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Serum Soluble Fas as a Seromarker of Disease Activity in Chronic Hepatitis C and the Possible Involvement of Peripheral Blood Mononuclear Cells in the Production of Serum Soluble Fas

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Summary. **Fas-mediated apoptosis is considered to be a major form of liver cell death in chronic hepatitis C. Recently, serum soluble Fas has become measurable although its clinical significance in chronic liver diseases is not fully understood. We measured serum soluble Fas (sFas) levels in 22 normal volunteers, in 10 cases with autoimmune hepatitis type 1 (AIH), in 18 cases with chronic hepatitis C with autoimmune features (C-AIH), in 34 cases with chronic hepatitis without such features (C-CH) and in 17 cases with systemic lupus erythematosus. The mean sFas level in C-AIH was significantly higher ($p<0.05$) than C-CH and normal volunteers. As a whole, the serum sFas level positively correlated with aspartate aminotransferase (AST) ($p<0.05$) and alanine aminotransferase (ALT) ($p<0.05$), and inversely with peripheral platelet counts ($p<0.05$). Only C-CH showed a striking correlation between serum sFas and AST/ALT levels while other disease groups with autoimmune features did not, suggesting the possible involvement of lymphocytes in sFas production in these groups. The serum sFas level also significantly correlated with the histological activity indexes of chronic inflammation in liver sections. Fas and sFas mRNAs were both detectable by reverse-transcription-polymerase chain reaction amplification in peripheral blood mononuclear cells (PBMC) that were collected from these patients. The intensity of sFas mRNA expression was higher than that of full-length Fas mRNA in half of the cases examined. Among these, the expression intensity of both mRNAs was highest in AIH. After the stimulation of PBMC with phytohemoagglutinin and interleukin-2, the intensity of Fas mRNA expression was consistently higher than that of sFas mRNA. The Fas and sFas mRNA expressions in 3 C-CH liver samples were both more up-regulated than in the normal liver. Nuclear DNA fragmentation visualized by terminal deoxyuridine transferase-mediated nick end labeling was detectable in mononuclear cells infiltrating portal triads of the AIH, C-AIH and C-CH liver. In conclusion, serum sFas levels can be a possible seromarker of disease activity in chronic hepatitis C. Moreover, peripheral blood mononuclear cells are possibly involved in the production of serum sFas, especially in chronic hepatitis C with autoimmune features.**

Key words—Fas, soluble Fas, chronic hepatitis C, autoimmune hepatitis, apoptosis.

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Abbreviations—AICD, activation-induced cell death; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, antinuclear antigen; AST, aspartate aminotransferase; C-AIH, chronic hepatitis C with autoimmune features; C-CH, chronic hepatitis C without autoimmune features; cDNA, cloned DNA; CH-C, chronic hepatitis C; DNA, deoxyribonucleic acid, ELISA, enzyme-linked immunosorbent assay, HAI, histological activity index; HCV, hepatitis C virus; HLA, histocompatibility leukocyte antigen, IL-2, interleukin-2; mRNA, messenger RNA, PAS, periodic acid Schiff; PBMC, peripheral blood mononuclear cells, PBS, phosphate buffer saline; PHA, phytohemagglutinin; RNA, ribonucleic acid, RT-PCR, reverse transcription-polymerase chain reaction amplification, sFas, soluble Fas; SLE, systemic lupus erythematosus; TUNEL, terminal deoxyuridine nick end labeling; WBC, white blood cell.

INTRODUCTION

The apoptosis of hepatocytes is implicated as a major form of liver cell death in viral hepatitis[1]. Both the Fas-Fas ligand and the perforin-granzyme systems in cytotoxic T lymphocytes are involved in this apoptosis[2,3]. Fas-ligands bound on the cell membrane of cytotoxic T cells recognize Fas molecules expressed on the liver cell surface, and the binding of these two molecules triggers the intracellular cascade system for the apoptosis of hepatocytes[4]. In fact, the degree of Fas expression in liver tissue correlates well with the severity and magnitude of hepatocyte apoptosis in both chronic hepatitis B[5] and C[6]. The HLA-restricted recognition of partial structural polypeptides of hepatitis viruses by immunocompetent cells is also important for this apoptosis[7,8] rather than the direct cytopathic effect of the viruses[9,10]. However, little is known concerning the apoptosis of lymphocytes that infiltrate the inflamed liver. In autoimmune disorders, the impaired apoptosis of autoreactive lymphocytes has been implicated as a possible pathogenesis of the disease[11].

