Immunosuppression and the infection in patients with early SAP

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1. ABSTRACT

Few data are available on the relationship between immune response and the infection caused by gut mucosal barrier dysfunction in patients with severe acute pancreatitis (SAP). The aim of this study was to investigate the immune response to gut mucosal barrier dysfunction in patients with early SAP. The results showed that the levels of endotoxin, the lactulose/mannitol (L/M) ratio, the D(-)-lactate concentration, the proportion of HLA-DR-positive monocytes, and the expression levels of TNF-α, IL-6 and IL-10 all decreased from a high level while the frequency of Tregs increased during the first 14 days. The Th1/Th2 ratio was decreased, with a decreased Th1 and an increased Th2 profile, in the beginning, but it was subsequently increased, with an increased Th1 profile. The data from this study showed that immunosuppression, the shift of the Th1/Th2 balance toward a Th2 response, increased Tregs, and related inflammatory cytokines are involved in the complex process of inflammation and infection caused by gut mucosal barrier dysfunction in patients with early SAP.

2. INTRODUCTION

Approximately 15%-20% of patients with acute pancreatitis will develop severe acute pancreatitis (SAP) (1), and the mortality rate of SAP patients is close to 30%-40% (2-4). The major cause of death is organ failure, which complicates SAP in 20%-80% of cases (5, 6). Infected pancreatic necrosis is the most severe complication in patients with SAP and is associated with systemic inflammatory response syndrome (SIRS), sepsis, and multiple organ failure (MOF). The failure of intestinal barrier function is most likely responsible for the occurrence of these phenomena (7-10). Previous studies have shown that translocated intestinal bacteria can cause necrotic pancreas. Furthermore, bacterial endotoxins and antigens invade the portal circulation and generate cytokines, causing multiple organ failure syndrome (MODS) (11, 12). Thus, the dysfunction of the gut mucosal barrier is crucial in the systemic inflammation of SAP patients.

Systemic inflammation is characterized by a decrease in the monocyte surface expression of HLA-DR

antigens (13-15), which leads to immune suppression (16), and the activation of circulating cells of the immune system, including monocytes (17) and CD4+ T-helper (Th) lymphocytes (18). Both experimental (19) and clinical (20) studies suggest that the host's defense against infection is depressed because Th1 cells become more strongly suppressed than Th2 cells in the course of AP, leading to Th1/Th2 cytokine imbalance. In addition, a previous study found that in the peripheral blood of patients with autoimmune pancreatitis, circulatory naïve (CD45RA+) regulatory T-cells (Tregs) are significantly decreased, whereas memory (CD45RA-) Tregs in most of the population are significantly increased (21). In patients with different stages of SAP, the expression of Tregs continues to increase (22). However, the dynamic changes of Tregs levels at the early stage of SAP have not been investigated. The relationship between Tregs and infection related to gut mucosal barrier dysfunction has also not been investigated.

The activation of those immune cells results in the systemic production of pro- and anti-inflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-10 (23). IL-10 promotes immune suppression, increasing the risk of secondary infections and MODS (24-27). These cytokines could reflect inflammation in patients.

Both pro- and anti-inflammatory factors, including immune cells and cytokines, participate in the pathogenesis of SAP. However, the relationship between the immune response in inflammation and infection caused by gut mucosal barrier dysfunction is still unclear in the early stage of SAP. Thus, the aim of this study was to investigate the immune response to the infection caused by gut mucosal barrier dysfunction in SAP.

3. MATERIALS AND METHODS

3.1. Study subjects

The study population included SAP patients admitted to the intensive care unit (ICU) from January 2010 to December 2011. SAP was diagnosed using criteria based on the Consensus of the International Symposium on Acute Pancreatitis (Atlanta definition). The inclusion criteria for the study were defined as follows: (1) the age of the patients ranged from 18–80 years and (2) the interval between the onset of typical abdominal symptoms and study inclusion was 24 hours or less. Patients were excluded if they (1) had evidence or a known history of renal dysfunction (creatinine >1.5 mg/dl); (2) were pregnant or lactating; (3) were expected to receive an intervention involving dialysis, plasmapheresis, or other physiologic support requiring extracorporeal blood removal; (4) were suffering from inflammatory bowel disease; (5) had infections at the time of admission to the hospital; or (6) received recent nonsteroidal anti-inflammatory drugs. This study protocol was approved by the Ethics Committee of Medical School of Nantong University, and informed consent was obtained from all study subjects.

