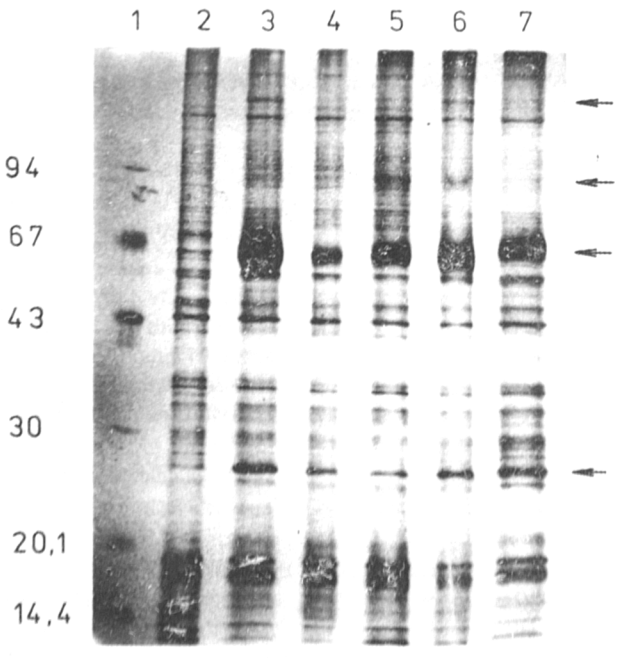

Fig. 1
SDS-PAGE of proteins induced by patients' sera in PLC/PRF/5 cells
Control cells (lane 2). Cells treated with sera of patient No. 5 (lane 3), No. 8 (lane 4), No. 2 (lane 5), No. 3 (lane 6) and No. 10 (lane 7). Size marker proteins M_r (K) values (lane 1). Arrows indicate increased synthesis of cells proteins.

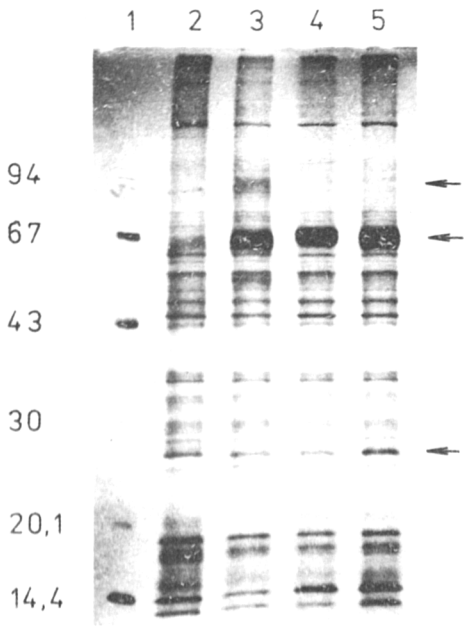

Fig. 3
SDS-PAGE of proteins induced by patients' sera in PLC/PRF/5 cells
Control cells (lane 2). Cells treated with sera of patient No. 9 (lane 3), No. 7 (lane 4) and No. 11 (lane 5). For the rest of the legend see Fig. 1.

Results

Effect of patients' sera on the synthesis of PLC/PRF/5 cell proteins

Cell monolayers were treated with 1.5 ml of cultivation medium and 0.5 ml of patients' sera per well for 48 hrs. Control cells were incubated with standard heat-inactivated calf serum.

Earlier experiments showed that cells treated with control (non-cirrhotic) human sera gave the same results. The results of SDS-PAGE of soluble cell proteins are shown in Figs. 1, 2 and 3. Increased synthesis of several proteins was observed. Their M_r were found to be about 25, 65, 90 and 130 K. The treatment of PLC/PRF/5 cells by individual sera resulted in the induction of different proteins.

