

High Levels of Serum Interleukin-10 and Tumor Necrosis Factor- α Are Associated with Fatality in Fulminant Hepatitis

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Serum pro- and anti-inflammatory mediators in patients with acute liver diseases were assessed to clarify the clinical significance of these measurements in relation to disease severity. Concentrations of circulating tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-10, IL-12, and soluble TNF receptors (sTNFR) p55 and p75 were measured at admission in patients with fulminant hepatitis (FH; $n = 19$), severe acute hepatitis (AHS, $n = 15$), or acute hepatitis (AH, $n = 7$). Serum concentrations of TNF- α , IL-10, and sTNFR-55 were significantly higher in patients with FH than in those with AHS ($P < .05$, $< .05$, and $< .01$, respectively) or AH ($P < .05$). Serum IL-10 and TNF- α levels were higher in patients who died of FH ($n = 13$) than in FH survivors ($n = 6$; $P < .05$). The ratios between TNF- α and IL-10 and sTNFR-55 or sTNFR-75 were not valuable in predicting mortality and disease severity. However, both proinflammatory cytokine TNF- α and anti-inflammatory cytokine IL-10 levels at admission were associated with fatal outcome among patients with FH.

Fulminant hepatitis (FH) is associated with high mortality, despite advances in medical management [1]. Several cytokines seem to play major roles in the initiation and maintenance of the immune response in FH. Moreover, these cytokines select the type of immune response and the effector mechanisms that mediate resistance to pathogens. However, certain cytokines, particularly when produced in excess, can induce pathogenesis. High plasma concentrations of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 in patients with sepsis or septic shock correlate with high severity of disease and a very poor prognosis [2–4]. Patients with acute hepatic failure have higher circulating concentrations of these cytokines than do persons with acute hepatitis (AH) or healthy volunteers [5–7]. However, considerable new evidence indicates that, in addition to a massive proinflammatory reaction, a compensatory anti-inflammatory response also contributes to the onset of these disorders. At a local site of injury or infection and during the initial appearance of pro- and anti-inflammatory mediators in the circulation, the beneficial effects of these mediators outweigh their harmful effects. Only when these 2 forces become imbalanced do these mediators become harmful [8]. Soluble TNF

receptors (sTNFR)-I and -II prevent the inflammatory effects of TNF- α by binding to TNF- α [9, 10]. High sTNFR/TNF plasma ratios are associated with a better prognosis in severe meningococemia [11, 12]. Recent studies of severe bacterial infections have focused on the suppressive effects of IL-10 on the synthesis of proinflammatory cytokines. IL-10 inhibits the production of TNF- α , IL-1, IL-6, IL-8, interferon- γ , and colony-stimulating factor by human monocytes [13] and protects mice from lethality in models of endotoxemia [14, 15] and hepatic failure [16, 17]. Hypersecretion of IL-10 in plasma from patients with septicemia and septic shock has been proposed to be involved in the control of the inflammatory response induced by bacterial products [18].

In Japan, FH is diagnosed if 2 criteria are met: hepatic coma of grade II or higher that develops <8 weeks after the onset of symptoms of presumed AH and prothrombin activity <40% of normal [7, 19]. FH develops in persons with AH in whom coagulation factors (e.g., prothrombin time [PT]) are markedly decreased (<40% of normal). However, some patients with high concentrations of transaminases and markedly decreased PT values, in addition to severe subjective symptoms such as general fatigue and anorexia, recover without having hepatic coma. This disease is classified as a severe form of acute hepatitis (AHS) [7, 20]. Little investigation has been done on the differences in concentrations of the circulating pro- and anti-inflammatory mediators in persons with FH, compared with those in patients with AHS, although both groups have severely impaired liver function.

Here we report studies of serum pro- and anti-inflammatory mediators in FH and in severe and ordinary AH, to clarify the clinical significance of their concentrations in relation to the severity of these diseases. By analyzing the hypothesis that serum pro- and anti-inflammatory cytokine measurements can

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Table 1. Clinical characteristics and laboratory values of patients with fulminant hepatitis (FH).