Several soluble Fas molecules exist in serum. These soluble forms of Fas are designated as soluble Fas (sFas), and are generated either by alternative splicing of the full-length Fas mRNA[12-15] or by the direct proteolysis of cell membrane-bound full-length Fas molecules. Both forms of sFas lack the transmembrane (TM) domain and are considered to inhibit Fas-mediated apoptosis by their affinity to Fas ligands[12,13]. Thus, the serum sFas level may influence the Fas-Fas ligand-mediated cell apoptosis or may reflect the overall status of apoptosis in inflamed tissues[16]. There have been several reports concerning sFas levels in liver diseases[17-18], although a lack of data remains.

In this study, we measured serum sFas levels in chronic hepatitis C (CH-C) with and without autoimmune features (C-AIH and C-CH), autoimmune hepatitis type 1 (AIH) and systemic lupus erythematosus (SLE), and correlated these data with several clinicopathological parameters and with histological findings. Moreover, we detected Fas and sFas messenger RNA (mRNA) in unstimulated and stimulated peripheral blood mononuclear cells (PBMC) of CH-C by reverse transcriptase-polymerase chain reaction (RT-PCR) amplification. Nuclear DNA fragmentation of infiltrating mononuclear cells in inflamed portal triads was visualized in C-CH, C-AIH and AIH.

MATERIALS AND METHODS

Subjects of the study

Soluble Fas (sFas) in pretreatment sera was quantified by using a commercial enzyme-linked immunosorbent assay (the sFas ELISA kit, Orient Yeast Co., Ltd., Osaka, Japan) according to the manufacturers' instruction in 22 normal volunteers, in 10 cases with AIH type 1, in 52 cases with CH-C, and in 17 cases with systemic lupus erythematosus (SLE). Patients with CH-C were subdivided into two groups, 18 cases with CH-C associated with autoimmune features (C-AIH) and 34 cases with CH-C without such features (C-CH), to determine whether sFas might contribute to the pathophysiology of C-AIH. AIH was diagnosed according to the diagnostic criteria proposed by the Japanese Autoimmune Hepatitis Study Group[19]. The requisite for these criteria was a positive antinuclear antibody (ANA) by immunofluorescence associated with hypergammaglobulinemia of more than 2.0 g/dl and/or serum IgG of more than 2,000 mg/dl. Hep2 cells were used for nuclear substances when ANA was evaluated. A titer greater than 160 fold dilution of tested sera was judged positive. The autoimmune features associated with CH-C were defined as in AIH other than positive anti-HCV. The international scoring system for the diagnosis of AIH[20] was also used to confirm the diagnosis of AIH. Anti-HCV was determined by using the 2nd generation assay kit (Abbott Laboratories, Abbott Park, IL, USA). AIH patients were all women (aged 42-71 years, mean 56 years). C-AIH patients comprised 6 men, aged 55-65 years, and 12 women, aged 49-69 years (mean 56 years). C-CH patients comprised 19 men, aged 31-67 years and 15 women, aged 42-71 years (mean 53 years). Normal volunteers (N) were 16 men, aged 27-62 years, and 7 women, aged 27-67 years (mean 45 years). Normal volunteers were all negative for serum HBsAg and anti-HCV antibody, and their ALT levels were within a normal range. SLE was diagnosed by the criteria of Tan EM, et al[21]. All SLE sera were collected from the patients before steroid treatment. Serum samples were collected at the day of liver biopsy and stored at -20℃ until use. Liver biopsy was done in all patients other than normal volunteers and patients with SLE under the assistance of ultrasonography or laparoscopy. Informed consent was obtained from all patients upon the approval of the Institutional Committee for Human Subjects prior to serum collection and liver biopsy. Liver specimens were processed for routine light microscopic examination for his-

tological diagnosis. These specimens were stained by silver impregnation, hematoxylin-eosin, Azan-Mallory, periodic acid Schiff (PAS) and diastase-resistant PAS.

