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Significant elevation of serum interleukin-18 levels in patients with acute pancreatitis

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Background. We have reported that peripheral lymphocyte reduction due to apoptosis is linked to the development of subsequent infectious complications in patients with severe acute pancreatitis and that Th1 (helper T cell type 1)/Th2 (helper T cell type 2) balance tends to cause Th1 suppression in experimental severe acute pancreatitis. It has been reported that interleukin (IL)-18 is a cytokine produced from Kupffer cells and activated macrophages, and that IL-18 acts on Th1 cells and in combination with IL-12 strongly induces production of interferon-γ. However, the role of IL-18 in acute pancreatitis has not yet been fully understood. ***Methods.*** Serum IL-18 concentrations were determined by an enzyme-linked immunosorbent assay in 43 patients with acute pancreatitis at the time of admission. The relationships with etiology, pancreatic necrosis, severity, blood biochemical parameters on admission, infection, and organ dysfunction during the clinical course and prognosis were analyzed. ***Results.*** Serum IL-18 levels in patients with acute pancreatitis (656 ± 115 pg/ml) were significantly higher than those in healthy volunteers (126 ± 7 pg/ml). Serum IL-18 levels were significantly positively correlated with the Ranson score and Japanese severity score. Among the blood biochemical parameters on admission, base excess and total protein were significantly negatively correlated with serum IL-18 levels. Moreover, the CD4/CD8 rate of lymphocytes, serum IL-6 levels, and serum IL-8 levels were significantly positively correlated with serum IL-18 levels. On day 7 after admission, the CD4/CD8 rate of lymphocytes and the rate of CD4-positive lymphocytes were significantly positively correlated with serum IL-18 levels. Furthermore, serum IL-18 levels in patients with hepatic dysfunction (980 ± 258 pg/ml) were significantly higher than those without hepatic dysfunction (464 ± 86 pg/ml). Serum IL-18 levels were not related to infection or prognosis. Elevation of serum IL-18 levels continued during 4 weeks after admission. ***Conclusions.*** These results suggest that serum IL-18 levels are significantly elevated and are correlated with severity in patients with acute pancreatitis and that IL-18 may be closely related to helper T cell response and hepatic dysfunction in this disease.

Key words: acute pancreatitis, IL-18, Th1/Th2 balance, helper T cell, hepatic dysfunction

Introduction

Sepsis resulting from infected pancreatic necrosis is the most serious complication in the late phase of severe acute pancreatitis and contributes to the high mortality of this disease.[1–3] Immunological impairment in the early phase may be linked to increased susceptibility to subsequent infection and the development of septic complications. Several investigators have reported the reduction in peripheral lymphocyte count,[4–6] and it may reflect the immunological impairment. Particularly, Curley et al. reported a significant decrease in the proportion of helper T cells in severe acute pancreatitis.[5] Moreover, we have reported that thymic atrophy and splenic atrophy occur in rat experimental severe acute pancreatitis[7,8] and that peripheral lymphocyte reduction due to apoptosis is linked to the development of subsequent infectious complications in patients with severe acute pancreatitis.[9,10]

Recently, the concept of "Th1 (helper T cell type 1)/Th2 (helper T cell type 2) balance" has been introduced for understanding the pathophysiological response during many diseases.[11–13] Upon antigen exposure, Thp

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(precursor of helper T cell) cells undergo differentiation to Th0 cells, the population of cells secreting multiple varieties of cytokines. Interleukin (IL)-12 facilitates conversion to the Th1 phenotype, and IL-4 promotes conversion to the Th2 phenotype. Th1 cells produce interferon (IFN)-γ and IL-2, activate cytotoxic T cells, and initiate cellular immunity. Th2 cells secrete IL-4 and IL-6, activate B cells, and stimulate production of certain antibodies. Concerning the functional changes of lymphocytes, we have clarified that splenocyte function is markedly suppressed and that Th1/Th2 balance tends to Th1 suppression in experimental severe acute pancreatitis.[14]

IL-18 is a cytokine that is identified as a costimulatory factor for production of IFN-γ in response to toxic shock.[15,16] It shares functional similarities with IL-12. It has been reported that IL-18 is produced from various cells, such as Kupffer cells and activated macrophages, and that IL-18 acts on Th1 cells and in combination with IL-12 strongly induces production of IFN-γ.[17] However, IL-18 has many other functions, including induction of proinflammatory cytokines, upregulation of adhesion molecules, and activation of natural killer cell activity.[18,19] IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases, in a variety of cancers, and in numerous infectious diseases. IL-18 is now considered to be an important regulator of inflammation, immunological reactions, and tissue injury.