Type of hepatitis	<i>n</i>	Viruses, A, B/others	Age, years	Albumin, g/dL	Total bilirubin, mg/dL	AST, IU/L	ALT, IU/L	PT, %	NH ₃ , μg/dL
FH	19	9/10 ^a	60 (20–75)	3.2 ^b (1.8–3.7)	12.4 (4.5–25.9)	667 (42–17,226)	941 (76–12,550)	21.4 ^{c,d} (6.0–45.0)	210 ^{b,d} (74–325)
Nonsurvivor	13	6/7	63 (24–75)	3.0 (2.2–3.7)	14.5 (4.5–25.9)	548 (118–17,226)	875 (114–12,550)	23.7 (6.0–45.0)	210 (121–311)
Survivor	6	3/3	48 (20–63)	3.3 (1.8–3.5)	9.4 (5.1–18.2)	1052 (42–14,770)	1171 (76–7655)	7.2 (6.2–27.0)	133 (74–325)
AHS	15	13/2	47 (16–61)	3.1 ^c (2.5–4.0)	8.6 (3.2–19.2)	756 (180–22,054)	2420 (405–6990)	33.0 ^b (14.4–39.0)	121 (55–247)
AH	7	5/2	34 (22–69)	3.7 (3.2–4.3)	4.9 (0.6–23.3)	1075 (18–1836)	1083 (11–2449)	70.0 (46.6–90.5)	104 (46–164)

NOTE. Data are median (range), except where noted. AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; NH₃, ammonia; AHS, severe form of acute hepatitis; AH, acute hepatitis.

^a $P < .05$ vs. patients with AHS (χ^2 test).

^b $P < .01$ vs. patients with AH (analysis of variance [ANOVA]).

^c $P < .001$ vs. patients with AH (ANOVA).

^d $P < .01$ vs. patients with AHS (ANOVA).

^e $P < .05$ vs. patients with AH (ANOVA).

be used to assess or confirm the severity of acute liver diseases, we can better understand how organ dysfunction develops and how we may someday be able to prevent it.

Patients and Methods

Patients. We studied 19 patients with FH (6 survived, 13 died), 15 with AHS (all survived), and 7 with AH (all survived). All were admitted to the First Department of Internal Medicine at Gifu University School of Medicine, Gifu, Japan. FH was defined by 2 criteria: the development of hepatic coma of grade II or higher within 8 weeks of the onset of symptoms of presumed AH and prothrombin activity <40% of normal. AHS was diagnosed if prothrombin activity was <40% of normal and hepatic encephalopathy did not develop. All patients with FH and AHS were hospitalized within a few days of diagnosis. Patient age ranges (in years) were as follows: FH patients, 20–75 (median, 60); AHS patients, 16–61 (median, 47); and AH patients, 22–69 (median, 34). FH was caused by viral hepatitis in 18 patients (9 with type non-A, non-B; 5 with type A; and 4 with type B) and was drug related in 1; AHS was caused by viral hepatitis in 15 patients (2 with type non-A, non-B; 10 with type A; and 3 with type B); and the cause of AH was viral hepatitis in 7 patients (1 with type non-A, non-B; 2 with type A; 3 with type B; and 1 with type C). Plasma exchange was performed by use of a membrane plasma separator in all 19 patients with FH; these patients also had the standard procedures for hepatic failure, including ventilatory support, hemodynamic monitoring, mannitol therapy for cerebral edema, H₂ antagonists, and nutritional support. AHS patients received similar intensive liver care management except for extracorporeal circulation.

Blood collection. Blood samples were collected into dry tubes at admission. Serial blood samples were obtained from selected patients with FH. Serum was separated by centrifugation at 900 g for 10 min and was stored in aliquot portions at -80°C until assay.

Cytokine assays. Serum concentrations of TNF- α , IL-6, and IL-10 were determined with human cytokine immunoassay kits (Genzyme, Cambridge, MA), and serum concentrations of IL-1 β and IL-12 were measured by immunoassay kits (R&D Systems, Minneapolis). Serum concentrations of sTNFR-55 and sTNFR-75 were quantitated by immunoassay kits (Hycult Biotechnology, Uden, Netherlands). The assays were performed according to the instructions of the manufacturers. The limits of detection of these immunoassays were 1.5 pg/mL of TNF- α , 3.5 pg/mL of IL-1 β , 3.4

pg/mL of IL-6, 1.3 pg/mL of IL-10, 0.8 pg/mL of IL-12, 24 pg/mL of sTNFR-55, and 25 pg/mL of sTNFR-75. Serum samples were used at a dilution of 1:2. IL-6, sTNFR-55, and sTNFR-75 levels at admission and the serial cytokine analysis were studied in a limited number of patients with FH, because of limited sample amounts. To minimize analytical errors, all samples were analyzed in the same run.

