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Association between TRAIL expression on peripheral blood lymphocytes and liver damage in chronic hepatitis B

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Abstract

AIM: To explore a novel mechanism for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), upregulation of CD4⁺ and CD8⁺ T lymphocytes participating in the pathophysiological process of chronic hepatitis B (CHB).

METHODS: The levels of serum soluble TRAIL (sTRAIL), serum IFN- γ and membrane-bound TRAIL expression on peripheral leucocytes from 58 CHB patients were examined by ELISA and flow cytometry respectively. The levels of TRAIL were compared with the baseline levels of 17 healthy controls, and correlation analysis was performed between ALT, TBIL, PT, morphological change in hepatic tissues, and serum IFN- γ .

RESULTS: The results showed that TRAIL levels on membranes of CD4⁺, CD8⁺ T cells in CHB patients were much higher than those in healthy controls ($P < 0.001$), and were correlated with serum TBIL ($r = 0.354$, $P = 0.008$ for CD4⁺ and $r = 0.522$, $P = 0.000$ for CD8⁺, respectively), ALT ($r = 0.393$, $P = 0.003$ for CD8⁺), PT ($r = 0.385$, $P = 0.004$ for CD8⁺) and serum IFN- γ level ($r = 0.302$, $P = 0.011$ for CD4⁺ and $r = 0.307$, $P = 0.009$ for CD8⁺). On the contrary to membrane-bound TRAIL expression, serum level of sTRAIL was not correlated with that of TBIL and PT, though it was higher than that of the normal population and was positively correlated with serum HBeAg expression ($r = 0.695$, $P = 0.001$).

CONCLUSION: The expression level of TRAIL on the membrane of lymphocytes was upregulated and associated with the liver injury in CHB patients. These findings suggest that upregulation of TRAIL expression may be induced by virus antigen and inflammatory cytokine IFN- γ .

Key words: HBV; CD8⁺ lymphocyte; CD4⁺ lymphocyte; TRAIL; Liver function

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INTRODUCTION

The TNF- α and Fas systems as well as the perforin/granzyme system have been implicated in hepatocyte apoptotic processes in viral hepatitis. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a type II transmembrane protein^[1], is a recently identified member of the TNF family and is capable of inducing apoptosis in virus-infected cells. This suggests that TRAIL may play an important role in HBV immunopathology. Human CD4⁺ T cell clones exhibit TRAIL-mediated cytotoxicity against certain target cells^[2], indicating that TRAIL constitutes an additional pathway of T cell-mediated cytotoxicity. The aim of this study was to study a possible novel mechanism for TRAIL upregulation of CD4⁺ and CD8⁺ T lymphocytes participating in the pathophysiological process of chronic hepatitis B (CHB).

MATERIALS AND METHODS

Patients

Fifty-eight patients seropositive for HBV marker (HBsAg) for more than 6 mo were the subjects of this study. Thirty were seropositive for HBV DNA, and all were negative for other hepatitis virus and HIV markers in sera. None had received any antiviral therapy or immunotherapy in the preceding 6 mo. They were classified into four groups^[3]: 13 cases with mild chronic hepatitis (CH1), 14 cases with moderate chronic hepatitis (CH2-3), 15 with liver cirrhosis (LC) and 16 with chronic severe hepatitis (CSH). Seventeen healthy laboratory workers negative for HBsAg and normal in liver functions were assessed at the same time and served as controls. The demographic data of all participants are summarized in Table 1. Serum specimens for antibody to HAV, HCV, HDV, HEV, as well as antibody to HIV were tested by enzyme immunoassay. Immunoassay for the *in vitro* qualitative determination of serum HBsAg and HBeAg was tested on Roche Elecsys immunoassay analyzers, HBV DNA was assayed by quantitative PCR.

Leukocyte and flow cytometry

Citrated blood samples were obtained. Aliquots of 100 μ L

were incubated for 15 min with 20 μ L antibody against TRAIL-PE (eBioscience, Clone number RIK-2), CD4-FITC, CD8-FITC, or isotype control (CALTAG, USA) at room temperature. After labeling with the antibody, leukocyte suspensions were washed and erythrocytes were lysed using FACS brand lysing solution (Becton Dickinson, USA). Washed cells were resuspended in 300 μ L of PBS and analyzed. FACS scan flow cytometer (Becton Dickinson, USA) and cell quest software were used for acquisition and analysis of the data. Populations of lymphocytes were identified from forward and side scatter characteristics on dot plot profiles and were analyzed for fluorescence intensity using fixed defined gate. At least 10 000 cells per sample were acquired.

ELISA

Serum specimens collected were stored at -80°C , and IFN- γ and soluble TRAIL (sTRAIL) protein levels were determined using immunoassay kit according to manufacturer's specifications.