In this study, to clarify whether IL-18 is implicated in acute pancreatitis, serum IL-18 concentrations were determined in patients with acute pancreatitis at the time of admission. Relationships between their serum IL-18 levels and various clinical factors for acute pancreatitis were analyzed.

Methods

Patients and samples

Serum samples were obtained from 10 healthy volunteers (7 males and 3 females; age, 48 ± 17 years) and 43 patients with acute pancreatitis in our department (30 males and 13 females; age, 51 ± 14 years) at the time of admission (within the initial 72 h after the onset of disease). Time interval between onset and admission was 40 ± 3 hours (0–24 h in 25 patients, 24–48 h in 7 patients, and 48–72 h in 11 patients). The samples were stored at –80°C until they were assayed. The diagnosis and evaluation of the severity of acute pancreatitis were made according to the criteria for clinical diagnosis and grading severity (1990) formulated by the Intractable Diseases of the Pancreas, Japanese Ministry of Health, Labour and Welfare, at the time of admission.[20] Clinical staging for acute pancreatitis was performed according to the Japanese staging system.[21] Accordingly, there were 38 severe cases and 5 moderate cases. Etiology of acute pancreatitis was presumed to be alcohol in 27 patients, gallstone in 9 patients, idiopathy in 5 patients, and other in 2 patients. Pancreatic necrosis was evaluated by dynamic computed tomography (CT) on admission. Ranson score and APACHE II score were estimated on admission as described.[22,23] Infection was defined as infected pancreatic necrosis or sepsis. Organ dysfunctions were defined as follows: liver, serum glutamate oxaloacetic transaminase (GOT) or glutamate pyruvic transaminase (GPT) >250 IU/l or total bilirubin >5.0 mg/dl; kidney, serum creatinine (Cr) >2.0 mg/dl or blood urea nitrogen (BUN) >30 mg/dl; lung, PaO_2 < 60 mm Hg (room air) or hypoxia requiring respirator-assisted ventilation. The serum IL-18 concentrations were determined. The relationships with etiology, pancreatic necrosis, severity, blood biochemical parameters on admission, infection and organ dysfunction during the clinical course, and prognosis were analyzed.

Assay for IL-18 concentration

The serum IL-18 concentrations were determined with an established available enzyme-linked immunosorbent assay (ELISA) kit (MBL, Nagoya, Japan). This kit measures human IL-18 by sandwich ELISA. The assay uses two monoclonal antibodies against two different epitopes of human IL-18. Briefly, in the wells coated with antihuman IL-18 monoclonal antibody, samples to be measured or standards are incubated for 60 min at room temperature. After washing the well 4 times, a peroxidase conjugated antihuman IL-18 monoclonal antibody is added into the microwell and incubated for 60 min at room temperature. After washing the well 4 times, the peroxidase substrate is mixed with the chromogen and allowed to incubate for 30 min at room temperature. An acid solution is then added to each well to terminate the enzyme reaction and to stabilize the developed color. The optical density of each well is then measured at 450 nm using a microplate reader. The concentration of human IL-18 is calibrated from a dose–response curve based on reference standards. The assay was performed in duplicate. The sensitivity of the assay is 12.5 pg/ml. In this ELISA, both intraassay coefficient of validation (CV) and interassay CV are <10%.