Seventy-seven patients who met the inclusion criteria were recruited in the study. Thirty-one participants in this trial died within two weeks after admission. Thus, in total, 46 patients (male=31, female=15) aged 21-68 years (mean=43.5 years) were included in the study.

All SAP patients were treated according to our standard protocol for the management of pancreatitis and the practice guidelines for SAP (28). The levels of endotoxin, TNF-α, IL-6, IL-10, and Th1/Th2, the percentage of HLA-DR and CD4+CD25+Tregs, the ratio of urinary lactulose/mannitol (L/M), and the concentration of D-lactate in the peripheral blood were measured on the 1st (Day 1), 3rd (Day 3), 7th (Day 7) and 14th (Day 14) day after admission.

3.2. Endotoxin analysis

For the chromogenic substrate limulus amebocyte lysate (LAL) assay, 1 ml peripheral blood was extracted from each patient. Peripheral blood samples were diluted 1:10 with pyrogen-free (PF) water containing 100 μg heparin. Endotoxin concentrations in the samples were calculated according to a standard curve. Samples were centrifuged (10 min at 2000 rpm), and supernatants were transferred into PF cryotubes.

The standard curve ranged from 0.05 to 5 endotoxin units (EU)/ml of standard endotoxin. The absorbance in each well was measured at 405 nm every 30 s for 90 min (SpectraMax 340; Molecular Devices, Inc., Sunnyvale, CA). Endotoxin determinations were based upon the maximum slope of the absorbance-versus-time plot for each well. The endotoxin value for a sample was calculated from the arithmetic mean of dilutions that fell in the middle two thirds of the standard curve.

3.3. Urinary lactulose/mannitol (L/M)

To evaluate small intestinal permeability to lactulose and mannitol, the urinary excretion of lactulose and mannitol of participating patients was measured by high-performance liquid chromatography (HPLC). For the permeability test, an oral dose of 5 g lactulose and 1 g mannitol in a 20-ml solution was followed by a 6-hour urine collection of 3 ml per patient. The concentrations of urinary lactulose and mannitol were measured by HPLC as described by Barboza *et al.* (29).

3.4. D(-)-lactate determination

The plasma from systemic blood samples was obtained and subjected to a deproteination and neutralization process by acid/base precipitation using perchloric acid and potassium hydroxide. The protein-free plasma was then assayed for D(-)-lactate concentration by an enzymatic-spectrophotometric method with minor modifications (30).

3.5. ELISA

The concentrations of TNF-α, IL-6, and IL-10 in peripheral blood were measured by ELISAs in accordance with the manufacturer's instructions (eBioscience).

3.6. Flow cytometry

For the analysis of Th1 and Th2 cells, the cell suspension was stimulated with 20 ng/ml phorbol 12-myristate-13-acetate and 1 μg/ml ionomycin in the presence
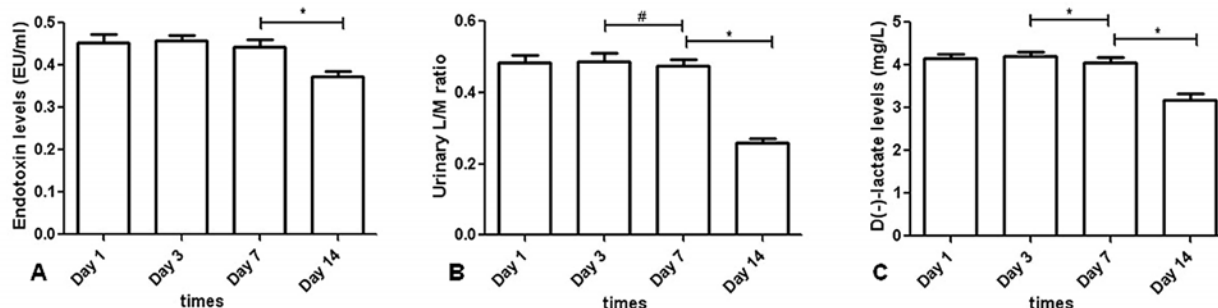
Figure 1. The levels of endotoxin, the urinary L/M ratio and D(-)-lactate concentration. (A) The endotoxin level was high from Day 1 to Day 7 and then decreased on Day 14. (B) The urinary L/M ratio was higher on Day 1 and Day 3 than Day 7; on Day 14, the ratio was significantly decreased. (C) The D(-)-lactate level was increased on Day 1 and Day 3 compared with Day 7; on Day 14, the level continued to decrease. * P<0.01, # P<0.05.