Statistical analysis. For data that were not normally distributed, we used the Mann-Whitney U test only for comparison of 2 groups. For comparison of ≥ 2 groups, we used the Kruskal-Wallis 1-way analysis of variance, followed by the Bonferroni-Dunn test. The χ^2 test was used for 2×2 contingency table analysis. Correlations were tested using Pearson's rank correlation coefficient.

Results

Determination of circulating pro- and anti-inflammatory mediators at admission. The major clinical characteristics and laboratory values of the participating patients are summarized in table 1. Age and sex did not differ significantly among patients with FH, AHS, and AH. The etiologic composition of the acute liver diseases showed a lower percentage of patients with hepatitis A and B in FH than in AHS ($P < .05$), but there was no difference between patients with FH who survived and those with FH who died. Consistent with this result is a study of Sekiyama et al. [6]. Persons with FH had significantly higher ammonia concentrations than did those with AHS or AH ($P < .01$). PT activity and albumin concentrations were lower in patients with FH ($P < .001$ and $P < .01$, respectively) or AHS ($P < .01$ and $P < .05$, respectively) than in those with AH. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities did not differ significantly among patients with FH, AHS, and AH. No differences were observed among patients with FH who survived and those with FH who died.

Serum concentrations of TNF- α at admission were significantly higher in persons with FH (median, 15.7 pg/mL [range, 2.3–170.1 pg/mL]) than in those with AHS (median, 1.5 pg/mL [range, not detectable to 22.3 pg/mL]; $P < .05$) or AH (median, 1.9 pg/mL [range, not detectable to 11.0 pg/mL]; $P < .05$) (figure 1). Serum IL-10 at admission was elevated in patients with FH (median, 36.2 pg/mL [range, 4.2–226.3 pg/mL]), compared with

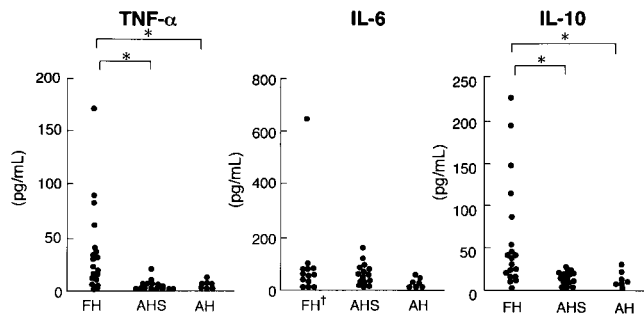


Figure 1. Circulating serum tumor necrosis factor (TNF)- α , interleukin (IL)-10, and IL-6 concentrations in patients with fulminant hepatitis (FH), severe form of acute hepatitis (AHS), or acute hepatitis (AH). * $P < .05$ (analysis of variance followed by Bonferroni-Dunn test). $^{\dagger}n = 13$.

levels in patients with AHS (median, 15.4 pg/mL [range, 1.3–20.1 pg/mL]; $P < .05$) or AH (median, 9.2 pg/mL [range, 1.9–31.1 pg/mL]; $P < .05$). There were no significant differences in serum IL-6 among patients with FH, AHS, and AH (figure 1). Circulating IL-1 β or IL-12 was below detection levels in all samples. Serum sTNFR-55 on admission was significantly increased in patients with FH (median, 5393 pg/mL [range, 1098–15,493 pg/mL]), compared with patients with AHS (median, 1637 pg/mL [range, 806–2886 pg/mL]; $P < .01$) or AH (median, 1641 pg/mL [range, 914–6265 pg/mL]; $P < .05$; figure 2). Serum sTNFR-75 in patients with FH was slightly increased but not significantly different from levels in patients with AHS or AH.

Serum IL-10 levels correlated with IL-6 ($P < .05$), sTNFR-55 ($P < .05$), and sTNFR-75 ($P < .01$) concentrations. Serum sTNFR-55 correlated with serum sTNFR-75 ($P < .001$). No correlation was observed between serum TNF and any cytokine. Serum IL-6 and sTNFR-55 or sTNFR-75 levels, but not TNF- α nor IL-10 levels, correlated with ALT activities ($P < .0001$, $< .05$, and $< .05$, respectively). Serum TNF- α , IL-10, sTNFR-55, and sTNFR-75 correlated with ammonia concentrations ($P < .001$, $< .001$, $< .05$, and $< .05$, respectively). We found no correlation between pro- or anti-inflammatory mediator concentrations and patient age.

