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Plasma Fas Ligand, an Inducer of Apoptosis, and Plasma Soluble Fas, an Inhibitor of Apoptosis, in Patients With Chronic Congestive Heart Failure

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Objectives. **This study sought to examine plasma levels of soluble Fas/APO-1 receptor (sFas), an inhibitor of apoptosis, and soluble Fas ligand (sFas-L), an inducer of apoptosis, and their relation to each other and to other clinical variables, such as New York Heart Association functional class, tumor necrosis factor (TNF) and interleukin-6 (IL-6) in congestive heart failure (CHF).**

Background. **It has been recently reported that apoptotic cell death occurs in myocytes of dogs with CHF. Hypoxia is frequently seen in advanced CHF and can stimulate Fas/APO-1 receptors (Fas) to induce apoptosis in cultured myocytes. Fas and Fas ligand (Fas-L) are cell-surface proteins and representative apoptosis-signaling molecules. Fas on the cell membrane induces apoptosis when it binds Fas-L or sFas-L. However, plasma sFas, a molecule lacking the transmembrane domain of Fas, blocks apoptosis by inhibiting binding between Fas and Fas-L or sFas-L on the cell membrane. At present, it is unknown whether plasma sFas-L and plasma sFas increase in the presence of cardiac disease.**

Methods. **The study included 70 patients (mean [±SEM] age 65 ± 2 years, range 21 to 93) with chronic CHF (coronary artery disease in 28, dilated cardiomyopathy in 27, valvular heart disease in 15) and 62 age- and gender-matched normal control subjects. Plasma levels of sFas, sFas-L, TNF-alpha and IL-6 were measured by enzyme-linked immunosorbent assays using monoclonal anti-human antibodies.**

Results. **There was no significant difference in sFas-L levels between normal subjects and patients in functional classes I to IV; however, sFas increased with severity of functional classification, independent of the underlying disease. sFas levels were significantly higher even in patients in functional class II than in normal subjects and those in functional class I, and were highest in patients in functional class IV (normal subjects: 2.2 ± 0.1 ng/ml; functional class I: 2.2 ± 0.2 ng/ml; functional class II: 3.1 ± 0.2 ng/ml; functional class III: 3.9 ± 0.3 ng/ml; functional class IV: 5.1 ± 0.6 ng/ml). Plasma sFas levels were significantly higher in patients with elevated pulmonary artery wedge pressure and a decreased cardiac index than in those with values in the normal range. In patients in functional class IV, there was no significant difference in plasma sFas levels between the survivors and nonsurvivors during 6-month follow-up. However, plasma levels of sFas tended to decrease in nine patients with clinical improvement (baseline sFas: 5.2 ± 0.8 ng/ml; 6-month sFas: 4.3 ± 0.5 ng/ml, p = 0.07) but were similar in patients with no change in functional class. TNF-alpha and IL-6 were increased significantly only in patients in functional class IV, as previously reported, but were not related to sFas.**

Conclusions. **We found elevated levels of plasma sFas and no increase in plasma sFas-L in human CHF. The increase in sFas may play an important role in the pathophysiologic mechanisms of CHF.**

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Apoptosis, or programmed cell death, was recognized by Kerr (1) as a distinctive morphologic entity that he called "shrinkage necrosis." Kerr et al. (2) then provided evidence of apoptosis as a basic biologic phenomenon with wide-ranging implications in tissue kinetics. Apoptosis differs from necrosis in terms of ultrastructural and biochemical features, which include cytoplasmic and nuclear condensation, subsequent formation of membrane-bound apoptotic bodies (segmentation of the nucleus) and extensive degeneration of chromosomal DNA into oligomers of ~180 base pairs caused by activation of endogenous endonuclease (3–6). Apoptosis is not characteristically associated with inflammatory cell infiltration, unlike necrosis (7). It has been reported (8–10) that apoptosis in myocytes is induced by postnatal morphogenesis, especially in the conduction system, and by hypoxia and reperfusion after ischemia. Recently, Sharov et al. (11) found morphologic and histologic

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Abbreviations and Acronyms

CHF = congestive heart failure
ELISA = enzyme-linked immunosorbent assay
Fas = Fas/APO-1 receptor
Fas-L = Fas ligand
Ig = immunoglobulin
IL-6 = interleukin-6
PBS = phosphate-buffered saline
sFas = soluble Fas
sFas-L = soluble Fas ligand
TNF = tumor necrosis factor

evidence of myocyte apoptosis in the left ventricular myocardium of dogs with congestive heart failure (CHF). Hypoxia, which can occur in advanced CHF, can induce apoptosis in cultured myocytes and liver cells (9,12,13). However, the relation between apoptosis and CHF is still unclear in humans.