Statistical analysis

The results are expressed as mean ± SEM. The Mann–Whitney *U* test was used to evaluate differences between two groups. The Kruskal–Wallis test was used to evaluate differences among more than two groups. Correlations were evaluated with the Spearman rank

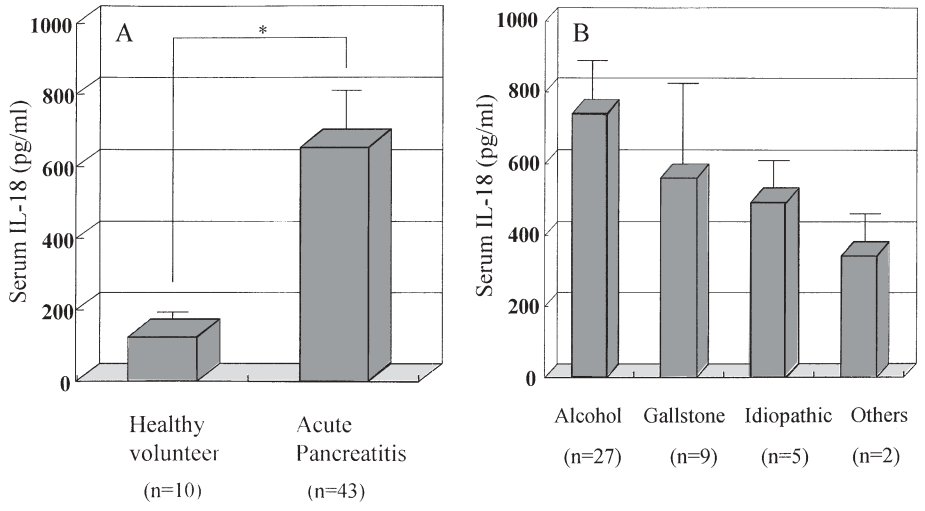

Fig. 1. A Serum interleukin (IL)-18 levels in patients with acute pancreatitis. Serum IL-18 concentrations at the time of admission in 43 patients with acute pancreatitis were determined by enzyme-linked immunosorbent assay (ELISA). *, Significantly different between two groups ($P < 0.05$). **B** Relationship between etiology and serum IL-18 levels

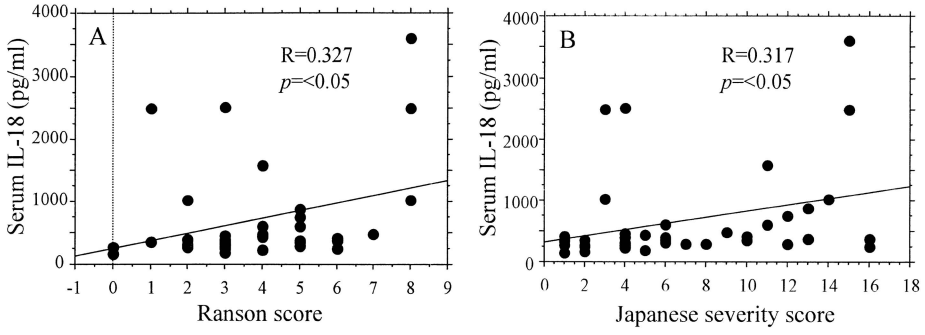

Fig. 2A,B. Correlations of serum IL-18 levels with severity indexes at the time of admission. **A** Correlation with Ranson score. **B** Correlation with Japanese severity score

test. A P value <0.05 was considered statistically significant.

Results

Serum IL-18 levels in patients with acute pancreatitis

The mean value of serum IL-18 levels in patients with acute pancreatitis at the time of admission was 656 ± 115 pg/ml and was significantly higher than that in healthy volunteers (126 ± 7 pg/ml) ($P < 0.05$) (Fig. 1A). In 40 patients, serum IL-18 levels were found to be abnormal (normal range, 37–215 pg/ml), with an incidence of 93%. The mean value of serum IL-18 levels in male patients (734 ± 153 pg/ml) was higher than that in female patients (548 ± 179 pg/ml), although a significant difference was not observed between the two groups.

Relationship between etiology and serum IL-18 levels

The mean values of serum IL-18 levels in patients with acute pancreatitis because of alcohol, gallstone, idiopathy, and other reasons were 740 ± 163, 562 ± 245, 495 ± 96, and 345 ± 67 pg/ml, respectively (Fig. 1B). There was no significant difference among these groups.