of 2 mmol/ml monensin (Sigma-Aldrich, USA) in 24-well plates. After 4 hours of culture (37°C; 5% CO_2), the cells were transferred to tubes and washed once in phosphate-buffered saline (PBS). The cells were then incubated with phycoerythrin-cy5 (PE-cy5)-conjugated antihuman CD4 (BD Pharmingen, USA) at 4°C for 30 minutes. After surface staining, the cells were fixed and permeabilized according to the manufacturer's instructions and stained with fluorescein isothiocyanate (FITC)-conjugated antihuman interferon (IFN)-γ (BD Pharmingen) plus phycoerythrin-conjugated antihuman interleukin (IL)-4 (BD Pharmingen).

For the analysis of Treg cells, the cell suspension was transferred into tubes and washed once in PBS. The cells were stained with FITC-conjugated antihuman CD4 and allophycocyanin-conjugated antihuman CD25 at 4°C for 30 min. The cells were then incubated with PE-conjugated antihuman Foxp3 after fixation and permeabilized according to the manufacturer's instructions. All of the antibodies and reagents were purchased from eBioscience.

The monocyte surface expression of HLA-DR, expressed as the proportion (%) of monocytes that were positive for HLA-DR fluorescence, was determined as described previously (31).

3.7. Statistical analysis

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) 16.0 (SPSS Inc., Chicago, IL, USA). Group data are expressed as the mean±std. deviation (SD). A paired samples *t*-test was used to evaluate differences in blood and urine sample parameters on different days. Correlation coefficients were calculated using Spearman's rank method. P values <0.05 were considered to be statistically significant.

4. RESULTS

4.1. Endotoxin, urinary L/M ratio and D(-)-lactate concentration

The level of endotoxin was high from Day 1 to Day 7 (0.45±0.13 (EU/ml) on Day 1, 0.46±0.09 (EU/ml) on Day 3, and 0.44±0.12 (EU/ml) on Day 7), and it was

decreased on Day 14 (0.37±0.08 (EU/ml); Day 14 versus Day 7, t=8.387, P<0.001) (Figure 1-A).

The urinary L/M ratio was higher on Day 1 (0.48±0.15) and Day 3 (0.49±0.15) than on Day 7 (0.47±0.13) (Day 3 versus Day 7, t=2.508, P=0.016). On Day 14, the ratio (0.26±0.07) was significantly decreased (Day 7 versus Day 14, t=18.982, P<0.001) (Figure 1-B).

The concentration of D(-)-lactate was increased on Day 1 (4.16±0.67 (mg/L)) and Day 3 (4.19±0.72 (mg/L)) compared with Day 7 (4.07±0.77 (mg/L); Day 3 versus Day 7, t=4.603, P=0.016). On Day 14, the D(-)-lactate concentration (3.18±0.94 (mg/L)) continued to decrease (Day 14 versus Day 7, t=10.322, P<0.001) (Figure 1-C).

When all of the subjects were considered over 14 days, the correlations between endotoxin level and the L/M ratio (R=0.879, P<0.001) (Figure 6-A), endotoxin and D(-)-lactate concentration (R=0.831, P<0.001) (Figure 6-B), and L/M ratio and D(-)-lactate concentration (R=0.833, P<0.001) (Figure 6-C) were positive.