At present, there are many inducers and inhibitors of apoptosis. The inducers of apoptosis, such as tumor necrosis factor (TNF)-alpha, a TNF family, and interleukin 6 (IL-6), a cytokine-inducing transcription of the Fas/APO-1 receptor (Fas) gene, are increased in the plasma of patients with severe CHF (14–17). We recently reported (12) that Bax, an inducer of apoptosis, was expressed in the myocytes of autopsied human hearts with an old myocardial infarction and in most of which CHF was present (12). The Fas–Fas ligand (Fas-L) system is the representative system of apoptosis-signaling receptor molecules. Fas (CD95) is a cell surface protein consisting of 319 amino acids with a single transmembrane domain, belonging to the nerve growth factor receptor/TNF receptor family (type I membrane protein) (18–22). Molecular cloning and nucleotide sequence analysis have revealed a human Fas messenger RNA variant that encodes a soluble Fas (sFas) molecule lacking the transmembrane domain because of the deletion of an exon encoding this region (23). sFas (soluble form of Fas) is found in the culture supernatants of different human B- and T-cell lines (24). Fas-L is a type II membrane protein that belongs to the TNF family (22,25,26). Membrane-bound human Fas-L is converted to a soluble form by a matrix metalloproteinase-like enzyme (27,28). Human soluble Fas ligand (sFas-L), a 26-kDa glycoprotein, consists of the extracellular region of the Fas-L. Fas-L is predominantly expressed in activated T cells, whereas Fas is expressed in various tissues, including the thymus, heart, liver and ovary but not in the brain or spleen in mice (29,30). Binding of Fas-L, sFas-L or agonistic anti-Fas antibodies to Fas induces apoptosis (26,29,30). However, extrinsic sFas blocks apoptosis in cells by inhibiting the binding of the Fas-L or sFas-L to Fas on the cell membrane (23,31). Recently, it has been reported that sFas is elevated in the serum of patients with B- and T-cell leukemias and systemic lupus erythematosus (23,24), as are sFas-L levels in patients with large granular lymphocytic leukemia and natural killer cell lymphoma (31). A recent report (9) showed that Fas messenger RNA is induced by hypoxia in cultured neonatal rat myocytes. However, whether plasma levels of sFas and sFas-L are elevated in patients with CHF remains unknown.

The present study was designed to assess 1) whether the plasma concentrations of sFas, an inhibitor of apoptosis, and sFas-L, an inducer of apoptosis, increase with New York Heart Association functional class in patients with CHF; and 2) whether they are related to other clinical variables such as hemodynamic data. Plasma levels of TNF-alpha and IL-6, which are elevated in patients with severe CHF and are Fas–Fas-L system-related factors (13–16), were also measured and compared with plasma levels of sFas-L and sFas.

Methods

Subjects. Seventy patients (mean [±SEM] 65 ± 2 years, range 21 to 93) with chronic CHF (coronary artery disease in 36, dilated cardiomyopathy in 24, valvular heart disease in 10) were diagnosed by physical examination, echocardiography and cardiac catheterization (Table 1). Patients with significant concomitant diseases, such as pulmonary disease, malignancy, autoimmune disorders, neurodegenerative disorders, diabetes mellitus, thyroid disease or concurrent viral disease, were excluded from the study. Patients were grouped according to their functional class, based on their clinical characteristics (Table 1). Most patients in functional classes II to IV had received cardiac glycosides, diuretic drugs or coronary vasodilating agents. All serum creatinine and aminotransferases levels were within the normal range. Sixty-two age- and gender-matched subjects with no disease and no medication were included as a control group (Table 1).

All subjects studied here gave their informed consent to the study, which was approved by the local ethics committee on human research (Gifu University).