Relationship between pancreatic necrosis and serum IL-18 levels

Serum IL-18 levels in patients with pancreatic necrosis and without pancreatic necrosis at the time of admission were 726 ± 170 pg/ml and 575 ± 154 pg/ml, respectively. There was no significant difference between the two groups.

Relationship between severity on admission and serum IL-18 levels

Serum IL-18 levels in moderate cases ($n = 5$) and severe cases ($n = 38$) were 309 ± 47 pg/ml and 701 ± 128 pg/ml, respectively. Serum IL-18 levels were significantly positively correlated with Ranson score ($R = 0.327$, $P < 0.05$) (Fig. 2A) and Japanese severity score ($R = 0.317$, $P < 0.05$) (Fig. 2B). Significant correlation was not noticed with APACHE II score ($R = 0.177$), but serum IL-18 levels in patients whose APACHE II scores were 7 and

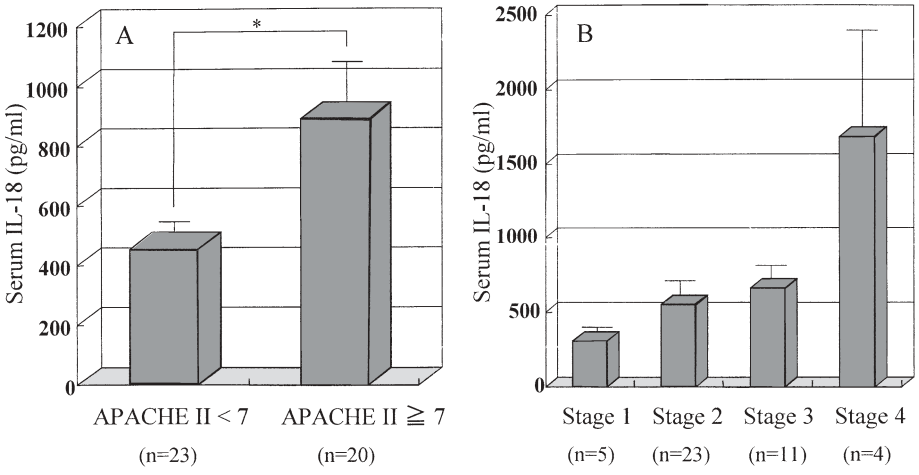

Fig. 3A,B. Relationships between severity indexes and serum IL-18 levels. **A** Relationship with APACHE II score. *, Significantly different between two groups ($P < 0.05$). **B** Relationship with Japanese clinical staging. Significant difference was observed by Kruskal–Wallis test ($P < 0.05$)

Table 1. Correlations of serum IL-18 levels with blood biochemical parameters at the time of admission in patients with acute pancreatitis

Parameter	Correlation coefficient	*P* value
Base excess	–0.576	<0.001
Total protein	–0.408	0.02
GOT	0.345	0.05
Platelet	–0.304	0.07
LDH	0.298	0.09
Prothrombin time	–0.243	0.41
Creatinine	0.240	0.17
BUN	0.222	0.22
Amylase	–0.211	0.27
WBC	–0.201	0.23
CRP	0.177	0.33
Total bilirubin	0.173	0.36
GPT	0.153	0.42
Hematocrit	–0.147	0.40
PaO_2	0.130	0.48
Fasting blood sugar	0.125	0.50
Ca	0.118	0.52

GOT, glutamate oxaloacetic transaminase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; WBC, white blood cell; CRP, c-reactive protein; GPT, glutamate pyruvic transaminase

Table 2. Correlations of serum IL-18 levels with subtype of blood lymphocytes and serum levels of other cytokines at the time of admission in patients with acute pancreatitis

Parameter	Correlation coefficient	*P* value
Lymphocyte (%)	–0.219	0.22
CD4 lymphocyte (%)	0.153	0.44
CD8 lymphocyte (%)	–0.290	0.13
CD20 lymphocyte (%)	–0.192	0.34
CD4/8 rate	0.412	0.03
IL-1β	–0.163	0.46
IL-6	0.742	<0.001
IL-8	0.904	<0.001
IL-10	–0.001	0.99
TNF-α	–0.191	0.39
IFN-γ	0.120	0.58