4.2. Decreased expression of HLA-DR-positive monocytes

The proportion of HLA-DR-positive monocytes in all patients on Day 3 (68.17%±19.52%) was lower than on Day 1 (79.11%±19.67%) (t=10.891, P<0.001), and the proportion on Day 7 (57.50%±18.90%) continued to decrease (Day 7 versus Day 3, t=17.692, P<0.001). The proportion on Day 14 (56.99%±18.82%) showed a trend of decrease, although it was not significant (P>0.05 compared with Day 7). These results showed that the proportion of HLA-DR-positive monocytes decreased significantly until Day 7, while the trend of decrease gradually lessened after Day 7 (Figure 2).

When all of the subjects were considered over 14 days, the correlations between the L/M ratio and the proportion of HLA-DR-positive monocytes (R=0.752, P<0.001) (Figure 6-D) and D(-)-lactate concentration and the proportion of HLA-DR-positive monocytes (R=0.759, P<0.001) (Figure 6-E) were both negative.
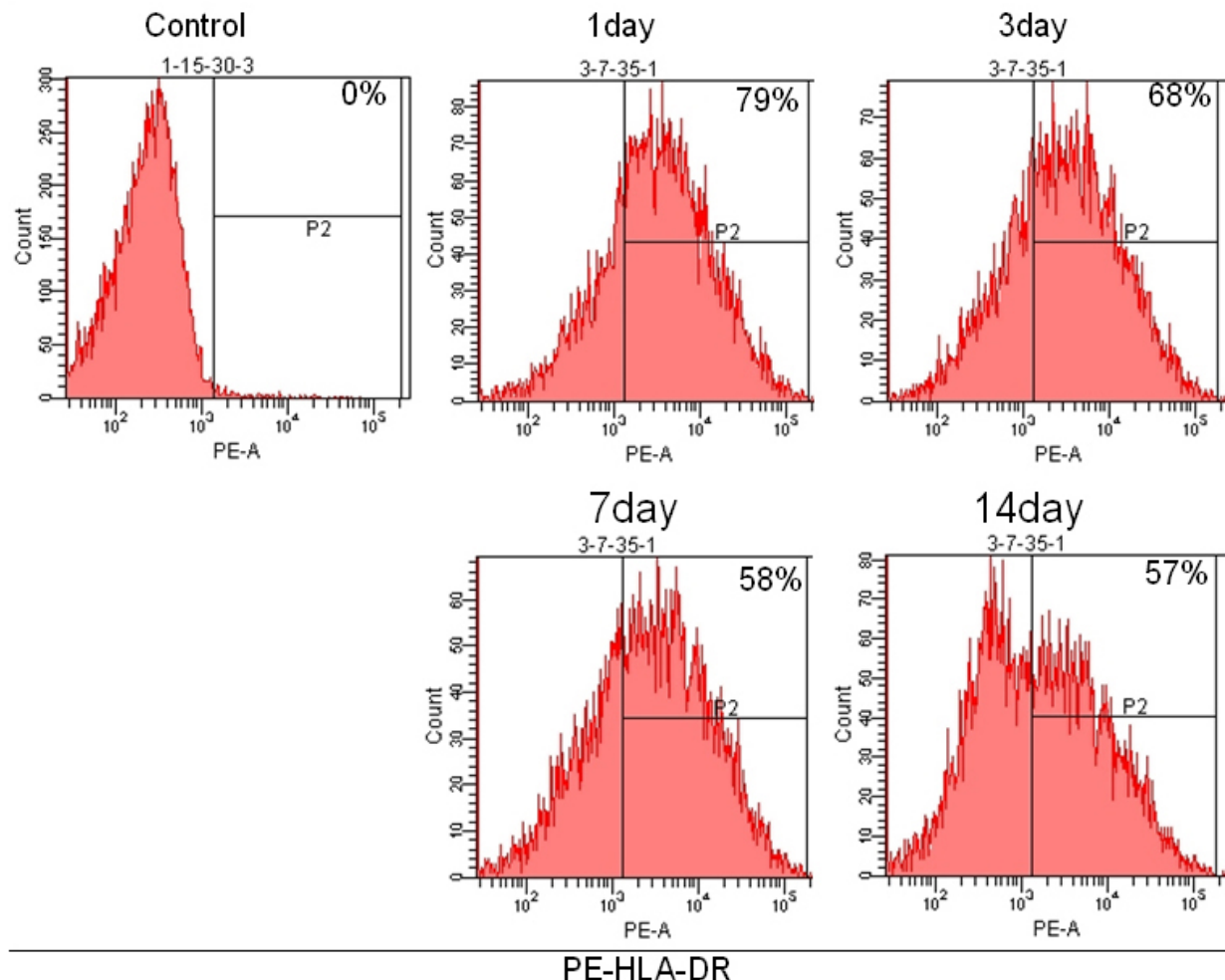
Figure 2. The expression of HLA-DR-positive monocytes in patients with SAP. The proportion of HLA-DR-positive monocytes in all patients on Day 3 was lower than on Day 1 (t=10.891, P<0.001), and the proportion on Day 7 continued to decrease (Day 7 versus Day 3, t=17.692, P<0.001). The proportion on Day 14 showed a trend of decrease, although it was not significant (P>0.05 compared with Day 7).

