who received a liver graft showed temporary evidence of systemic and cerebral homodynamic stability during the anhepatic phase[13]. In a similar case, it was reported that removal of the liver was associated with a sharp and sustained reduction in circulating proinflammatory cytokine concentrations, suggesting that liver-derived proinflammatory cytokines may be important in the pathogenesis of intracranial hypertension in patients with FH[13].

The aim of this study was to investigate the relationship between circulating inflammatory cytokines and the severity of encephalopathy in FH patients. We also investigated the association between ICP and serum concentrations of TNF-α, IL-1β, and IL-6 in patients with FH.

PATIENTS AND METHODS

Patients

Between June 1997 and May 2003, 21 patients with FH were admitted to the intensive care unit (ICU) at Chiba University Hospital. Serum concentrations of TNF-α, IL-1β, and IL-6 were determined in 19 of these 21 patients (9 survived, and 10 died). The criteria for FH included: the development of hepatic coma grade $>$II; a prothrombin activity at$<$40% of normal; and $\leq$8 wks after the onset of symptoms of presumed acute hepatitis[14]. All patients were admitted within a few days after diagnosis. The patients were aged 12 to 64 yrs (median, 45 yrs). FH was caused by viral hepatitis in 12 patients (2 with type A, and 10 with type B), was drug related in 2 patients, was autoimmune in one patient, and was of unknown etiology in 4 patients.

The grade of hepatic encephalopathy was determined using the following criteria: patients exhibiting slowness of mentation and altered sleep habits were classified as grade I; those exhibiting drowsiness and confusion were classified as grade II; those who were stuporous and slept most of time, but could be aroused were classified as grade III; those who were comatose and did not always respond to noxious stimuli were classified as grade IV; and those who were in a deep coma and did not respond to noxious stimuli were grade V.

Informed consent for participation in this study was obtained from the patients' next of kin. The study was approved by an institutional review board.

Management in the ICU

Plasma exchange (PE) with continuous hemodiafiltration (CHDF) or high-flow dialysate continuous hemodiafiltration (HF-CHDF) was performed for all 19 patients. PE and CHDF were connected in a series, with the plasma separator installed proximal to the extracorporeal circulation line and the hemofilter placed distally. CHDF was performed simultaneously during PE implementation, and when PE was completed after 6-8 hrs of operation, only the PE line was withdrawn while CHDF was continued[15].

These patients also received standard treatment for hepatic failure and complicating organ failures. Broad-spectrum antibiotics were administered prophylactically.

ICP was continuously monitored in 13 patients with an epidural fiber-optic system (Codman & Shurtleff, Raynham, MA, USA) that was inserted after the correction of coagulation abnormalities with fresh-frozen plasma and platelets.

Study design

Patients were divided into three groups according to the development, recovery, or deterioration pattern of encephalopathy during their ICU stay: no deterioration of the encephalopathy (ND group), the recovery and deterioration of the encephalopathy (RD group), and the progressive deterioration of the encephalopathy (PD group). The ND group included patients with more than grade II of encephalopathy at admission but no further deterioration of encephalopathy. The RD group included patients who initially showed deteriorated encephalopathy by 2 grade or more, and then recovered by 2 grades or more, and finally redeveloped encephalopathy by 2 grades or more. The PD group included patients who progressively exhibited deteriorated encephalopathy.

We compared serum cytokine and ammonia concentrations during first 7 ICU days among the ND, RD, and PD groups.

FH patients in the RD group showed a relatively long ICU stay (median 31 days; range 30-34days). To clarify the contribution of cytokines and ammonia to the clinical presentations (grade of encephalopathy), we divided the entire ICU stay for the RD group into the following 3 periods: period A, or the development of encephalopathy (median 10 days; range 7-18 days); period B, the recovery of encephalopathy (median 16 days; range 10-21 days) ; period C, the redevelopment and deterioration of encephalopathy (median 5 days; range 4-7 days). We compared the changes in cytokines and ammonia concentrations during the above 3 periods. The development, recovery, or redevelopment of encephalopathy was defined as a change of grade of encephalopathy by 2 grades or more. The

concentration of cytokines and ammonia during each period was taken as its median value.

We also assessed the correlation between serum concentrations of cytokines and ICP as measured with an ICP monitor.

Blood sampling and cytokine measurements

Arterial blood was sampled every morning (6 am) and collected into dry tubes and pyrogen-free heparinized tubes. Serum was separated by centrifugation at 2000 rpm for 5 min, and aliquots were stored at −80℃ until assay.