Immunoblot analysis of induced proteins

Using the immunoblot technique and antibodies against alpha-1 foetoprotein, CRP and IL-6 we tried to detect these proteins among those induced in PLC/PRF/5 cells treated with patients' sera. Alpha-1 foetoprotein (about 65 K) was identified in cells treated with all sera while CRP (about 25 K) was found only in one case (serum of patient No. 9). IL-6 was not detected among the induced cell proteins in any case (data not shown).

Discussion

Cytokines can be detected in sera of the patients suffering from a variety of serious diseases (Waage *et al.*, 1987). It is presumed that in body fluids only over-production, e.i. surplus of these circulating proteins after their adsorbtion by producing and/or target cells is usually detected (Cavaillon *et al.*, 1992). Other biologically active factors influencing activity of individual cytokines may be present in patients' sera but remain unrecognized by *in vitro* testing of the purified substances. This presumption formed the basis of our experimental approach.

The aim of our work was to describe a putative regulatory effect of the substances present in sera of patients with liver cirrhosis on the proteosynthesis in hepatoma cell line. Liver cirrhosis in the majority of our selected patients was related to ongoing or passed chronic viral hepatitis B. Four of them had cryptogenic cirrhosis.

Increased synthesis of some cellular proteins was observed 48 hrs after the treatment of hepatoma PLC/PRF/5 cells with sera from cirrhotic patients. M_r of the induced proteins was approximatelly 25, 65, 90 and 130 K. No correlation was found between this effect and the HBV-positivity or HBV-negativity of the sera. An attempt was made to identify CRP, IL-6 and alfa-1 foetoprotein using Western blot technique and specific antibodies. High induction of alpha-1 foetoprotein in PLC/PRF/5 cells was observed with all patients' sera while increased production of CRP was observed only in one case. A comparison of the cellular proteins profiles disclosed that the profile of proteins of M_r around 25 K induced by the serum of the patient No. 9 was almost identical with that of the control. On the other hand, the serum of the patient No. 6 induced a massive synthesis of 25 K protein with reacted neither with the anti-CRP antibody nor with the anti-IL-6 antibody. It is not clear so far whether this protein is a surface or a cytoplasmic cellular protein.

The individual bands may be composed of more than one protein. Thus a better picture of the influence of patients' sera on the synthesis of cellular proteins could be obtained through their analysis by two-dimensional electrophoresis.

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References

Abb, J., Zachoval, R., Eisenberg, J., Pape, G. R., Zachoval, V., and Deinhardt, F. (1985): Production of interferon alpha and interferon gamma by peripheral blood leucocytes from patients with chronic hepatitis B virus infection. J. *Med. Virol.* **16**, 171–176.

Alexander, J. J., Bey, E. M., Geddes, E. W., and Lecatsas, G. (1976): Establishment of a continuously growing cell-line from primary carcinoma of the liver. S. *Afr. med. J.* **50**, 2124-2128.

Bissell, D. M., and Roll, J. (1990): Connective tissue metabolism and hepatic fibrosis, pp. 424–444. In Zakim, D., Boyer, T. D. (Eds) *Hepatology: a text book of liver disease,* 2nd, ed., W. B. Saunders, Philadelphia.

Castilla, A., Prieto, J., and Fausto, N. (1991): Transforming growth factors beta 1 and alpha in chronic liver disease. Effects of interferon alpha therapy. *N. E. J. Med.* **324**, 933–940.

Cavaillon, J.-M., Munoz, C., Fitting, C., Misset, B., Carlet, J. (1992): Circulating cytokines: The tip of the iceberg? *Circulatory Shock* **38**, 145–152.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Naure* **277**, 680–688.

Liaw, Y.-F., Tai, D.-I., Chu, C-M., and Chen, T. J. (1988): The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Hepatology* **8**, 493–496.

Towbin, J., Staehelin, T., and Gordon, J. (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natn. Acad. Sci. USA* **76**, 4350–4354.

Waage, A., Halstensen, A., and Espevik, T. (1987): Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet* **i**, 355–357.