TNF, tumor necrosis factor; IFN, interferon

Table 3. Correlations of serum IL-18 levels with subtype of blood lymphocytes on day 7 after admission in patients with acute pancreatitis

Parameter	Correlation coefficient	*P* value
Lymphocyte (%)	0.174	0.48
CD4 lymphocyte (%)	0.680	0.04
CD8 lymphocyte (%)	–0.467	0.22
CD20 lymphocyte (%)	–0.295	0.50
CD4/8 rate	0.790	0.009

above (890 ± 210 pg/ml) were significantly higher than those whose APACHE II scores were below 7 (452 ± 100 pg/ml) (Fig. 3A) ($P < 0.05$). Moreover, serum IL-18 levels in patients with stage 1, 2, 3, and 4 according to the Japanese staging system were 309 ± 47 pg/ml, 551 ± 134 pg/ml, 660 ± 115 pg/ml, and 1683 ± 825 pg/ml, respectively (Fig. 3B). A significant difference was observed among the four groups ($P < 0.05$).

Relationships between blood biochemical parameters and serum IL-18 levels

Correlation efficient of serum IL-18 levels with various blood biochemical parameters at the time of admission are shown in Table 1. Base excess ($R = –0.576$) and total protein ($R = –0.408$) were significantly negatively correlated with serum IL-18 levels ($P < 0.05$). Table 2 shows the correlation coefficient of serum IL-18 levels with subtype of blood lymphocytes and serum levels of other cytokines at the time of admission. CD4/CD8 rate of lymphocytes ($R = 0.412$), serum IL-6 levels ($R = 0.742$), and serum IL-8 levels ($R = 0.904$) were significantly positively correlated with serum IL-18 levels ($P < 0.05$). Other cytokines [IL-1β, IL-10, tumor necrosis factor (TNF)-α, and IFN-γ] were not correlated with IL-18. IL-4 was not detected (<2 pg/ml) in serum samples of 43

patients. Table 3 shows the correlation efficient of serum IL-18 levels with subtype of lymphocytes on day 7 after admission (day 8–9 after the onset). Rate of CD4-positive lymphocytes ($R = 0.680$) and CD4/CD8 rate of lymphocytes ($R = 0.790$) were significantly positively correlated with serum IL-18 levels ($P < 0.05$).

Relationships between organ dysfunction during hospitalization and serum IL-18 levels

Figure 4 shows the relationships between organ dysfunction during hospitalization and serum IL-18 levels. Serum IL-18 levels with hepatic dysfunction (980 ± 258 pg/ml) were significantly higher than those without hepatic dysfunction (464 ± 86 pg/ml) ($P < 0.05$). Serum IL-18 levels with renal dysfunction (767 ± 177 pg/ml) were higher than those without renal dysfunction (608 ± 147 pg/ml). Serum IL-18 levels with pulmonary dysfunction (749 ± 210 pg/ml) were higher than those without pulmonary dysfunction (588 ± 130 pg/ml).

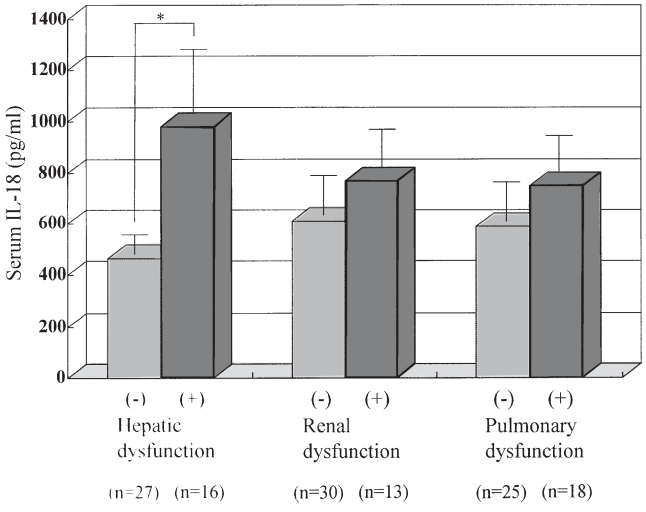

Fig. 4. Relationships between organ dysfunction during hospitalization and serum IL-18 levels at the time of admission. Organ dysfunctions were defined as described under Methods. *, Significantly different between two groups ($P < 0.05$)

Relationship between infection during hospitalization and serum IL-18 levels

Serum IL-18 levels in patients with infection and without infection were 591 ± 118 pg/ml and 666 ± 133 pg/ml, respectively (Fig. 5A). No significant difference was observed between the two groups.