Serum concentrations of TNF-α, IL-1β, and IL-6 were determined with human cytokine immunoassay kits (MEDGENIX, Biosource Europe S.A., Nivelles, Belgium). The assays were performed according to the manufacturer's instructions. The detection limits of the assays were 3 pg for TNF-α, 2 pg for IL-1β, and 15 pg for IL-6. Serum samples were tested at a dilution of 1 : 2.

Statistical analysis

Data are expressed as median (range) or mean± standard error of mean (SEM). For comparison among more than two groups, we used the Kruskal-Wallis analysis of variance, followed by the Mann-Whitney U test with the Bonferroni correction.

For serial measurements of more than 2 groups, we used repeated measure ANOVA followed by the Bonferroni -Dunn test. The correlation coefficient was calculated using linear regression analysis. A p value of less than 5% was considered significant ($p = 0.05$).

Table 1. Clinical characteristics and laboratory results of patients with fulminant hepatitis at admission to the intensive care unit

Group	ND group	RD group	PD group
No. of patients	7	7	6
Age (yr)	43.5 (17-60)	49 (12-64)	41 (17-51)
T-bil (g/dL)	11.7 (3.8-37.2)	18.9 (3.8-24.6)#	9.22 (4.9-26.5)
AST (IU/L)	1860 (109-15972)	911 (98-16720)	9283 (227-22740)
ALT (IU/L)	4739 (58-11490)	23065 (442-10120)	10035 (170-11800)
Ammonia (μg/dL)	228 (54-324)	124 (55-186)	164 (79-582)
PT (%)	22 (19-27)	20 (9-36)	27 (18-36)
Alb (g/dL)	3.4 (2.7-4.4)	3.8 (3.5-4.4)	3.9 (2.5-4.3)

Data is presented as the median (range). ND, no deterioration of encephalopathy; RD, recovery and deterioration of encephalopathy; PD, progressive deterioration of encephalopathy. T-bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; Alb, serum albumin. #indicates a significant difference from the other 2 groups (both $p<0.01$).

Table 2. Circulating serum interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 concentrations in patients with fulminant hepatitis at admission to the intensive care unit

	ND group	RD group	PD group
No. of patients	6	7	6
IL-1β (pg/mL)	30.6 (2.5-42.4)	1.7 (1.3-17.6)	7.2 (1.6-30.8)
TNF-α (pg/mL)	41.0 (14.7-105.1)	43.2 (6.1-169.7)	35.7 (4.0-152.9)
IL-6 (pg/mL)	29.9 (2.4-314.9)	21.1 (4.5-406.6)	184.4 (5.9-1190.5)

Data are presented as the median (range). ND, no deterioration of encephalopathy; RD, recovery and deterioration of encephalopathy; PD, progressive deterioration of encephalopathy.