The measurement of serum sFas levels

The sFas ELISA kit was based on a sandwich ELISA assay with two different antibodies, both of which recognized two independent extra-cellular domains of Fas molecules, respectively. Briefly, one hundred microliters of two-fold diluted sera were incubated in duplicate in a 96-well microtiter plate which was coated with rabbit polyclonal anti-Fas antibodies at 25℃ for 2 h. After washing 4 times, peroxidase-labeled mouse monoclonal anti-Fas antibodies were added to each well and incubated at 25℃ for 1 h. After rinsing, each well was incubated with *o*-phenylenediamine, a substrate for peroxidase reaction, and the plate was incubated for 20 min. The reaction was then stopped, the optical density at 450 nm was quantified, and the serum sFas concentration was calculated according to a standard curve made by measuring sera that contained known concentrations of sFas molecules.

Isolation and culture of PBMCs

PBMCs were isolated from heparinized whole blood from patients with AIH, C-AIH, C-CH, and normal volunteers by Ficoll gradient centrifugation according to a standardized method. Half of the isolated PBMCs were directly subjected to the subsequent total RNA extraction. The remaining part was cultured in a RPMI1640 medium with 10% human serum (blood type AB) under 5% O_2 and 95% CO_2 at 37℃ for 72 h with 1 μg/ml phytohemagglutinin (PHA, Amersham Pharmacia Biotech, Tokyo, Japan) and 10 U/ml human recombinant IL-2 (Lifetech Oriental, Tokyo, Japan). The stimulated peripheral blood mononuclear cells (PBMCs) were also subjected to the subsequent total RNA extraction.

Total RNA extraction from PBMCs and liver tissues

Non-cancerous liver tissues were obtained at surgery for metastatic liver tumors in 3 colon cancer patients upon full informed consent. Liver biopsy specimens were obtained from 3 C-CH patients for the same purpose. These liver samples were snap-frozen in liquid nitrogen and stored.

Total RNA was extracted from PBMCs and liver specimens by using ISOGEN-LS (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instruction. This instruction manual was based on the procedure first described by Chomczynski and Sacchi[22]. The RNA obtained was quantified by measuring absorbance at 260 nm and stored at -80℃ until use.

RT PCR for Fas and sFas mRNA determination

One microgram of the total RNA was reverse transcribed (RT) with Moloney leukemia virus-reverse transcriptase (LifeTech Oriental) with random hexamers. Reverse-transcribed cDNA was then subjected to PCR amplification with DNA polymerase (Boeringer Mannheim, Tokyo, Japan) and a pair of sense and antisense primers specific for amplifying human Fas. The nucleotide sequence of the sense primer was 5'-CATGGCTTAGAAGTGGAAAT-3' and that of the antisense primer was 5'-ATTTATTGCCACTG-TTTCAGG-3', respectively. A thermo-cycle consisted of initial denaturation at 94℃ for 5 min, 30 thermal cycles including denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, and polymerization at 72℃ for 2 min followed by a final extension at 72℃ for 7 min. Amplified PCR products were separated on a 3.0% agarose gel. The fractionated DNA was stained with ethidium bromide and visualized on a UV trans-illuminator. The expected size of the fractionated DNA for full-length Fas mRNA was 242 base pairs (bp) and its spliced form was 179 bp, respectively.

Terminal deoxyuridine nick end labeling (TUNEL) method

Liver sections obtained from 3 AIH, 3 C-AIH and 3 C-CH patients were used for this purpose. The TUNEL method was performed to demonstrate the nuclear DNA fragmentation *in situ* on formalin-fixed paraffin-embedded liver sections using an ApopTag Plus *in situ* apoptosis detection kit (S7101-Kit, Oncor Ltd., Gaithersburg, MD, USA) according to the manufacturer's instruction. Briefly, sections were incubated with terminal deoxynucleotidyl transferase at 37℃ for 1 h after deparaffinization and the blocking of endogeneous peroxidase activity by 3% hydrogen peroxide in phosphate buffer saline (PBS) at pH 7.2 for 30 min. Following a vigorous washing, the peroxidase-conjugated anti-digoxigenin antibody solution was added onto sections to recognize digoxigenin-nucleotides succeed to fragmented DNAs. Liver sections were then rinsed with PBS, and subjected to color development using 3,3'-diaminobenzidine tetrahydrochloride. Developed sec-

tions were counter-stained by hematoxylin, dehydrated, embedded in Permount, and observed in a light microscope.