Blood sampling. Peripheral venous blood samples were taken slowly from an antecubital vein and were transferred to chilled disposable tubes containing 2-natrium-ethylenediamine tetraacetic acid (1.5 mg/ml). Then, the disposable tubes were promptly centrifuged at 4°C, and aliquots of plasma were immediately stored at −80°C until analyzed.

In eight patients with CHF, a second plasma sample to measure the reproducibilities of plasma sFas and sFas-L levels was taken 1 day after the first sample. All patients were reevaluated for the severity of CHF 6 months after the first plasma sample was taken, and plasma sFas levels were remeasured.

To determine the main site of sFas expression, blood samples were taken from the pulmonary artery, coronary sinus, superior vena cava, right or left renal vein and femoral artery, in 8 patients in functional class II or III, using a 7F Swan-Ganz catheter and a 6F Judkins-type catheter for the right coronary artery.

sFas and sFas-L assay by enzyme-linked immunosorbent assay. We have developed and characterized a highly sensitive enzyme-linked immunosorbent assay (ELISA) for plasma sFas (32). Polystyrene plates were precoated with rabbit immunoglobulin (IgG) for anti-human Fas synthetic peptide (amino

Table 1. Clinical Characteristics, Plasma Concentrations of sFas, sFas-L and Cytokines and Hemodynamic Data

	Control Group (n = 62)	NYHA Functional Class			
		I (n = 20)	II (n = 20)	III (n = 15)	IV (n = 15)
Age (yrs)	62 ± 5	63 ± 4	66 ± 2	64 ± 4	66 ± 5
M/F	43/19	14/6	13/7	10/5	11/4
Cause of CHF					
DCM	—	6	10	6	6
CAD	—	10	6	5	6
VHD	—	4	4	4	3
Plasma conc					
sFas (ng/ml)	2.2 ± 0.1	2.2 ± 0.2	3.1 ± 0.2*†	3.9 ± 0.3*†‡	5.1 ± 0.6*†‡§
sFas-L (ng/ml)	0.43 ± 0.01	0.44 ± 0.01	0.49 ± 0.02	0.44 ± 0.02	0.48 ± 0.02
TNF-alpha (pg/ml)	4.2 ± 0.6	5.9 ± 2.0	10.0 ± 3.3	11.3 ± 5.5	121.0 ± 28.3*†‡§
IL-6 (pg/ml)	2.5 ± 0.2	4.1 ± 0.8	4.0 ± 1.0	10.0 ± 3.6	53.9 ± 19.3*†‡§
Hemodynamic data					
PAW (mm Hg)	—	10 ± 1	11 ± 1	20 ± 2†‡	25 ± 2†‡
CI (liters/min/per m^2)	—	3.1 ± 0.1	3.0 ± 0.2	2.3 ± 0.1†‡	2.0 ± 0.1†‡

*$p < 0.05$ versus Control Group. †$p < 0.05$ versus New York Heart Association (NYHA) functional class I. ‡$p < 0.05$ versus functional class II. §$p < 0.05$ versus functional class III. Data presented are mean value ± SEM or number of patients. CAD = coronary artery disease; CHF = congestive heart failure; CI = cardiac index; conc = concentration; DCM = dilated cardiomyopathy; F = female; IL-6 = interleukin-6; M = male; PAW = pulmonary artery wedge pressure; sFas = soluble Fas; sFas-L = soluble Fas ligand; TNF = tumor necrosis factor; VHD = valvular heart disease.

acids 305 to 319), and each well was blocked with 1% bovine serum albumin/phosphate-buffered saline (PBS). Plasma was diluted to a ratio of 1:5 in PBS containing 0% normal rabbit serum, and 100 μl of the diluted sample was applied to each well. The wells were incubated with mild shaking (200 rpm) at room temperature for 1 h. After the incubation, the wells were washed with PBS containing 0.05% Tween 20, and 100 μl of horseradish peroxidase conjugated anti-human Fas mouse monoclonal antibodies developed by Yonehara et al. (18) was added and incubated with shaking at room temperature for 1 h. The wells were then washed with PBS containing 0.05% Tween 20 and incubated for 30 min with 100 μl of freshly prepared tetramethylbenzidine (1.0 mmol/l) in 10 mmol/l citric acid buffer, pH 5.5, containing hydrogen peroxide (3.5 mmol/l) in each well. The reaction was terminated by the addition of 100 μl of 6 mol/liter phosphate. The absorbance was read at 450 nm using a microplate reader (SLT-210, Labinstruments, Glodig, Austria). The results were calculated from the best-fitting least-square parabola with an on-line microcomputer (NEC 9801-VM2, NEC Co., Tokyo). All samples were assayed in duplicate. The linear part of the calibration range was from 0.06 to 2.00 ng/ml, and the detection limit was 0.01 ng/ml. The average within- and between-run coefficients of variation were 3.4% and 5.7%, respectively. Recovery of the sFas added to the serum ranged between 93% and 118%.