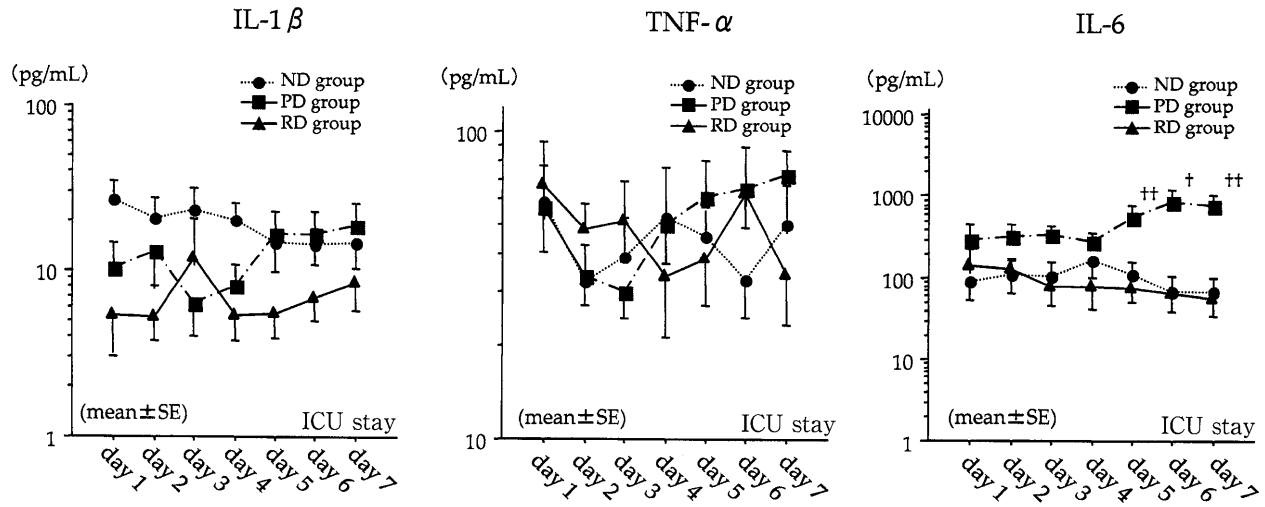

Fig. 1. Serial measurements of serum interleukin (IL)-1β, tumor necrosis factor (TNF)-α and IL-6 concentrations during 1 to 7 days of intensive care unit stay in patients with fulminant hepatitis, $^{+}$ and $^{++}$ indicate significant difference from the other 2 groups ($p<0.05$ and $p<0.01$, respectively), ND, no deterioration of encephalopathy (n=6); RD, recovery and deterioration of encephalopathy (n=7); PD, progressive deterioration of encephalopathy (n=6).

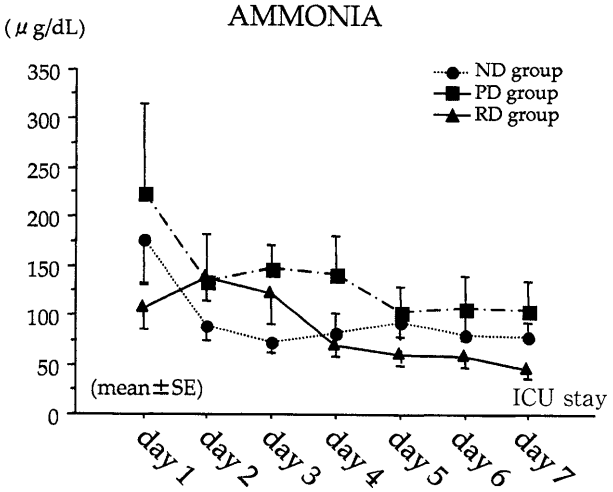

Fig. 2. Serial measurements of serum ammonia concentrations during 1 to 7 days of intensive care unit stay in patients with fulminant hepatitis. ND, no deterioration of encephalopathy (n=6); RD, recovery and deterioration of encephalopathy (n=7); PD, progressive deterioration of encephalopathy (n=6).

RESULTS

Major clinical characteristics and laboratory results of the participating patients are summarized in Table 1. There were no significant differences with regard to age, gender, or causes of FH among the three groups. At admission to the ICU, total bilirubin was significantly higher in the RD group compared with the other groups. Ammonia concentrations, serum alanine aminotransferase, and aspartate aminotransferase did not differ significantly among the three groups (Table 1).

Serum concentrations of TNF-α, IL-1β, and IL-6 at admission did not differ significantly among the three groups (Table 2). Serial analysis showed that serum levels of IL-6 were significantly higher in the PD group as compared with both the ND and the PD group at day 5 ($p<0.01$), day 6 ($p<0.05$), and day 7 ($p<0.01$) (Fig. 1). The concentration of TNF-α, IL-1β, and ammonia did not significantly differ among the three groups (Fig. 1 and 2, respectively). There were no significant intra-group differences in TNF-α, IL-1β, IL-6 and ammonia concentration as compared with the values at day 1 (Fig. 1 and 2, respectively).

Fig. 3 depicts the changes in IL-1β, TNF-α, IL-6, and ammonia during periods A, B, and C in the RD group. IL-1β did not significantly change among the three periods. TNF-α were significantly higher at period C as compared with the other two periods (both $p<0.05$); IL-6 significantly increased during period C as compared with period B ($p<0.05$). Ammonia concentrations were significantly higher during period A than period B ($p<0.05$).

We investigated the correlation between serum concentrations of TNF-α, IL-1β, and IL-6 and maxi-