Histological examination

Hiistological changes in liver sections were semi-quantified by the hisotological activity index (HAI) score by Knodell et al[23]. Each category of HAI represents an independent histological finding. We used this scoring system with minor modifications. Briefly, category I represents periportal interface hepatitis and bridging necrosis. Category II represents intralobular degeneration and the focal necrosis of liver cells. Category III represents portal inflammation. Category IV represents the extent of fibrosis. Category I is ranked into 7 grades: no interface hepatitis (score 0), mild (score 1), moderate (score 3), marked (score 4), moderate interface hepatitis plus bridging necrosis (score 5), marked interface hepatitis plus bridging necrosis (score 6) and multilobular necrosis (score 10). Each category of II, III and IV is ranked into 4 grades: none (score 0), mild (score 1), moderate (score 3) and marked (score 4). Obtained scores were correlated with serum sFas levels of the patients.

Statistical analysis

The serum sFas level in each disease group was expressed by a mean ± a standard deviation. When serum sFas levels were compared among study groups, one-way ANOVA with Scheffe's modification was employed. A Peason's correlation coefficient was calculated when serum sFas levels were correlated with serum AST, ALT, LDH, ALP levels, γ-gl, white blood cell counts (WBC) and platelet counts. Spearman's correlation test was carried out when serum sFas levels were correlated with each category of HAI scores. P-values of less than 0.05 were considered to be statistically significant.

RESULTS

Mean serum sFas levels in each disease group were as follows: AIH, 710±133 (mean ± standard deviation) ng/ml (min – max; 576–843), C-AIH 752±288 ng/ml (465–1,040), C-CH 514±260 ng/ml (255–774), SLE 555±286 ng/ml (270–841), normal volunteers, 495±176 ng/ml (320–671) (Fig. 1). The mean sFas level in C-AIH was significantly higher than that in C-CH and normal volunteers ($p<0.05$). In contrast, both the mean sFas levels in AIH and SLE before steroid treatment were not statistically different from that in normal volunteers.

When serum sFas levels were correlated with blood biochemical data and peripheral blood cell counts (Table 1), AST ($r=0.266$, $p=0.036$) and ALT ($r=0.310$, $p=0.014$) levels showed a statistically

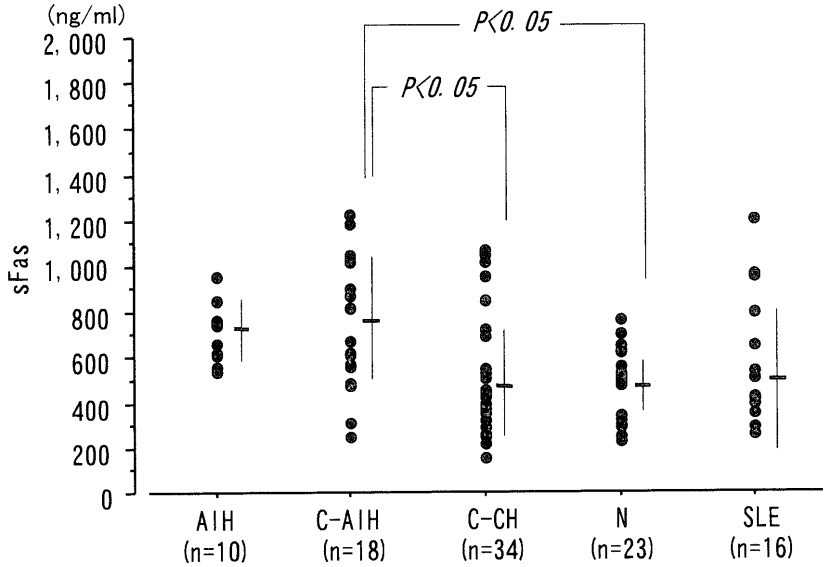

Fig. 1. Serum sFas levels in AIH, C-AIH, C-CH, normal (N) volunteers and SLE patients. The mean serum sFas levels in C-AIH were significantly higher than that of C-CH and normal volunteers (N).