4.3. Th1/Th2 imbalance in SAP

The frequency of Th1 (CD4+IFN-γ+ cells) was significantly decreased in the initial days of SAP (16.0%±2.7% (Day 1) versus 18.2%±2.3% (Day 3), t=2.424, P=0.019). The frequency on Day 7 (18.8%±2.2%) was not significantly different from Day 3, while the frequency on Day 14 (28.2%±2.2%) was higher than Day 7 (t=-13.466, P<0.001) and even higher than Day 1 (t=10.075, P=0.026). These results indicated that the rate of Th1 decline was sharp in the first days but gradually slowed down and even reversed over time (Figure 3).

In contrast, the frequency of Th2 cells (CD4+IL-4+ cells) was increased in the beginning of SAP (2.9%±1.0 % (Day 1) versus 3.1%±1.0% (Day 3), t=-9.429, P<0.001). The frequency on Day 7 (3.1%±1.0%) was not significantly different from Day 3, while the frequency on Day 14 (3.9%±1.2%) was higher than on Day 7 (t=-4.399P<0.001) and even higher than on Day 1 (t=-5.205, P<0.001). These results showed that the number of Th2 cells in peripheral blood was significantly increased in the beginning but lessened over the course of SAP (Figure 3).

An imbalance in Th1/Th2 cells was present in SAP patients, especially in the first week. The Th1/Th2 ratio was significantly lower on Day 3 (6.1%±0.4%) than on Day 1 (6.67%±0.5%) (t=4.262, P<0.001). However, there were no differences between Day 3 and Day 7 (6.1%±0.4%). On Day 14, the ratio was significantly increased (7.2%±0.5%) compared with Day 7 (t=-9.969, P<0.001). These results showed that the Th1/Th2 ratio was decreased, with a decreased Th1 and an increased Th2 profile, in the beginning but subsequently increased, with an increased Th1 profile.