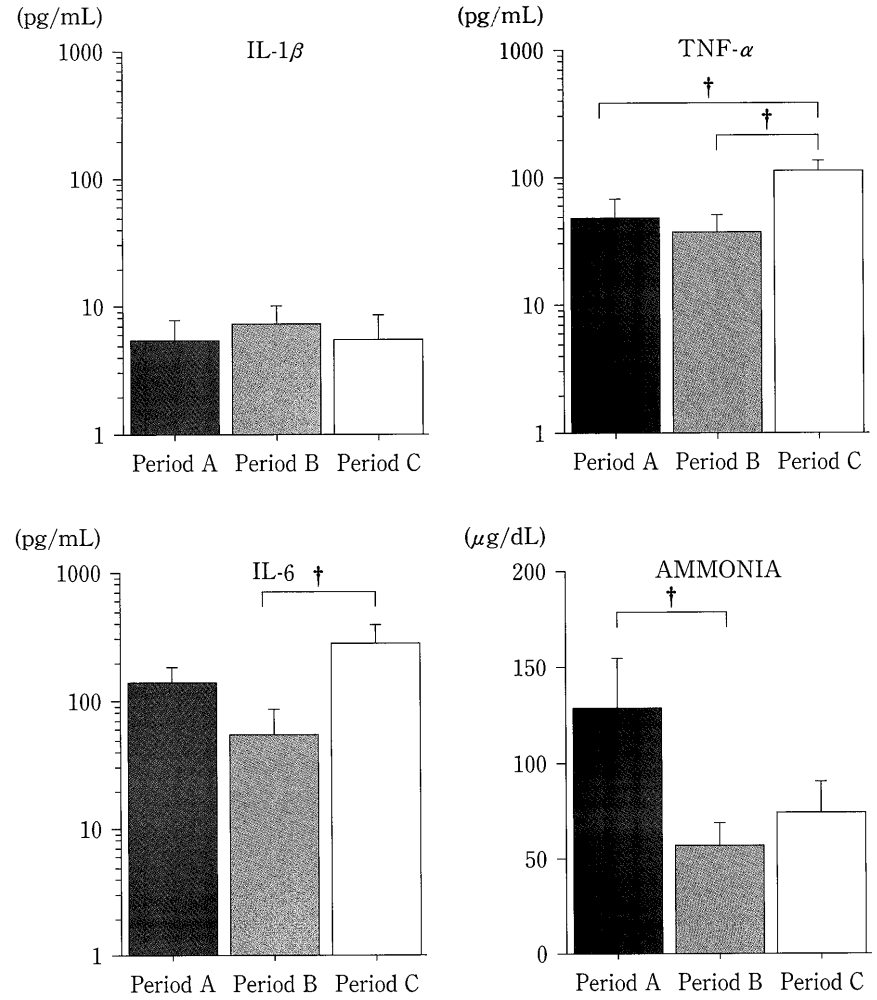

Fig. 3. Comparison of changes in serum interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6 concentrations, and ammonia concentrations during periods A, B, and C in the RD group, †indicates significant difference ($p<0.05$), Period A, development of encephalopathy; period B, recovery from encephalopathy; period C, redevelopment of encephalopathy.

mum ICP during the day. Both serum TNF-α and IL-6 levels positively correlated with maximum ICP ($r=0.272$, $p=0.00049$ and $r=0.517$, $p<0.0001$, respectively) (Fig. 4). IL-1β did not correlate with ICP.

DISCUSSION

The results of this study can be summarized as follows: 1) In serial measurements, serum concentrations of IL-6 were at significantly higher levels in the PD group as compared with other two groups from day 5 to day 7; 2) In the RD group, serum concentrations of TNF-α and IL-6 were significantly higher during the period of the redevelopment of encephalopathy (period C) than the recovery period (period B); 3) Both serum TNF-α and IL-6 levels were significantly correlated with ICP.

FH is a life-threatening illness that results from the nearly complete destruction of the liver. FH results in progressive multiple-organ failure, including the brain. Indeed, the development of intracranial hypertension is a leading cause of death in FH patients.

Two theories have independently emerged to explain the pathogenesis of cerebral edema and elevated ICP associated with FH[2-4]. The glutamine