significant positive correlation with serum sFas levels. In addition, peripheral platelet counts had a significant inverse correlation ($r=-0.314$, $p=0.010$) with serum sFas levels. In contrast, serum LDH, ALP, γ-gl, and WBC did not significantly correlate with serum sFas levels. Scattered plots of serum sFas and ALT levels in all examined cases (n=62) revealed a positive correlation between these two parameters (Fig. 2). The same plot of serum sFas and AST levels also revealed a good correlation (data not shown). When the serum sFas and AST/ALT levels were correlated within each disease group (Table 2), a strong correlation (sFas and AST: $p=0.006$, sFas and ALT: $p=0.004$) between these two values was found only in C-CH. In contrast, these two values did not show any significant correlation in the groups that were related to autoimmunity (the AIH, AIH+C-AIH and C-AIH groups, respectively).

Table 1. Correlation between serum sFas levels and blood biochemical parameters and categories of HAI scores in all cases examined (n=62).

	r[a]	p
AST	0.266	0.036
ALT	0.310	0.014
LDH	0.170	0.172
ALP	0.187	0.133
γ-gl	0.132	0.305
WBC	−0.230	0.636
Platelet	−0.314	0.010
HAI[b]	ρ[c]	p
I	0.296	0.019
II	0.231	0.066
III	0.346	0.006
IV	0.191	0.130
I+II+III	0.253	0.044
Total	0.267	0.034

Correlation coefficient, ρ value and corresponding p values are shown. Underlined letters are statistically significant. See text for details. a, A Peason's correlation coefficient; b, Histological activity index by Knodell et al.; c, ρ value in Spearman's rank correlation test.

When serum sFas levels were correlated with HAI scores (Table 1), a significant positive correlation was noted between serum sFas levels and the category I ($\rho=0.296$, $p=0.019$), III ($\rho=0.346$, $p=0.006$), I+II+III ($\rho=0.253$, $p=0.044$) and the total HAI score ($\rho=0.267$, $p=0.034$), respectively. In contrast, the category II and IV had no significant correlation with serum sFas levels.

RT-PCR of the total RNA extracted from PBMC with specific primers for amplifying Fas mRNA generated definite visible bands corresponding to at least two major Fas mRNA species on a 3.0% agarose gel (Fig. 3). A 242 bp band corresponded to the full-length Fas mRNA with TM portions. A shorter 179 bp band corresponded to the spliced form of Fas mRNA lacking the TM domain. Thus, the shorter 179 bp band corresponded to the mRNA that specifically encoded the soluble Fas antigen (sFas) in the sera examined. When PBMCs were not stimulated with

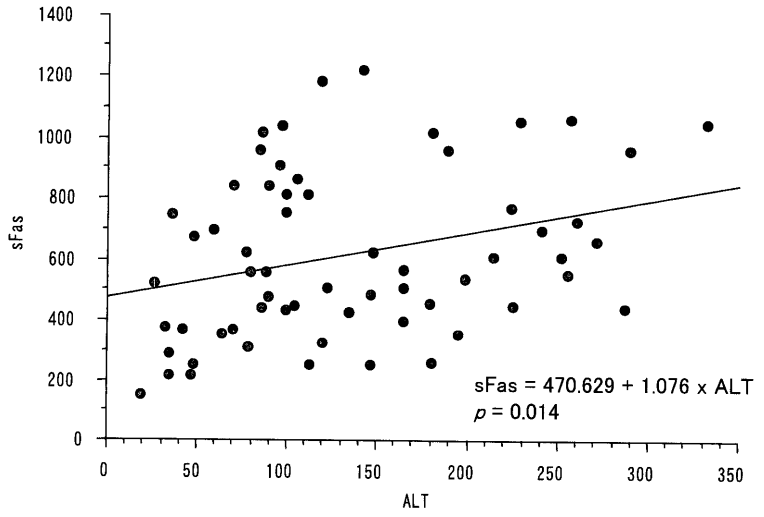

Fig. 2. Scattered plot of serum sFas and ALT levels in all cases examined (n=62).