When all of the subjects were considered over 14 days, the correlations between the L/M ratio and Th1/Th2 ratio (R=-0.815, P<0.001) (Figure 6-F) and D(-)-lactate concentration and Th1/Th2 ratio (R=-0.783, P<0.001) (Figure 6-G) were both negative.
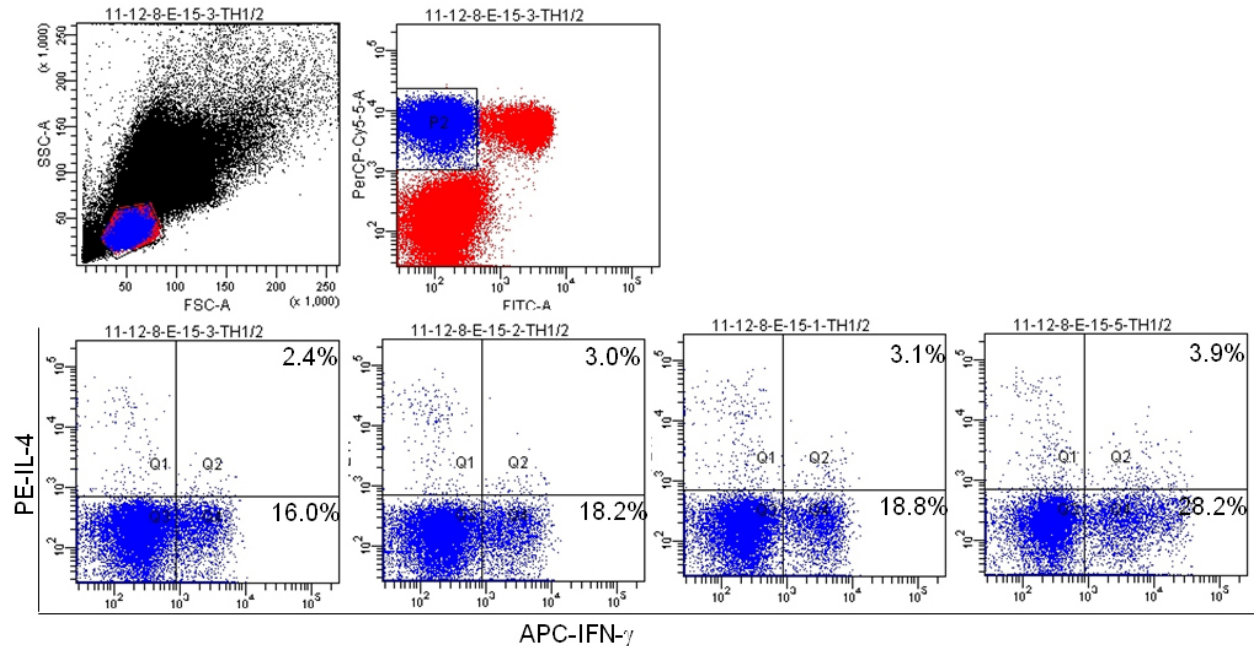
Figure 3. The number of Th1 and Th2 cells in patients with SAP. The frequency of Th1 cells (CD4+IFN-γ+ cells) in SAP was significantly decreased on Day 1 versus Day 3 (t=2.424, P=0.019). The frequency on Day 7 was not significantly different from Day 3, while the frequency on Day 14 was higher than on Day 7 (t=-13.466, P<0.001) and even higher than on Day 1 (t=10.075, P=0.026). These results indicated that the rate of Th1 decline was sharp in the first days but gradually decreased and even reversed over time. The frequency of Th2 cells (CD4+IL-4+ cells) in SAP was increased on Day 1 versus Day 3 (t=-9.429, P<0.001). The frequency on Day 7 was not significantly different from Day 3, while the frequency on Day 14 was higher than on Day 7 (t=-4.399P<0.001) and even higher than on Day 1 (t=-5.205, P<0.001). These results showed that the number of Th2 cells in peripheral blood was significantly increased in the beginning, but the rate decreased over the course of SAP.

4.4. High levels of Treg cells in SAP

The frequency of Tregs was determined in the population of CD4+CD25+ cells. The frequency of Tregs (CD4+CD25+Foxp3+ cells) was higher on Day 7 (30.9‰±7.8%) than on Day 1 (20.1‰±6.3%) and Day 3 (21.5‰±3.8%; Day 3 versus Day 7, t=-5.763, P<0.001). On Day 14, this frequency continued to increase (37.7%±7.1%; Day 7 versus Day 14, t=-15.455, P<0.001) (Figure 4).

When all of the subjects were considered over 14 days, the correlation between the L/M ratio and the proportion of Tregs (R=-0.189, P=0.010) (Figure 6-H) was negative.

4.5. Plasma TNF-α, IL-6 and IL-10 levels

The level of TNF-α was high on Day 1 (75.35±9.32 (pg/ml)) and Day 3 (75.20±9.27 (pg/ml)) and decreased on Day 7 (72.09±10.05 (pg/ml); Day 3 versus Day 7, t=7.619, P<0.001). On Day 14, the TNF-α level was significantly decreased (64.57±10.83 (pg/ml)) compared with Day 7 (t=19.027, P<0.001) (Figure 5-A).

