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Detection of serum TNF-α,IFN-γ,IL-6 and IL-8 in patients with hepatitis B *

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Subject headings hepatitis B; TNF-α; IFN-γ; IL-6; IL-8

Abstract

AIM To assess the possible roles of cytokines (TNF-α, IFN-γ, IL-6 and IL-8) in liver damage of hepatitis B.

METHODS The serum TNF-α, IFN-γ, IL-6 and IL-8 were detected by ELISA in 66 patients with hepatitis B and 20 healthy blood donors.

RESULTS TNF-α and IL-6 in all types of clinical hepatitis B were significantly higher than those in healthy blood donors ($P<0.05$); meanwhile the levels of TNF-α, IFN-γ, IL-6 and IL-8 in the patients with fulminant hepatitis B were much higher than those in the patients with acute hepatitis B($P<0.05$); the level of TNF-α was positively correlated with the levels of IFN-γ, Il-6 and IL-8 in all types of hepatitis B ($r_{IFN} = 0.24$, $r_{IL-6} = 0.35$, $r_{IL-8}=0.44$) and the TNF-α, IFN-γ, IL-6 and IL-8 were positively correlated with serum bilirubin ($P<0.05$). Dynamic changes of these cytokines were observed in the course of acute and fulminant hepatitis. The level of IFN-γ peaked in the initial period of acute hepatitis and early stage of hepatic coma in fulminant hepatitis; TNFα, IL-6 and IL-8 increased with exacerbation, and reached a peak when the liver damage was most serious, then decreased when patient conditions were improved.

CONCLUSION The increased cytokines were related to the inflamm ation of liver cells and multiple factors may play certain roles in liver damage.

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INTRODUCTION

Since Muto[1] reported that TNF-α and IL-1 were related to fulminant hepatitis, the studies on the relationship between cytokines and liver damage have been paid more and more attention especially in recent years. Most scholars now agree that TNF and IFN are related to liver damage, so are IL- 6 and IL-8. We detected the serum TNF-α, IFN-γ, IL-6 and IL-8 in the patients with different clinical types of hepatitis B by ELISA for asse ssing the relationship between the cytokines and the liver damage.

MATERIALS AND METHODS

Samples

A total of 66 patients with HBV infection and 20 healthy blood donors were studied. They were admitted to this college between 1993 and 1997. The patients (48 men and 18 women) ranged in age from 21 to 56 years. There were 22 cases of acute hepatitis (AH), 25 cases of chronic hepatitis (CH) and 19 cases of fulminant hepatitis (FH). The serological markers of HAV, HCV, HEV, CMV and EBV were negative, and HBsAg and other markers of HBV were positive in all the patients.

Healthy blood donors

Healthy blood donors, aged from 25 to 43 years, included 16 men and 4 women. They had no serological markers of HAV-HEV, CMV, EBV infection and liver functions were normal.

Detection of cytokines

The kits of the four cytokines were produced by the Genzyme Company, U. S. A. No. 1 9970214. The four cytokins were detected by ELISA according to the manufacturer's instructions. The first antibody was biotin-labelled and the second one was connected with horse radish peroxidase.

RESULTS

The serum TNF-α, IFN-γ, IL-6 and IL-8 in patients with different types of hepatitis B are shown in Table 1.

Table 1 TNF-α, TNF-α, IL-6 and IL-8 in various types of hepatitis B ($\bar{x}\pm s$, ng/L)

	n	TNF-α	IFN-γ	IL-6	IL-8
FH	19	359.0±17.2[ab]	234.7±16.5[ab]	347.5±31.3[ab]	181.1±19.6[ad]
AH	22	220.6±8.9[c]	174.9±12.0	285.8±16.5[c]	118.4±5.1
CH	25	322.1±13.0[c]	200.0±15.7[c]	329.5±25.2[c]	133.1±6.7[c]
CI	20	146.7±9.4	165.0±7.7	231.1±16.4	110.2±2.9

CI: healthy blood donor. [a]P<0.01, *vs* CI; [c]P<0.05, *vs* CI; [b]P<0.01, *vs* AH;[d]P<0.05, *vs* AH.

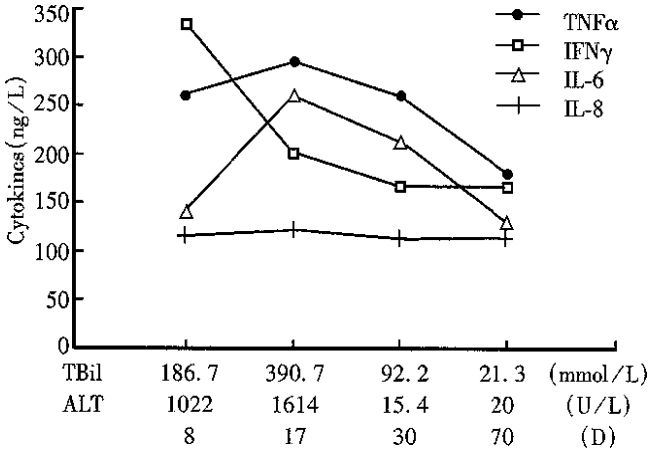

Figure 1 Dynamic changes of four cytokines in the course of AH.