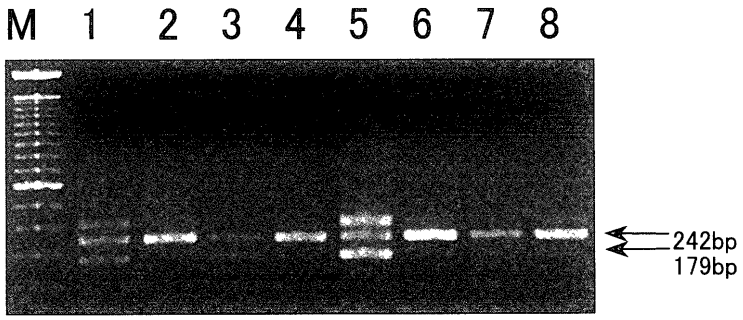

Fig. 3. RT-PCR of Fas and the spliced form of Fas messenger RNAs in PBMC and in liver tissues. M, Molecular weight marker. Lanes 1, 3, and 5, PBMC obtained from a normal control (lane 1), C-CH (lane 3) and AIH (lane 5) patients before the stimulation by IL-2 and PHA, respectively. Lanes 2, 4, and 6: PBMC from a normal control (lane 2), C-CH (lane 4) and AIH (lane 6) patients after 72 h incubation in RPMI-1640 with IL-2 and PHA. Lane 7: Liver tissue from a normal control. Lane 8: Liver tissue from a patient with C-CH. DNA bands of 242 and 179 bp correspond to Fas and sFas mRNA, respectively. See text for details.

PHA and IL-2, both the full and spliced forms of Fas mRNA were expressed in all disease groups, although band intensities of both Fas and sFas mRNA were apparently higher in AIH when compared with those of normal volunteers and C-CH as shown in Fig. 3. After the stimulation of PBMCs by PHA and IL-2, band intensities of the full-length Fas mRNA became exclusively higher than that of spliced Fas mRNA in all examined cases. RT-PCR of the total RNA extracted from non-cancerous liver tissues of metastatic liver tumors and those from C-CH also revealed full and spliced forms of Fas mRNA as shown in PBMCs. The band intensity of the full-length Fas mRNA in C-CH liver tissues was higher than that of non-cancerous liver tissues of metastatic liver tumors as exemplified by lanes 7 and 8 in Fig. 3.

The TUNEL method clearly demonstrated the presence of lymphocytes that had nuclear DNA fragmentation both in inflamed portal triads and hepatic lobules in all disease groups. These TUNEL-positive lymphocytes were easily discernible by definite dark brown deposits corresponding to nuclear DNA fragmentation. Most of these TUNEL-positive lymphocytes showed picnotic nuclei, revealing that these cells had undergone apoptosis. These cells were scattered among numerous mononuclear cells infiltrating inflamed portal triads (Fig. 4). In hepatic lobules, TUNEL-positive lymphocytes had migrated to hepatic sinusoids. The distribution, number, and morphological alterations of TUNEL-positive lymphocytes were essentially equally observed among all disease groups.

Table 2. The relationship between serum sFas and AST/ALT levels in disease groups

Disease subgroups	No. of cases	sFas and AST		sFas and ALT	
		r	p	r	p
Total cases	62	0.266	0.036	0.310	0.014
AIH	10	0.214	0.566	0.062	0.870
AIH+C-AIH	28	0.006	0.976	0.094	0.639
C-AIH	18	0.021	0.934	0.164	0.520
C-CH	34	0.461	0.006	0.473	0.004

Underlined data are statistically significant.

DISCUSSION

The significance and usefulness of the serum sFas level measurement in liver diseases have long been debated without a consensus. Arguments were made for the fact that serum sFas levels are elevated in chronic liver diseases and thus might predict outcome of the disease[16–18]. Serum sFas levels are elevated in chronic hepatitis C and so correlated with the degree of inflammation[16], and serum sFas levels are elevated in liver cirrhosis C[17]. Since serum sFas levels are elevated in hepatocellular carcinoma[18] and in other malignant solid tumors, they could possibly serve as a seromarker of activity and development of the disease[11,16–18,24,25].