The level of IL-6 was also increased on Day 1 (38.40±6.19 (pg/ml)) and Day 3 (38.42±0.93 (pg/ml)) compared with Day 7 (34.74±7.26 (pg/ml); Day 3 versus Day 7, t=6.294, P<0.001). On Day 14, the IL-6 level continued to decrease (28.79±6.74 (pg/ml); Day 7 versus Day 14, t=10.563, P<0.001) (Figure 5-B).

The level of IL-10 was higher on Day 1 (16.50±3.06 (pg/ml)) than on Day 3 (15.92±2.72 (pg/ml); Day 1 versus Day 3, t=6.907, P<0.001). During the course of SAP, the IL-10 level continued to decrease from 15.38±2.45 (pg/ml) on Day 7 to 13.38±2.61 (pg/ml) on Day 14 (Day 3 versus Day 7, t=6.256, P<0.001; Day 7 versus Day 14, t=19.117, P<0.001) (Figure 5-C).

When all of the subjects were considered over 14 days, the proportion of HLA-DR-positive monocytes was positively correlated with levels of TNF-α (R=0.826, P<0.001), IL-6 (R=0.840, P<0.001) and IL-10 (R=0.799, P<0.001).

5. DISCUSSION

In the present study, we monitored the gut mucosal barrier dysfunction, the relative abundance of HLA-DR-positive monocytes, Th1, Th2 and CD4+CD25+ Treg cells, and the secretion patterns of pro- and anti-inflammatory cytokines, including TNF-α, IL-6, and IL-10, in patients with SAP during the first two weeks of SAP occurrence. In all subjects, the endotoxin level, L/M ratio and D(-)-lactate concentration decreased continuously during the first two weeks of SAP. The proportion of HLA-DR-positive monocytes decreased while the frequency of Tregs increased from Day 1 to Day 14. The Th1/Th2 ratio decreased in the first week but increased by the end of the second week. The levels of TNF-α, IL-6, and IL-10 were
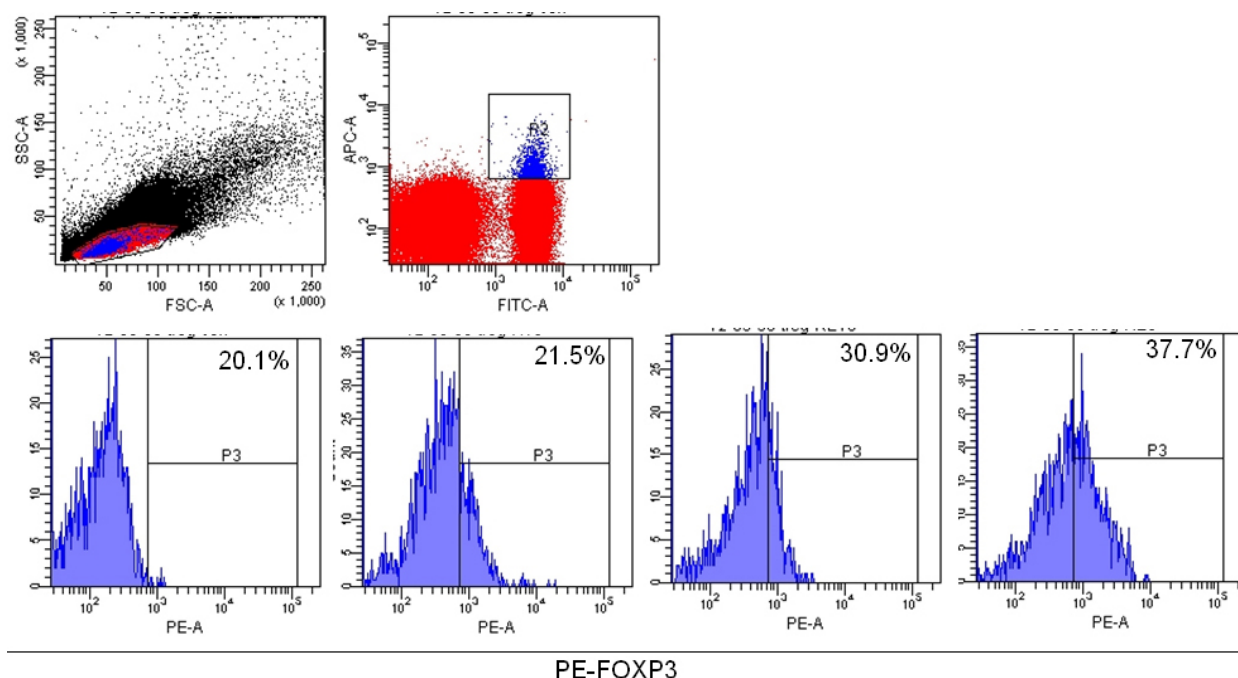
PE-FOXP3