Plasma levels of the sFas-L were also quantified by a sandwich ELISA using NOK-1 (mouse IgG1, k) and NOK-3 (mouse IgM, k), monoclonal antibodies purified from mice immunized through an intraperitoneal injection of human Fas-L (L5178Y, mouse T lymphoma cell lines, kindly provided by Dr. S. Yonehara, Kyoto University, Kyoto, Japan) (27). Briefly, Immulon-2 plates (Dynatech Lab Inc.), were incubated overnight with NOK-3 (10 μg/ml). After washing with 0.05% Tween/PBS, the wells were blocked with a skim milk solution (Block Ace, Snow Brand Milk Co., Sapporo, Japan) for 2 h at 37°C; 50 μl of the sample was added and incubated for 1 h at room temperature; biotinylayed NOK-1 (5 μg/ml) containing 10% murine serum was then added and incubated for another hour at room temperature; and, finally, 50 μl of ABC solution (Vectastain ABC kit, Vector Lab. Inc.), was added and incubated for 30 min at room temperature. The wells were developed with 100 μl of 1 mg/ml of orthophenylenediamine in 50 mmol/liter citrate-phosphate buffer (pH 5.0) containing 0.03% H_2O_2 and stopped by the addition of 100 μl of 2N H_2SO_4. The optical density at 490 nm was measured on a microplate reader. Serial dilutions of purified sFas-L that had been affinity purified from the human Fas-L/L5178Y supernatant in a NOK-1 column were used as the standard. The detection limit of this assay was 0.2 ng/ml. We examined whether measuring soluble Fas ligand was influenced by sFas. We respectively incubated 1 or 10 ng/ml of human recombinant sFas-L with 10-fold doses (10 or 100 ng/ml) of human recombinant sFas at 37°C for 2 h. The result showed that the levels of sFas-L were not influenced by sFas. Therefore, increased levels of sFas do not mask an increase in sFas-L by interfering with the assay.

The differences in sFas and sFas-L between the two assays using plasma taken at the same time were 3.4 ± 3.9% and 4.9 ± 2.9%, respectively, and those between paired samples taken at a 1-day interval were 5.0 ± 2.2% and 4.3 ± 2.0%, respectively. These data were satisfactory and reliable.

Measurements of plasma TNF-alpha and IL-6 levels. Plasma levels of TNF-alpha were measured using a sandwich ELISA kit with two monoclonal anti-human TNF-alpha anti-

bodies (No. 1121, Immunotech, Marseille, France). The plasma levels reported for healthy subjects were <5 pg/ml (33). The sensitivity limit of the assay in the plasma was 5 pg/ml. No cross-reactivity or interference with either TNF-beta or the p55 and p75 forms of the TNF receptor was observed. Intraassay and Interassay coefficients of variation were 7.0% and 6.8%, respectively.

Plasma levels of IL-6 were also measured using a two-step sandwich ELISA kit with two monoclonal anti-human IL-6 antibodies and recombinant IL-6 standards (Human IL-6 ELISA Kit Fujirebio, Fujirebio Inc., Tokyo, Japan), as reported by Yamagishi et al. (34). The sensitivity limit of the assay was 3 pg/ml, and its analytic range was from 3 to 200 pg/ml. Its precision, reproducibility and sensitivity were satisfactory (34). Reproducibility with the same plasma was 1.3%; plasma IL-6 levels from 200 normal subjects were all <3 pg/ml.

Right-sided cardiac catheterization. Right-sided cardiac catheterization using a 7F Swan-Ganz thermodilution catheter (Baxter Healthcare Corporation) was performed in all patients for measurement of pulmonary artery wedge pressure and cardiac output within 3 days before or after blood sampling, according to standard techniques. Cardiac index was calculated as the ratio of cardiac output to body surface area.