Correlation between cytokines and outcome. Serum TNF- α was significantly elevated in FH patients who died (median, 27.9 pg/mL [range, 4.3–170.1 pg/mL]), compared with levels in those with FH who survived (median, 7.0 pg/mL [range, 2.3–34.5 pg/mL]; $P < .05$; figure 3, left). Serum IL-10 levels in patients with FH who died (median, 43.2 pg/mL [range, 5.2–226.3 pg/mL]) were significantly higher than in patients with FH who survived (median, 19.1 pg/mL [range, 4.2–36.9 pg/mL]; $P < .05$; figure 3, right). There were no differences in serum IL-6, sTNFR-55, or sTNFR-75 levels among patients with FH who did or did not survive (data not shown).

Results of serial analysis in a limited number of patients with FH during the course of illness showed that the highest serum

levels of TNF- α , IL-10, and IL-6 were present at admission and fell within a few days after admission (figure 4). Plasma exchange, which was performed in all patients with FH, might influence the circulating levels of these cytokines; in another study, we found that plasma exchange removes the inflammatory mediators from the circulation of patients with severe liver disease [7].

Imbalance between TNF- α and IL-10 or TNF receptors. Because of the inhibitory effects of IL-10 and those of the 2 TNF- α receptors, we wondered whether the ratios of TNF- α to IL-10, TNF- α to sTNFR-55, and TNF- α to sTNFR-75 would be a better indication of severity and mortality of liver diseases than were the cytokine or the cytokine receptor concentration alone. The ratios at admission were slightly higher in patients with FH but not significantly different among persons with the liver diseases (table 2). In persons with FH, the ratios were not significantly higher in patients who died than in those who survived.

Discussion

FH is a life-threatening illness that results ultimately from the nearly complete destruction of the liver by agents such as viruses or drugs. Despite advances in intensive care medicine, FH has a poor prognosis, whereas the outcomes of AHS and AH are not fatal, although persons with FH or AHS have severely impaired liver function (table 1) [7, 20]. Proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, are thought to play important roles in the pathophysiology of acute hepatic failure. Patients with FH have elevated circulating levels of proinflammatory cytokines, including TNF- α and IL-6 [6, 7]. As an approach to elucidating the involvement of anti-inflammatory mediators in the pathogenesis of FH, we measured serum concentrations of IL-10 and sTNFRs in patients with acute liver diseases. We found that at admission not only TNF- α but also IL-10 and sTNFR-55 were higher in persons with

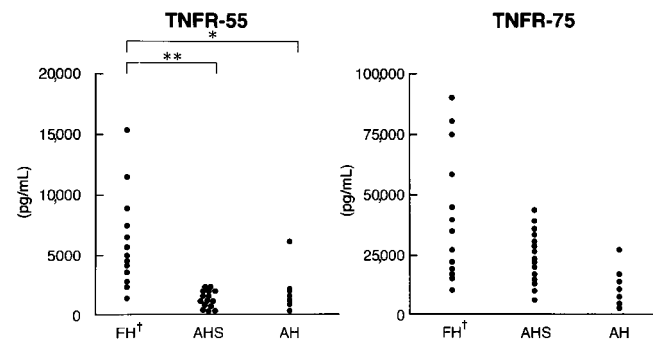


Figure 2. Circulating serum soluble tumor necrosis factor receptor (sTNFR)-55 and sTNFR-75 concentrations in patients with fulminant hepatitis (FH), severe form of acute hepatitis (AHS), or acute hepatitis (AH). * $P < .05$, ** $P < .01$ (analysis of variance followed by Bonferroni-Dunn test). $^{\dagger}n = 13$.

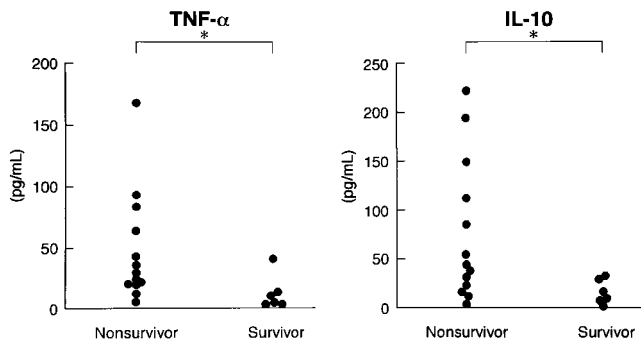


Figure 3. Circulating serum tumor necrosis factor (TNF)- α and interleukin (IL)-10 concentrations in survivors and nonsurvivors. * $P < .05$ (Mann-Whitney U test).