Statistical analysis

Statistically significant differences among groups of patients were determined by ANOVA, correlation coefficients were determined by Pearson (two-tailed) using SPSS10.0 software, and P values ≤ 0.05 were considered as significant.

RESULTS

Changes of serum soluble TRAIL and modulation of membrane-bound TRAIL expression on peripheral T lymphocytes in chronic hepatitis

The results showed that TRAIL levels in membranes of

CD4⁺ and CD8⁺ T lymphocytes and the serum levels of sTRAIL in CHB patients were much higher than those in healthy controls ($P < 0.001$). Membrane-bound TRAIL expression was shown to correlate with the activity of viral hepatitis, that of CD4⁺ and CD8⁺ T lymphocytes correlated with serum TBIL ($r = 0.354$, $P = 0.008$ for TRAIL of CD4⁺ *vs* TBIL and $r = 0.522$, $P = 0.000$ for TRAIL of CD8⁺ *vs* TBIL) and that of CD8⁺ T lymphocytes was correlated with serum ALT ($r = 0.393$, $P = 0.003$), and PT ($r = 0.385$, $P = 0.004$). A positive relationship between serum IFN- γ level and the degree of TRAIL antigen expressions of CD4⁺ and CD8⁺ T lymphocytes ($r = 0.302$, $P = 0.011$ for TRAIL of CD4⁺ *vs* IFN- γ ; $r = 0.307$, $P = 0.009$ for TRAIL of CD8⁺ *vs* IFN- γ) was shown. Serum level of sTRAIL was not correlated with TBIL and PT, though it was higher than that in normal population. The related results are shown in Table 2.

Positive correlation between liver histological findings and membrane-bound TRAIL expression

In 30 samples from 58 HBV patients, with the aggravation of liver inflammation grade, membrane-bound TRAIL expression in peripheral T lymphocytes in chronic hepatitis was upregulated. There was a positive correlation between the inflammation grade and level of TRAIL antigen expression ($r = 0.677$, $P < 0.01$). Statistical analysis is shown in Table 3.

Positive correlation between serum IFN- γ level and membrane-bound TRAIL expression

The serum level of IFN- γ in patients with CHB was 24.04 ± 19.03 pg/mL for CH1, 76.02 ± 84.35 pg/mL for

Table 1 Demographic data, HBV marker and histological findings of the liver in the study population

Characteristics	Normal control	Chronic HBV infection			
		CH1	CH2-3	CSH	LC
Number	17	13	14	16	15
Male:female	12:5	9:4	10:4	11:5	11:4
Median age (range)	33.5 (26-50)	34 (24-58)	36 (23-64)	33 (24-56)	44 (21-68)
ALT (U/L)	33 \pm 12	57.8 \pm 73.6	190.3 \pm 207.8	238.3 \pm 381.2	85.5 \pm 60.4
TBIL (mmol/L)	15 \pm 5.2	22.4 \pm 20.1	70.0 \pm 46.7	405.8 \pm 132.9	64.4 \pm 47.4
Alb (g/L)		40.78 \pm 5.3	35.4 \pm 6.8	31.8 \pm 3.6	34.9 \pm 4.5
PT (s)		13.7 \pm 3.4	17.4 \pm 5.2	32.1 \pm 10.9	21.8 \pm 6.4
HBV DNA (positive cases)	0	9	10	4	7
Liver histological findings					
Not done	(n) 17		8	11	9
G ₀₋₁	(n)	11			
G ₂₋₃	(n)	2	6		
G ₄	(n)			5	6

Table 2 Changes of serum sTRAIL and modulations of membrane-bound TRAIL expression on peripheral T lymphocytes in chronic hepatitis

Group	n	CD4 ⁺ T lymphocyte TRAIL expression (%)	CD8 ⁺ T lymphocyte TRAIL expression (%)	sTRAIL (pg/mL)
CH1	13	2.82 \pm 1.00 ^b	5.10 \pm 2.01 ^b	1 001.93 \pm 474.23
CH2-3	14	4.64 \pm 2.08 ^b	6.56 \pm 3.56 ^b	1 358.38 \pm 391.66 ^b
CSH	16	5.33 \pm 2.38 ^b	8.31 \pm 3.62 ^b	969.56 \pm 377.41
Cirrhosis	13	4.20 \pm 1.92 ^b	5.09 \pm 2.50 ^b	
Normal	17	1.21 \pm 0.57	3.04 \pm 1.72	800.24 \pm 322.9

^b $P < 0.001$ vs normal controls.

Table 3 Positive correlation between liver histological findings and membrane-bound TRAIL expression

Inflammation grade	n	CD4 ⁺ T lymphocyte TRAIL expression (%)	CD8 ⁺ T lymphocyte TRAIL expression (%)
G ₀₋₁	11	2.51±0.96	5.22±2.25
G ₂₋₃	8	4.66±2.30 ^a	6.43±3.00 ^a
G ₄	11	4.74±2.00 ^a	7.92±3.58 ^a

^aP<0.05 vs G₀₋₁.