Statistical analysis. Plasma levels of sFas, sFas-L, TNF and IL-6 and hemodynamic variables were compared among patient groups and normal subjects by one-way analysis of variance (Bonferroni/Dunn), and if the difference was significant, a modified unpaired *t* test was used to assess which group was significantly different. Correlations between plasma levels of sFas or sFas-L and cytokines or hemodynamic variables were examined using simple linear regression analysis by the least-squares method, and differences between regression lines were tested by analysis of covariance using sFas or sFas-L as a dependent variable and either a hemodynamic or echocardiographic variable as an independent variable. Results are expressed as mean value ± SEM; $p < 0.05$ was regarded as significant.

Results

Characteristics of patients and hemodynamic data. Table 1 shows the clinical characteristics and hemodynamic data for the four groups of patients and the normal subjects. There were no significant differences in age or gender ratio among the five groups. All patient groups were similar with respect to cause of heart failure (Table 1). Pulmonary artery wedge pressure was not significantly different between functional classes I and II but was significantly higher in functional classes III and IV than in functional classes I and II. Cardiac index decreased with increasing severity of CHF and was significantly lower in functional classes III and IV than in functional classes I and II (Table 1).

Plasma levels of sFas and sFas-L. There were no significant differences in plasma levels of sFas-L between any of the patient groups and normal subjects (Table 1). However, sFas increased levels significantly with severity of CHF (Fig. 1).

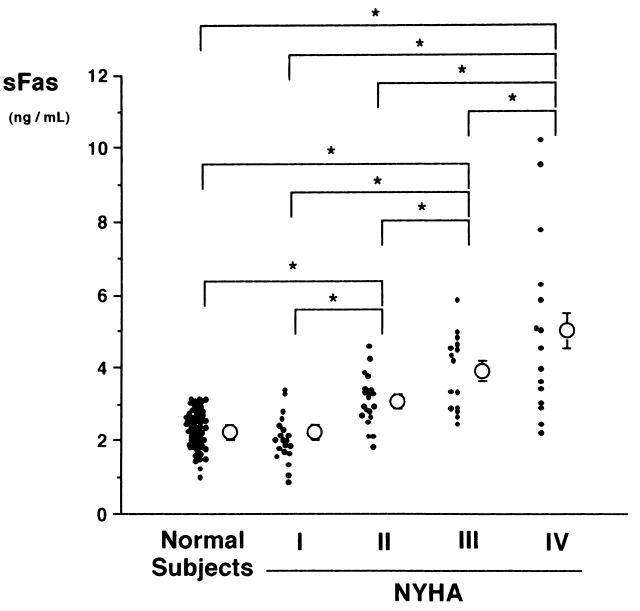

Figure 1. Plasma soluble Fas (sFas) levels in patients according to New York Heart Association (NYHA) functional class. Note that normal subjects and patients in functional classes I had similar plasma levels of sFas, but significant differences are seen between patients in functional classes II to and IV. **Open circles** = mean value ± SEM. *$p < 0.05$.

Plasma levels of sFas were similar in normal subjects and patients in functional classes I, but there were significant differences between functional classes II, III and IV. Plasma sFas levels in patients with CHF relative to those in normal subjects were 1.0 in functional classes I, 1.4 in functional classes II, 1.8 in functional classes III and 2.3 in functional classes IV. The underlying cardiac disease in patients with CHF was coronary heart disease, dilated cardiomyopathy or valvular heart disease (Table 1). There was no significant difference in plasma sFas levels among patients in functional classes I to III and even among those in functional classes IV. Plasma sFas levels were significantly higher in patients with an elevated pulmonary artery wedge pressure (>18 mm Hg) and a decreased cardiac index (<2.2 liters/min per m^2 body surface area) than in those with values in the normal ranges (Fig. 2).