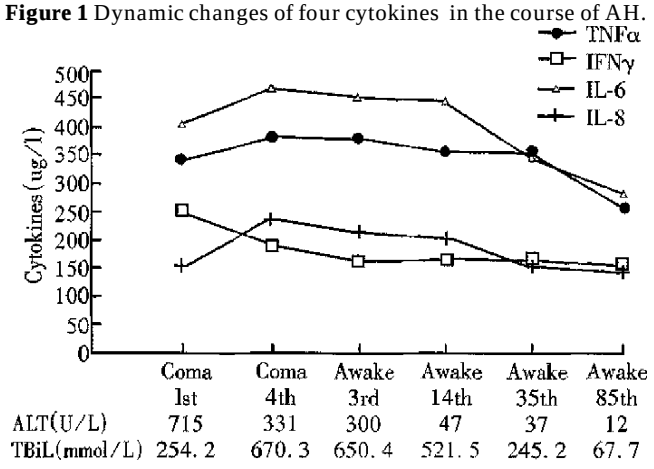

Figure 2 Dynamic changes of four cytokines in the co urse of FH.

The TNF-α and IL-6 in various clinical types of hepatitis B were significantly higher than those in healthy blood donors (P<0. 05). Except acute hepatitis B, the levels of IFN-γ and IL-8 were obviously higher in other types than those in healthy blood donors. The levels of four cytokines of patients with FH were much higher than those of patients with AH (P<0.05).

Correlation between TNF-α and IFN-γ, IL-6 and IL-8

The correlations between TNF-α and IFN-γ, IL-6 and IL-8 in HBV infections were analyzed. The results suggested that TNF-α and the other cytokines were positively correlated (r_{IFN} = 0.24, $r_{IL\text{-}6}$ = 0.35, $r_{IL\text{-}8}$ = 0.44).

Correlation between serum bilirubin and four cytokines

The correlation between serum billirubin and four cytokines was analyzed in HBV infections. The results suggested that bilirubin was positively related to the levels of four cytokines (P<0.05).

Dynamic changes of four cytokines in AH and FH

The dynamic changes of four cytokines in the course of AH and FH are shown in Figures 1, 2. At the early stage of AH, IFN-α peaked and decreased rapidly. TNF-α and IL-6 increased with exacerbation, reached a highest level when jaundice became most severe, and then decreased gradually. IL-8 level did not change during the course.

On the first day of hepatic encephalopathy of FH, IFN-γ reached a peak, then decreased rapidly. TNF-α, IL-6 and IL-8 increased when condition of the patients worsened. They reached the highest level during the peak of jaudice, then decreased gradually, and maintained abnormal for a long time.

DISCUSSION

Since it was reported that TNF and IL-1 in supernatant of cultured monocytes of peripheral blood in patients with FH were obviously higher than those with AH, the studies on cytokines related to liver damage advanced rapidly. Pei Liu[2] proved that TNF could cause liver necrosis in corynebacterium sensitized animals and the necrosis could be blocked by anti-TNF monoclonal antibody[3]. At present, the studies of hepatitis focused on the roles of cytokines in cell-mediated injury of tissues. Ferluga *et al*[4] reported that the liver injury of animal model induced by corynebacterium-endotoxin could be caused by the soluble factors produced by monocytes gathering at the hepatic lobules. Luca[5] proved that activation of CTL induced by IFN-γ resulted in CTL-mediated hepatocytic injury in the study of HBV transgenic mouse. IL-6 may induce the activation, differentiation and maturation of NK cells[6] and expression of monocytic IL-8 gene[7]. The IL-8 may cause degranulation of nutrophile granulocyte, leading to DIC within the l iver[8].

The serum levels of TNF-α, IFN-γ, IL-6 and IL-8 in patients with HBV infection were higher than those in healthy blood donors. The difference was obvious between the levels of cytokines in FH and those in AH. TNF- α and IFN-γ, IL-6 and IL-8 were positively correlated in various types of hepatitis B. The bilirubin was also positively related to the four cytokines. In the course of AH and FH, IFN-γ peaked in the early stage of AH and the 1st day of hepatic coma of FH. TNF-α, IL-6 and IL-8 in-

creased with exacerbation of condition of the patients with AH and FH except for IL-8 in AH. These suggested that the four cytokines were related to liver injury.

The roles of cytokines in the liver damage are complex. They affect each other to form a cytokine network, in which IFN-γ may be the chief cytokine and induce the immune cells to release other cytokines and improve cytotoxic activities mediated by immune cells.

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