Relationship between prognosis and serum IL-18 levels

The mean values of serum IL-18 levels in survivors and nonsurvivors were 651 ± 140 pg/ml and 668 ± 198 pg/ml, respectively (Fig. 5B). No significant difference was observed between the two groups.

Time course of serum IL-18 levels

The time course of serum IL-18 levels was examined in ten recent patients with severe acute pancreatitis (Fig. 6). Serum IL-18 levels in patients with organ dysfunction (liver, kidney, or lung) were significantly higher (907 ± 173 pg/ml) than those without organ dysfunction on admission. In patients with organ dysfunction, serum IL-18 increased on day 3 after admission and continued at higher levels during 4 weeks compared with patients without organ dysfunction. Even in patients without organ dysfunction, elevation of serum IL-18 levels continued during 4 weeks (373 ± 25 pg/ml on admission and 448 ± 37 pg/ml on day 28 after admission).

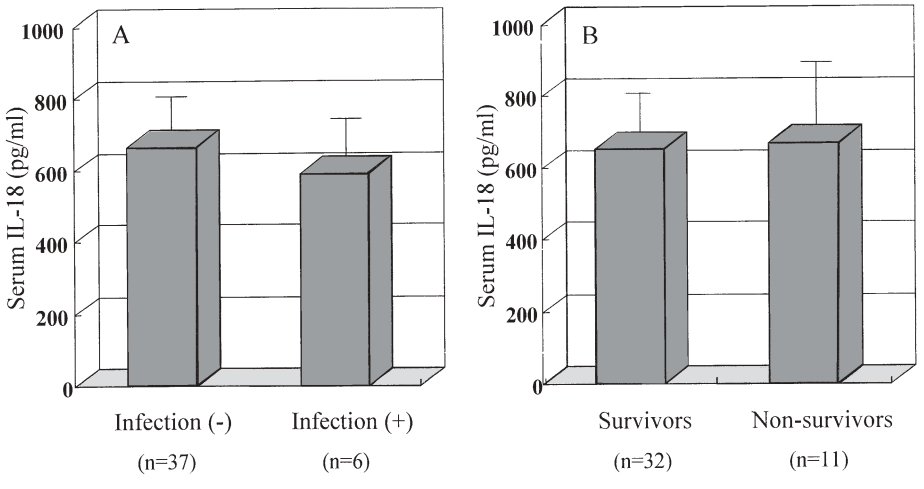

Fig. 5A,B. Relationships between clinical course and serum IL-18 levels at the time of admission. **A** Relationship with infection during hospitalization. Infection was defined as infected pancreatic necrosis or sepsis. **B** Relationship with prognosis