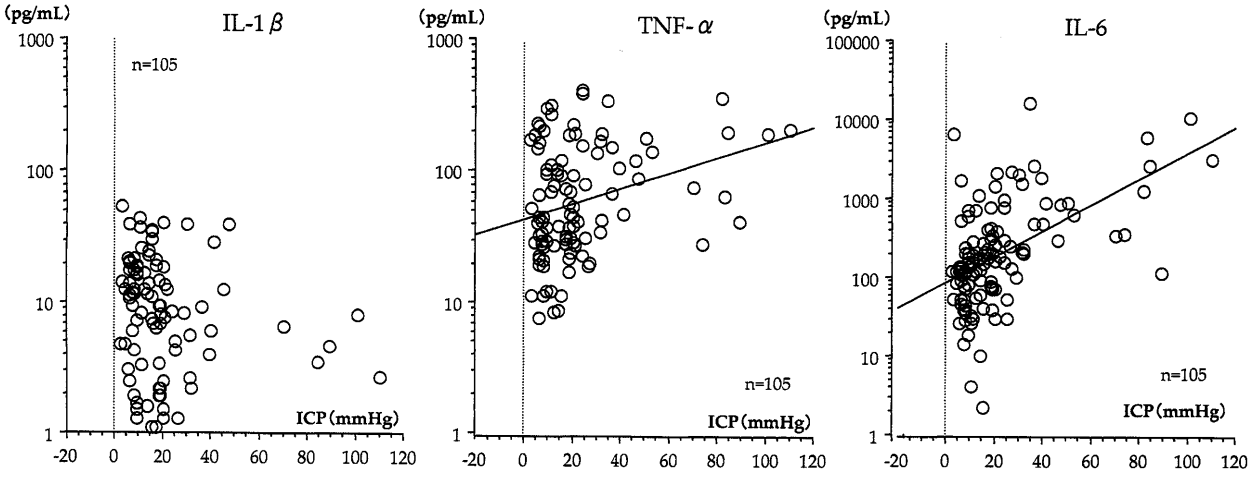

Fig. 4. Scatter plot of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 concentrations and intracranial pressure (ICP). There was a significant relationship between ICP and TNF-α ($y=1.672+0.005x$; $r=0.290$, $p<0.001$), and IL-6 ($y=1.917+0.016x$; $r=0.516$, $p<0.001$).

hypothesis is based on the fact that ammonia is detoxified in the brain to glutamine; osmotic effects in astrocytes may account for the development of brain edema[16]. Astrocytic swelling is a prominent neuropathological feature of FH[17]. In humans, hyperammonemia induces brain edema in several medical conditions[18]. Experimentally, the inhibition of glutamine synthesis prevents the development of ammonia-induced brain edema[19], decreases astrocytic swelling[20], and ameliorates brain edema[21]. A second hypothesis suggests that cerebral edema arises as a result of cerebral vasodilatation[22]. Physiological studies in patients with FH[23,24] and in experimental FH models[25] indicate that cerebral arterioles are dilated. Furthermore, patients with signs of cerebral edema and intracranial hypertension have a higher CBF than patients without brain swelling[26,27].

Recent studies suggest that the development of brain edema may depend both on glutamine accumulation in astrocytes and changes in CBF[28]. Master et al.[29] showed that the inhibition of glutamine synthesis ameliorates brain edema by decreasing glutamine accumulation and reducing cerebral vasodilatation.

A systemically released humoral mediator is thought to be associated with the development of cerebral vasodilatation. In patients with FH, leakage of endotoxins from the gut to portal blood may result in elevated systemic endotoxin levels as portal blood goes to the non-functioning liver. Endotoxin may increase the plasma concentration of TNF-α, IL-1β, and IL-6 in the inflammatory host defense response[30,31]. Cytokines are potent stimulators of nitric oxide synthesis. The hyper dynamic systemic circulation with high cardiac output and low systemic vascular resistance in FH patients may result from the production of excessive amounts of nitric oxide in the endothelium, as suggested by a study that examined cGMP levels in these patients[32].

This study suggests that proinflammatory cytokines have an effect on cerebral edema in FH patients. IL-6 and TNF-α seem to be some of the key factors in the development of hepatic encephalopathy with FH. In this study, the serum concentrations of IL-6 were significantly higher in the PD group from day 5 to day 7 of the ICU stay, but there were no significant differences in the serum concentrations of IL-1β or TNF-α among the groups. IL-1β and TNF-α have a relatively short half-life as compared with IL-6[33], possibly accounting for the present results.

In the RD group, IL-6 and TNF-α concentrations were significantly higher during the redevelopment period (period C) than the development period (period A). However, conversely, ammonia concentrations were higher during period A than period C. Therefore, it seems that ammonia plays a major role during the development of encephalopathy, and in contrast, IL-6 and TNF-α play a major role in the redevelopment of encephalopathy. In addition, serum IL-6 and TNF-α levels were significantly correlated with ICP, suggesting that IL-6 and TNF-α induce the redevelopment of encephalopathy by increasing ICP.

Generally, the blood-brain barrier prevents the entry of cytokines from the systemic circulation into the brain. Possible mechanisms for this action are as follows: first, direct signaling to peripheral tissues such as the liver; second, signaling through the brain

vasculature through the production of endothelial factors such as nitric oxide or prostanoids; and third, a direct action of the cytokines after crossing the blood-brain barrier[34]. Although it is not clear which mechanism is mainly responsible, it has recently been reported that TNF-α can itself induce a change in permeability of the blood-brain barrier[35], indicating that systemic cytokines may have a direct effect on the brain by crossing this barrier.

In conclusion, the present study suggests that IL-6 and TNF-α but not IL-1β may play important roles in the pathogenesis of intracranial hypertension and encephalopathy in FH patients. Future studies should address the possible mechanisms by which peripheral cytokines modulate intracranial hypertension in FH patients, presumably leading to the development of newer therapeutic strategies for this fatal complication of FH.

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