All patients were reevaluated for functional classes and plasma sFas levels 6 months after the first plasma sample was taken, and their prognosis (survival or death) was evaluated. Nine of 70 patients with CHF (1 of 15 in functional classes III; 8 of 15 in functional classes IV) died. Of patients in functional classes III and IV, the functional classes improved in nine (four in functional classes III, five in functional classes IV), worsened in one in functional classes III and had not changed in the other three (one in functional classes III, two in functional classes IV). In patients in functional classes IV, there was no significant difference in plasma sFas levels between the survivors (5.7 ± 0.9 ng/ml) and those who died (4.5 ± 0.9 ng/ml, p = 0.38). However, plasma levels of sFas tended to decrease in patients with clinical improvement (baseline 5.2 ± 0.8 ng/ml,

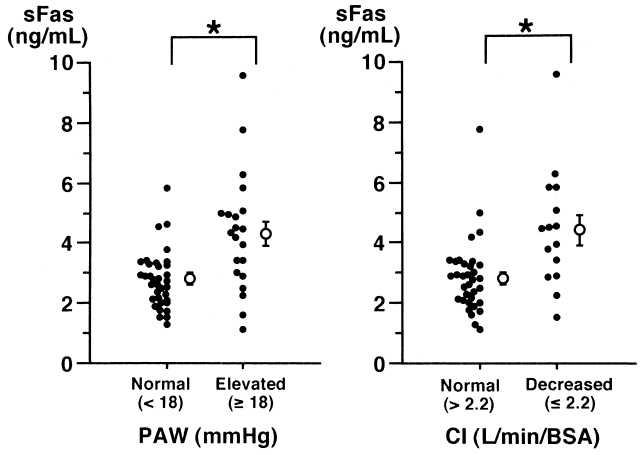

Figure 2. Comparison of plasma soluble Fas (sFas) levels between patients with and without elevated pulmonary artery wedge pressure (PAW) (>18 mm Hg) and those with and without a decreased cardiac index (CI) (<2.2 liters/min/per m^2 body surface area). **Open circles** = mean value ± SEM. *$p < 0.05$ versus normal subjects.

6-month follow-up 4.3 ± 0.5 ng/ml, $p = 0.07$) but were similar in those who had no change in functional classes (baseline 4.3 ± 0.5 ng/ml, 6-month follow-up 4.7 ± 0.3 ng/ml).

To determine the main site of sFas expression, blood samples were taken from the pulmonary artery, coronary sinus, superior vena cava, right or left renal vein and femoral artery in eight patients in functional classes II or III. There were no significant differences in sFas levels among the different sites: 3.6 ± 0.4 ng/ml for the pulmonary artery; 3.7 ± 0.3 ng/ml for the coronary sinus; 3.3 ± 0.5 ng/ml for the superior vena cava; 3.7 ± 0.4 ng/ml for the right or left renal vein; and 3.4 ± 0.5 ng/ml for the femoral artery.

Plasma levels of TNF-alpha and IL-6. Plasma levels of both TNF-alpha and IL-6 increased significantly only in patients in functional classes IV. There was no correlation between plasma levels of sFas and TNF-alpha or IL-6 (Table 1).

Discussion

The present study revealed that in patients with chronic CHF, plasma sFas-L levels did not increase, but plasma sFas levels increased relative to the severity of functional class.

Measurement of plasma sFas-L and sFas levels. The plasma level of sFas-L was 0.43 ± 0.01 ng/ml in normal subjects. There was no evidence of an increase in sFas-L in patients with CHF in this study.

Using the ELISA method we developed, the sFas level in the plasma of 155 healthy subjects was 2.3 ± 0.1 ng/ml; it was higher in men than in women and increased according to age (32). The precision, recovery and linearity were all excellent. The assay was not affected by possible interfering substances. In this study, the sFas level in the plasma was also 2.2 ± 0.1 ng/ml in 62 normal subjects. The variation between results from the same samples and between samples taken 1 day later were also very small, and the data were reliable. However, the sFas levels in the plasma were much lower than those previously reported by Cheng et al. (23) (<30 ng/ml), although the rate of elevation in CHF (approximately twofold) was similar to that in patients with systemic lupus erythematosus and B- and T-cell leukemias and lymphoma, which they reported (23,24). The reason for this difference is unknown at present. However, some possible explanations are 1) because they also used an ELISA system, there seems to be no major differences between the assays. Therefore, differences in the standard and the specificity of antibodies against sFas are likely, but they are not described in detail. 2) There may be population differences, but this is not likely because the difference between their results and ours are more than 10-fold. Their study was also a small study (n = 10), and they did not mention gender and age differences in their healthy subjects.