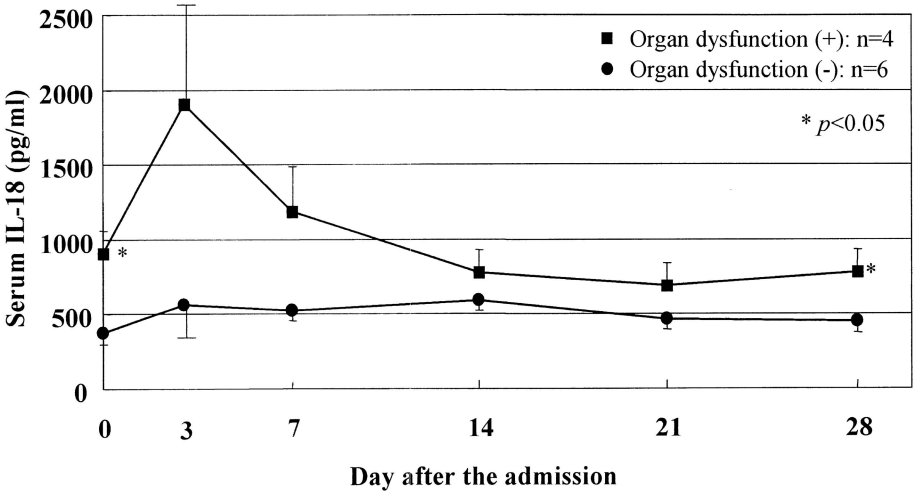

Fig. 6. Time course of serum IL-18 levels in ten patients with severe acute pancreatitis: ■, patients with organ dysfunction (liver, kidney, or lung); ●, patients without organ dysfunction. *, Significantly different versus organ dysfunction (–) group ($P < 0.05$)

Discussion

In this article, we have shown that serum IL-18 levels are significantly elevated and are correlated with the severity indexes in patients with acute pancreatitis. Moreover, we have first demonstrated that serum IL-18 levels are significantly higher in patients with hepatic dysfunction and are correlated with CD4/CD8 rate of lymphocyte. These results suggest that serum IL-18 levels may reflect severity and organ dysfunction in acute pancreatitis and that IL-18 may be closely related to helper T cell response and hepatic dysfunction in this disease.

Concerning IL-18 during acute pancreatitis, there have been four reports so far.[24–27] However, the role of IL-18 in acute pancreatitis has not yet been fully understood. Endo et al. showed that serum IL-18 levels reflected the severity and were significantly correlated with the serum TNF-α and bilirubin levels.[24] It was also demonstrated that serum IL-18 levels reflected the severity and were significantly correlated with plasma polymorphonuclear leukocyte elastase and serum C-reactive protein (CRP) levels.[25,26] In our study, although serum IL-18 levels reflected severity, they were not correlated with TNF-α, bilirubin, or CRP levels. Rau et al. reported that serum IL-18 concentrations were significantly elevated in patients with pancreatic necrosis and remote organ (pulmonary and renal) failure, whereas no correlations were found with serum IFN-γ concentrations or the development of pancreatic infection.[27] These results are consistent with ours obtained in this study. However, the relationship with hepatic dysfunction was not mentioned, and correlations with subtype of lymphocytes and other cytokines were not analyzed in previous papers.

IL-18, which is found first as an IFN-γ-inducing factor because of its ability to induce IFN-γ production in T cells and natural killer cells, plays an important role in the Th1 response.[15–17] We have clarified that Th1/Th2 balance tends to Th1 suppression in experimental severe acute pancreatitis.[14] In the light of these observations, we analyzed correlations of serum IL-18 levels with subtype of lymphocytes and serum levels of other cytokines. In the results obtained, CD4/CD8 rate of lymphocytes on admission, CD4/CD8 rate of lymphocytes, and the rate of CD4-positive lymphocytes on day 7 after admission were significantly positively correlated with serum IL-18 levels, whereas serum IFN-γ levels (Th1 cytokines) were not correlated with serum IL-18 levels. Rau et al. discussed that a possible reason for the low IFN-γ production in the presence of elevated IL-18 levels might be the lack of significant amounts of IL-12.[27]

Until the discovery of IL-18, the dominant inducer of IFN-γ production was IL-12, which is still a necessary costimulant for enhanced generation of IFN-γ.[28,29] A previous report on the course of IL-12 in human acute pancreatitis revealed a significant reduction of the bioactive heterodimer IL-12p70 after an initial increase.[30] In the absence of increased IFN-γ levels, IL-18 does not seem to contribute to Th1 induction, but may implicate Th1 response via other Th1 cytokines such as IL-2 or IL-15. On the other hand, because IL-4 (Th2 cytokine) was not detected in serum samples where IL-18 levels were increased, elevation of serum IL-18 levels in acute pancreatitis does not seem to be related to the Th2 response. At least, the results in this study suggest that IL-18 promotes CD4-positive lymphocyte (helper T cell) response.