In the research of organ disorders other than the liver, there has been a growing body of evidence that

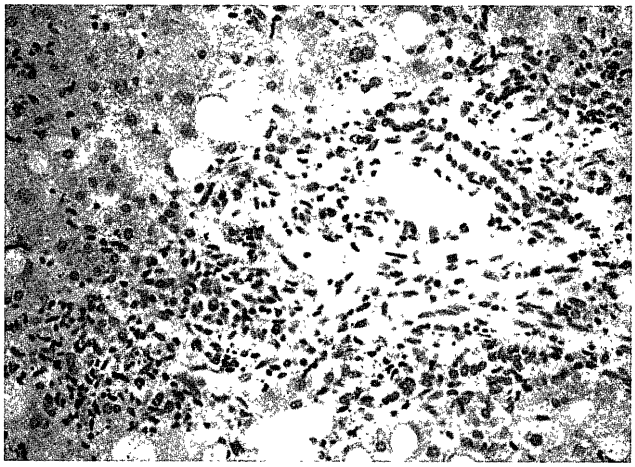

Fig. 4. TUNEL staining of liver specimens obtained from a patient with chronic hepatitis C. TUNEL-positive cells were scattered among numerous mononuclear cells present in inflamed portal triads. Original magnification, ×25.

the measurement of serum sFas levels well reflects disease activity. This is especially so in those disorders caused by autoimmunity. Since the serum sFas is known to be secreted by activated lymphocytes that abundantly infiltrate the local tissue area of inflammation, it may reflect the depth of lymphocyte activation[14]. In systemic autoimmune disorders typically exemplified by SLE, serum sFas levels can be significantly high, presumably reflecting the activity of autoimmune processes[26].

The phenomenon called activation-induced cell death (AICD) is considered to be an elimination system of activated lymphocytes for avoiding the catastrophe of a host due to uncontrolled over-inflammation[27-32]. Since the impairment in apoptosis of auto-reactive lymphocytes is supposed to be a possible pathogenesis of autoimmune disorders, the high serum sFas levels may, at least in part, play a role in this impairment by preventing lymphocyte apoptosis through its binding activity to Fas ligands.

However, the currently available body of data on the expression of Fas and its spliced variants in PBMC of CH-C is limited. This study has first disclosed a new finding that serum sFas levels were significantly higher in C-AIH than those in C-CH and normal volunteers. This fact may raise the possibility that activated auto-reactive lymphocytes are more involved in the autoimmune features accompanying C-CH than AIH, the most representative disorder induced by autoimmunity in the field of liver diseases. The elevated serum sFas may suppress the apoptosis of auto-reactive lymphocytes and may play a role in inducing the autoimmune features observed in C-AIH. Hepatocytes are also known to express Fas molecules on their cell surface. The expressed Fas molecule mediates hepatocyte apoptosis through its binding with Fas ligands present on the cell surface of activated cytotoxic T lymphocytes[2-4]. Since the hepatocyte is another candidate for the production of serum sFas molecules[17,33], the sFas produced not only by activated T lymphocytes but also by hepatocytes may contribute to the elevated serum sFas levels. The apparent positive correlation between serum sFas and AST/ALT levels as a whole greatly rests on the C-CH group without autoimmune features since the striking correlation was only evident in this group, as shown in Table 2. The reason why the AIH, AIH+C-AIH and C-AIH groups did not show any meaningful correlation between serum sFas and AST/ALT levels may be explained by the autoimmune features that accompany these disease groups. In the C-CH group, the serum sFas level may simply reflect the extent of hepatocyte apoptosis that is represented by AST/ALT levels in serum. In other groups that are accompanied by autoimmune features, however, serum sFas levels may reflect not only the extent of hepatocyte but also lymphocyte apoptosis. A survey of Fas and sFas expression in activated PBMC is needed to demonstrate this possibility.

AIH and SLE, both of which have an autoimmune nature, failed to show a significant increase in serum sFas levels in our study. In turn, C-AIH revealed significantly elevated serum sFas levels when compared with those in C-CH and normal volunteers. It may be interesting to consider that this elevation is

characteristic and specific to the autoimmune features that could accompany CH-C since it might give clues to elucidate the mechanism of this virus-associated autoimmunity.

Another interesting finding in our study is a significant inverse correlation between serum sFas levels and blood platelet counts. Since blood platelet counts inversely represent a degree of chronicity and cirrhotic change of liver diseases[34] in general, the serum sFas levels may be an inverse seromarker of this.