Plasma sFas and CHF. In the present study, plasma sFas increased significantly, even in patients in functional classes II, and was highest in those in functional classes IV. Plasma sFas also increased significantly in patients with elevated pulmonary artery wedge pressure and a depressed cardiac index but tended to decrease in patients with an improvement in functional classes ($p = 0.07$) and remained the same in patients with no change in functional class. Plasma sFas levels were similar between survivors and nonsurvivors. A significant elevation of plasma sFas levels >2 SD of that in normal subjects was seen in 20% of patients in functional classes II, 53% of those in functional classes III and 67% of those in functional classes IV. Plasma sFas levels varied considerably from those in the normal range to very high levels, even in patients in functional classes IV. There was no significant difference in plasma sFas levels with regard to underlying cardiac disease (coronary artery disease, dilated cardiomyopathy, valvular heart disease).

Thus, the increase in plasma sFas occurs independently of underlying cardiac disease but reflects the severity of CHF despite the presence of limitations. Therefore, the increase in plasma sFas may be related to the pathophysiologic mechanisms of CHF, at least, in patients in whom plasma sFas levels are elevated.

Increased plasma sFas and apoptosis in CHF. In CHF, the heart cannot pump blood at a rate commensurate with the requirements of the metabolizing tissue. Therefore, relative hypoxia or ischemia, congestion, hypertrophy, cell death and fibrosis occur in various organs, such as the heart, lungs, liver and spleen. Progressive deterioration of left ventricular function is also a characteristic feature of CHF. This progressive deterioration occurs despite the absence of intercurrent adverse clinical events. Recent studies using canine heart failure models and human dilated cardiomyopathy with advanced CHF showed that ongoing myocyte loss due to apoptosis is important in the pathogenesis (11,35). Although the factors that may trigger apoptosis in the failed myocardium are unclear, increased cytosolic calcium concentration, formation of oxygen free radicals, temporary ischemia and hypoxia may all trigger apoptosis (9,11,12,36). In patients with CHF, abnormalities of various apoptosis-related factors have been re-

ported. Levels of cytokines, such as plasma TNF-alpha, IL-1 and IL-6, all inducers of apoptosis, are elevated (14–17). The cytolytic activity of TNF is mediated by Fas in animal models (36).

In the present study, we found an elevation of approximately twice that of the normal range in plasma sFas, an inhibitor of apoptosis, in patients with advanced CHF. As Cheng et al. (23) described, sFas injected at approximately twice the control level in mice is active in vivo and inhibits cell apoptosis; the increased plasma sFas in CHF may have an inhibiting effect against apoptosis. However, plasma sFas-L, an inducer of apoptosis, was not increased in the present study. To date, there have been no reports of overexpression of Fas and Fas-L, inducers of apoptosis, in patients with CHF. In addition, cells stimulated toward apoptosis might cleave and release or synthesize sFas as a consequence of apoptosis. That is, there is no evidence to suggest the Fas–Fas-L system contributes to apoptosis in human CHF. Therefore, it remains unknown whether the elevation of plasma sFas in CHF is protective against apoptosis.

Recently, Wada et al. (37) reported that the nuclear factor for IL-6 is activated on influenza virus infection of HeLa cells through posttranslational modification, and that the modified factor stimulates the transcription of the human Fas gene. However, plasma levels of IL-6 were not related to plasma sFas levels. Several investigators (9) have reported that hypoxia induced expression of Fas messenger RNA in cultured neonatal rat cardiomyocytes, which suggests that hypoxia in CHF may also induce an increase in sFas in humans. An alternative explanation for our findings is that the increase in sFas is indicative of ongoing "immune activation" in CHF.

It has been reported (30) that Fas is present in the heart, thymus, liver, kidney, ovary and other organs. To determine whether increased levels of sFas are released from the heart, lung or kidney, we measured plasma levels in the coronary sinus, superior vena cava, pulmonary artery, right or left renal vein and femoral artery. The differences in plasma sFas among these sampling sites were slight (from 0 to 0.4 ng/ml). However, plasma levels are determined both by rate of release and half-life. The circulating half-life of sFas may be long or the release rate may be very low such that it would not be detectable as a difference in regional drainage.

Conclusions. Plasma levels of sFas increase in relation to the severity of CHF. The increase in plasma sFas may be an important role in the pathophysiology of CHF, although the specific mechanism remains unclear.

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