CH2-3, 81.81±63.11 pg/mL for CSH. There was a positive relationship between serum levels of IFN- γ and TRAIL antigen expression in CD4⁺T ($r = 0.302$, $P = 0.011$) and CD8⁺T ($r = 0.307$, $P = 0.009$). No relationship between serum levels of IFN- γ and sTRAIL was found.

DISCUSSION

In this paper, a possible novel mechanism for TRAIL upregulation of CD4⁺, CD8⁺ T lymphocytes participating in the pathophysiological process of CHB was studied in patients with CHB. HBV is the main etiologic agent for viral hepatitis. Cytotoxic T lymphocytes (CTLs) recognize viral peptides, which have been intracellularly processed and expressed on cell surface of hepatocytes in conjunction with MHC class I molecules. CTL-induced immunological tissue destruction is one of the hallmarks of liver injury in viral hepatitis. Apoptosis is now recognized to play a significant role in the pathogenesis. Different pathways, which include the Fas and TNF- α system as well as the perforin/granzyme system, have been implicated in hepatocyte apoptotic processes^[4]. Fas has been shown to be correlated with the activity of viral hepatitis^[5], and Fas expression can be induced either by virus-specific protein expression or by inflammatory cytokines such as interleukin-1. Activated CTLs express FasL and induce hepatocyte apoptosis via Fas signaling cascades. TNF-R1 expression has also been shown to be enhanced in HBV, and the increased production of TNF- α has been demonstrated in peripheral blood mononuclear cells in hepatitis B. Additionally, HBx has been shown to sensitize cultured and transfected hepatocytes to TNF- α -induced apoptosis^[6]. In contrast to Fas and TNF- α signaling, the potential hepatotoxicity of TRAIL is still controversial, especially its ability to induce hepatocyte apoptosis. A recombinant soluble version of TRAIL ligand fused to a trimerizing leucine zipper failed to induce hepatotoxicity in mice^[7], whereas a polyhistidine-tagged recombinant soluble form of TRAIL was reported to induce apoptosis in cultured human hepatocytes^[8]. Because of the rapid dedifferentiation of cultured hepatocytes, it is difficult to extrapolate these experiments to *in vivo* condition. Furthermore, these discrepancies may be attributed to the different TRAIL agonists. Although there is expression of TRAIL receptors 1 and 2 mRNA in human liver, a more recent report^[9] demonstrated that TRAIL-R2 protein was minimally expressed in human hepatocytes and a specific TRAIL-R2 agonist did not induce apoptosis in cultured human hepatocytes. Therefore, TRAIL signaling seems to play a less important role in pathophysiological process of hepatocyte apoptosis than Fas or TNF- α . In this paper we

found that even serum sTRAIL in healthy individuals is very high, which also supports this theory. However, since TRAIL-R expression can be induced, for example, by DNA damage or by bile acid^[10,11], it is also possible that hepatocyte sensitivity to TRAIL may increase in disease states. Viral infection triggering sensitivity to TRAIL-mediated apoptosis has been reported. Yoon *et al.*^[4], have recently observed that in HBV-transfected HepG2 cells, TRAIL-R1 expression level was higher than in their parent cell line, HepG2 cells. Moreover, the HBV-transfected cells were more sensitive to TRAIL-induced apoptosis than the untransfected ones, and this sensitization was reduced by anti-viral treatment. Our group also demonstrated that in HBV-transfected HepG2 cells, TRAIL expression level was higher than in their parent cell line (unpublished data). Mundt *et al.*^[12], also reported that HBV-mediated acute liver failure resulted in upregulation of protein expression of TRAIL and TRAIL receptors, thereby activating the TRAIL-specific death pathway in HBV-infected hepatocytes. Our study is the first report that membrane-bound TRAIL expression on peripheral T lymphocytes in CHB was upregulated and was correlated with the activity of viral hepatitis. Serum level of sTRAIL was not correlated with TBIL and PT, and same results were also reported by Janssen *et al.*^[13]. It suggested that serum level of sTRAIL was unable to accurately reflect the severity of disease.

Recently it was reported that the expression level of sTRAIL was positively correlated with the level of HBeAg. Furthermore, we found that there existed a positive correlation between the degree of TRAIL antigen expression and serum levels of IFN- γ . The IFN-induced gene family is now known to comprise the death ligand TRAIL, the dsRNA-dependent protein kinase, interferon regulatory factors and the promyelocytic leukemia gene, all of which have been reported to be the mediators of cell death^[14]. Our study suggests that interaction of TRAIL signaling and IFN- γ participates in the pathophysiological process in CHB patients.

TRAIL expression in liver tissues, especially in lymphocytes needs to be analyzed further.

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