FH than in those with AHS or AH, indicating that, in addition to a massive proinflammatory reaction, a compensatory anti-inflammatory response contributes to the onset of FH. Green et al. [21] found that, in other viral diseases, plasma levels of TNF- α and sTNFR were higher in children who developed dengue hemorrhagic fever than in those with dengue fever. IL-10 and sTNFR protect mice from lipopolysaccharide-induced hepatic damage and lethal shock [14–17, 22]. Moreover, IL-10

is produced in a murine model of concanavalin A–induced hepatitis [23]. Serum levels of IL-10 are increased in patients with chronic hepatitis C [24, 25] or hepatosplenic candidiasis [26].

Increased serum concentrations of sTNFRs have been reported in persons with chronic liver disease and acute viral hepatitis [24, 27]. However, there have been few studies on patients with fulminant hepatic failure that measured circulating levels of anti-inflammatory mediators, and only Keane et al. [28] reported that plasma sTNFR-55 and sTNFR-75 were elevated in persons with fulminant hepatic failure, compared with those of normal control subjects. In the present study, sTNFR-55 was significantly higher in persons with FH than in those with AHS or AH. However, sTNFR-75 levels in patients with FH were slightly increased but not significantly different from those in the other 2 groups. The reasons for this discrepancy are unclear. This result suggests that circulating sTNFR-55 may be associated more with the severity of acute liver diseases, which is in agreement with the work of Goldie et al. [29]. Goldie et al., by logistic regression analysis of concentrations of circulating mediators and their antagonists, found that sTNFR-55 was significantly predictive of mortality in the sepsis syndrome, but sTNFR-75, which was of borderline significance, was not significantly associated with mortality.

Elevated levels of circulating TNF- α and IL-10 were asso-

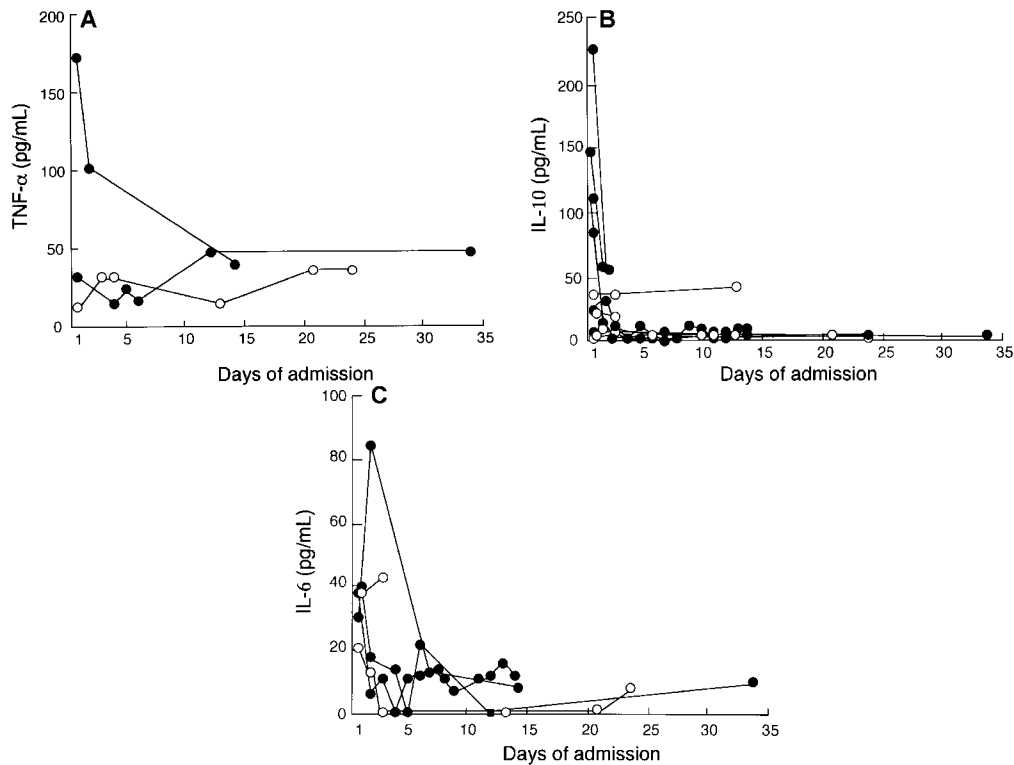


Figure 4. Serial serum tumor necrosis factor (TNF)- α (A), interleukin (IL)-10 (B), and IL-6 (C) concentrations in selected patients with fulminant hepatitis. Survivors (○); nonsurvivors (●). TNF- α : 1 survivor, 2 nonsurvivors; IL-10: 4 survivors, 6 nonsurvivors; IL-6: 2 survivors, 3 nonsurvivors. Lack of completeness was due to incomplete sampling.