Serum sFas levels may also be influenced by sex and age, since males rather than females, and elder individuals rather than younger ones show higher sFas levels[35]. In this context, the serum sFas levels in SLE patients in our study might be underestimated since most SLE patients in our study were young women.

The serum sFas levels were reported to correlate with categories I and II of the HAI scores in CH-C[17]. In our study, however, serum sFas levels in CH-C were significantly correlated with the categories I, III, I+II+III and the total score of HAI scores. Among these, the correlation between sFas levels and category III was the strongest. Category III is known to reflect the degree of inflammatory cell infiltration in portal triads rather than the degree of lobular necroinflammation that is reflected by category II. This finding suggests that serum sFas may be involved in the apoptosis not only of hepatocytes but also activated lymphocytes, since lymphocytes infiltrating inflamed portal triads both express and secrete it. Our previous report pointed out a significant correlation between the enhanced expression of IL-6 and category II in CH-C[36], offering an intriguing hypothesis that different cytokines and serum factors may reflect or influence the degree of necroinflammatory changes in different areas of the liver.

The expressions of the full-length and spliced form of Fas mRNAs were enhanced in the unstimulated PBMC of AIH. The enhanced expression of the full-length Fas mRNA suggests the presence of ongoing PBMC apoptosis in AIH. This ongoing apoptosis of AIH lymphocytes may reflect AICD of auto-reactive activated lymphocytes in this disorder. In contrast, the simultaneously enhanced expression of spliced Fas mRNA may in turn suppress the apoptosis of auto-reactive activated lymphocytes by producing greater amounts of sFas molecules in serum, although the accelerated consumption and/or more rapid turnover of serum sFas in AIH may mask an actual elevation of the serum sFas level as shown in this study.

An enhanced expression of the full-length Fas mRNA in the liver tissue of CH-C when compared with that in the normal liver may also reflect the ongoing apoptosis of both hepatocytes and activated lymphocytes in the CH-C liver.

The TUNEL method actually disclosed the presence of lymphocytes showing nuclear DNA fragmentation in hepatic sinusoids and in inflamed portal triads of diseased livers. This finding is direct evidence of activated lymphocyte apoptosis taking place in liver parenchyma and inflamed portal triads of the liver. We previously reported that activated polymorphonuclear leukocytes (PMNs) infiltrating the liver parenchyma of alcoholic hepatitis showed an augmented nuclear DNA fragmentation, indicating that, as in activated lymphocytes, an AICD-like phenomenon is also present in PMNs[37]. The apoptosis of tissue-infiltrating cells functions as an eliminating system for activated cells. This system is exclusively necessary for avoiding a catastrophe of the host evoked by uncontrolled over-inflammation. In this context, the downregulation of sFas coupled with the upregulation of full-length Fas observed in stimulated lymphocytes as shown in our study may show the existence of a system like AICD for eliminating and controlling activated lymphocytes in CH-C. Failure to demonstrate a close correlation between serum sFas levels and the expression of sFas mRNA in PBMC in C-AIH may be due to the paucity of C-AIH cases. Toubi et al. revealed in their recent study that peripheral T-cell apoptosis was enhanced in CH-C and was associated with liver disease severity and autoimmunity[38]. They simultaneously demonstrated that a decreased expression of nuclear factor kappa B was important in the development of this apoptosis. More recently, Ohkawa et al. revealed that high production levels of soluble Fas antigen might be associated with a poor response to interferon therapy in CH-C patients[39]. These results are all in accordance with ours in that enhanced lymphocyte apoptosis and elevated serum soluble Fas levels are more or less related to the disease activity and severity.

It has recently been demonstrated that the liver is a site of lymphocyte apoptosis in the entire body[40–46]. Activated $CD8^+$ lymphocytes that are accumulated or recruited from the body undergo apoptosis in the liver, much like a graveyard (graveyard hypothesis). These new findings -- mainly obtained in transgenic mice or transplantation experiments -- may also support our data concerning the apoptosis of lymphocytes in the liver. A more detailed description of the mechanism of lymphocyte apoptosis in liver that may occur in various disease conditions includ-

ing CH-C must be further verified in future studies.

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