IL-18, a recently described proinflammatory cytokine, has striking similarities concerning structure and function with IL-1β. As well as IL-1β, IL-18 has comparable primary amino acid sequences and secondary structures, and is cleaved into its biologically active

form by caspase-1.[18] In addition, IL-18 induces the production of TNF-α, IL-1β, IL-6, and several chemokines such as IL-8.[18] Therefore, IL-18 is now recognized as one of the key mediators of inflammation and may be of similar relevance as IL-1β in the pathomechanism of acute pancreatitis. In the present study, serum IL-18 levels were not correlated with serum IL-1β levels but were significantly positively correlated with serum IL-6 and IL-8 levels. Together with IL-6 and IL-8, IL-18 may be useful as one of the early markers for the severity of acute pancreatitis.

Concerning the elevation of serum IL-18 levels in patients with hepatic dysfunction, following mechanism can be assumed. Shibata et al. reported increased IL-18 concentrations in patients with hepatic dysfunction after hepatectomy.[31] IL-18 is metabolized in the liver and excreted into bile, and an increase of IL-18 in patients with hepatic dysfunction reflects the decreased metabolism in the liver. In our study, because serum IL-18 levels on admission were related to hepatic dysfunction during hospitalization, it is conceivable that an increase of IL-18 preceded the hepatic dysfunction. Kupffer cells have the potential to induce liver injury by production of IL-18. Lipopolysaccharide (LPS)-induced liver injury is the prototype of IL-18-induced tissue injury, in which IL-18 secreted by Kupffer cells acts on natural killer cells to increase Fas ligand, which causes liver injury by induction of Fas-dependent hepatocyte apoptosis.[32] Finotto et al. also reported severe hepatic injury caused by Fas-dependent hepatocytes apoptosis in IL-18 transgenic mice.[33] Takeuchi et al. showed that IL-18 caused neutrophil-dependent hepatic ischemia/reperfusion injury through suppressing antiinflammatory cytokine expression.[34] We previously found that apoptotic cell death occurred in hepatocytes in rat severe acute pancreatitis.[35] We also demonstrated that strong expression of TGF-β1 was detected in the liver and peritoneal macrophages early in the course of rat severe acute pancreatitis and that macrophage-derived TGF-β_1 induced hepatocellular injury via apoptosis.[36] Moreover, we clarified that hematin was one of the cytotoxic factors in pancreatitis-associated ascitic fluid that caused hepatocellular injury.[37]

However, TGF-β_1 and hematin have not explained all of apoptosis-inducing activity. From these observations, it is possible that IL-18 may be one of the hepatocyte apoptosis-inducing factors that cause hepatic dysfunction in severe acute pancreatitis. On the other hand, Marino et al. reported that IL-18 increased the susceptibility of liver endothelial cells to apoptosis mediated by TNF-α.[38] In acute pancreatitis, it is widely accepted that endothelial cell injury occurs and contributes to the development of organ injury. Therefore, it is supposed that IL-18 may facilitate hepatic dysfunction through promoting the liver endothelial cell apoptosis in acute pancreatitis. Further investigations are required to elucidate the role of IL-18 in the mechanism of hepatic dysfunction.

Multiple organ failure in the early phase and infectious complications in the late phase contribute to the high mortality of severe acute pancreatitis. According to the report by Rau et al., serum IL-18 levels increased in the early phase and remained high until the end of the observation period in patients with pancreatic necrosis and systemic complications.[27] Our study also revealed that elevation of serum IL-18 levels continued during 4 weeks after admission. In severe acute pancreatitis, IL-18 may be a key regulator of the inflammatory response including hepatocellular injury in the early phase and an important modulator of innate and acquired immune responses (mainly helper T cell response) in the late phase. Melnikov et al. demonstrated that impaired IL-18 processing protected caspase-1-deficient mice from ischemic acute renal failure.[39] Blockade of IL-18 in the early phase may be useful as a new therapeutic option against not only hepatic dysfunction but also renal dysfunction in severe acute pancreatitis. Furthermore, administration of IL-18 or reinforcement of production of IL-18 in the late phase may be of therapeutic importance for immunostimulation against subsequent infectious complications in this disease.

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