Table 2. Evolution of the ratios of tumor necrosis factor (TNF)- α to interleukin (IL)-10, soluble tumor necrosis factor receptor (sTNFR)-55, and sTNFR-75 in acute liver diseases.

Type of hepatitis	n	TNF- α /IL-10	TNF- α /sTNFR-55 $\times 10^2$	TNF- α /sTNFR-75 $\times 10^2$
FH	19	0.52 (0.09–7.43)	3.91 (0.26–41.13)	0.74 (0.06–14.28)
Nonsurvivor	13	0.49 (0.02–7.43)	4.82 (2.54–41.43)	0.95 (0.17–14.28)
Survivor	6	0.46 (0.16–2.16)	1.64 (0.26–13.07)	0.39 (0.06–1.32)
AHS	15	0.17 (0.07–7.96)	2.28 (0.49–18.52)	0.18 (0.03–1.18)
AH	7	0.22 (0.07–1.67)	1.53 (0.73–2.74)	0.14 (0.05–0.40)

NOTE. Data are median (range), except where noted. FH, fulminant hepatitis; AHS, severe form of acute hepatitis; AH, acute hepatitis.

ciated with high mortality in patients with FH (figure 3). TNF- α and IL-10 blood levels were directly related to the development of liver failure, as indicated by the correlation with NH₃, but not with liver injury (indicated by ALT and AST levels). These cytokines possibly could be used as additional prognostic markers of FH. In several studies of patients with sepsis and multiple organ dysfunction syndrome, persistently elevated levels of pro- and anti-inflammatory mediators were associated with increased mortality [11, 12, 30, 31]. Thus, increased production of inflammatory cytokines in septic shock and fulminant hepatic failure is associated with IL-10 hypersecretion, and both pro- and anti-inflammatory mediators may be influenced by each other. Numerous clinical trials in sepsis and septic shock have been conducted using monoclonal antibodies to TNF- α , dimeric TNF receptors, a recombinant IL-1 receptor antagonist, and platelet-activating factor antagonists [8]. However, the results of these trials were uniformly disappointing. The fact that patients with FH or septic shock have high levels of circulating anti-inflammatory mediators may be one possible explanation for the results of these trials.

In our study patients, the ratios of TNF- α to IL-10, sTNFR-55, or sTNFR-75 were not useful in predicting mortality and clinical disease severity, although all ratios appeared to be higher in persons with FH, especially in nonsurvivors with FH (table 2). Consistent with this result is a recent study of patients with sepsis syndrome, in which the ratio of TNF- α to sTNFR did not correlate with fatal outcome [29]. Several studies, however, reported that the ratios of TNF- α to sTNFR at the time of hospital admission were higher in persons who died than in survivors of bacterial infections [11, 12], yet a recent study indicated that the ratio of IL-10 to TNF- α was higher in nonsurvivors than in survivors [31]. These conflicting results may indicate that, when massive amounts of both types of mediators are released in persons with multiple organ dysfunction syndrome, the balance between these forces cannot be restored. How these agents interact is not yet understood, but it is clear that they create a complex, often overlapping, network of interactions.

Bone [8] explained that the final stage of multiple organ dysfunction syndrome is reached when a person develops what he dubbed “immunologic dissonance”—that is, a pathophysiologic response that is out of balance and inappropriate for the person’s biologic needs. He suggested that, at this stage of im-

munologic dissonance, the balance between pro- and anti-inflammatory mediators is lost, and subsequently some persons may have persistent massive inflammation; others may have ongoing immunosuppression and secondary infections; still others may oscillate between periods of inflammation and immunosuppression. This hypothesis may well explain our results. Further studies will be required to elucidate how proinflammatory and anti-inflammatory cytokines can interact in the initiation and progression of the liver cell damage.

In conclusion, IL-10 serum levels were high, even in the presence of elevated concentrations of TNF- α , in patients with FH, in contrast to levels of this cytokine in patients with AHS or AH. The individual cytokine levels of IL-10 or TNF- α were better correlated with mortality than were the ratios of TNF- α to anti-inflammatory mediators.

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