Figure 4. The level of Tregs in patients with SAP. The frequency of Tregs (CD4+CD25+Foxp3+ cells) in the population of CD4+CD25+ cells was higher on Day 7 than Day 1 and Day 3 (Day 3 versus Day 7, t=-5.763, P<0.001). On Day 14, this frequency continued to increase (Day 7 versus Day 14, t=-15.455, P<0.001).

continuously decreased. These results suggested that immunosuppression and immune imbalance could play a role in systemic inflammation, which is related to the infection caused by dysfunction of the gut mucosal barrier.

The gut serves as an "intestinal barrier" to resist antigens (32, 33). When this barrier is broken down or overwhelmed, pathogenic bacteria and their antigens enter the blood, resulting in sepsis, the initiation of cytokine-mediated SIRS, MODS and even death (34-36). The present study showed that the L/M ratio and D(-)-lactate concentration were continuously increased, especially at the end of the first week. The endotoxin level, L/M ratio and D(-)-lactate concentration were positively correlated. These parameters reflect intestinal permeability and gut barrier dysfunction, which could facilitate bacterial translocation. The high levels of endotoxin in the first week confirmed bacterial translocation in these SAP patients. In these subjects, the L/M ratio, D (-)-lactate concentration and endotoxin level peaked on the third day, were maintained on the 7th day, and then decreased by the end of the second week. These results reflected the development of intestinal permeability, gut barrier dysfunction and bacterial translocation at the beginning of SAP.

Once endotoxins cross the mucosal barrier, they can cause systemic inflammation and trigger the immune response. Systemic inflammation in SAP is concomitantly associated with rapidly strengthening compensatory anti-inflammatory response syndrome (CARS) (37, 38). However, CARS can lead to immune suppression, which renders the host susceptible to secondary infections. In immunosuppression, monocytes are characterized by

markedly reduced human HLA-DR expression. A clinical study reported that among 25 patients with SAP, monocyte HLA-DR expression was consistently decreased at the early stage of the disease during 11 days of follow-up and recovered during the follow-up in survivors but not in non-survivors (39). Our results confirmed and further demonstrated immunosuppression in SAP patients by showing a decreased expression of HLA-DR-positive monocytes. The HLA-DR-positive monocyte frequency was positively correlated with the dysfunction of the gut mucosal barrier. This result suggested that immunosuppression in these patients may have already occurred on admission or rapidly developed after admission to the hospital. Immunosuppression developed in the space of the dysfunction of the gut mucosal barrier.

In the present study, we observed an imbalance in Th1/Th2. In the first week, the frequency of Th1 was decreased and researched its minimum at the 7th day, while the frequency of Th2 was increased. On the 14th day, the decrease in Th1 frequency was reversed; the frequency of Th2 continued increasing but not as significantly as Th1. In the first 3 days, the ratio of Th1/Th2 decreased quickly. Then, the rate gradually decreased, and the ratio was increased in the second week. These results suggested a shift from Th1 to Th2 in patients with SAP in the first week, and then the Th1 response became strong. This shift was correlated with dysfunction of the gut mucosal barrier.

In addition, we observed the expression of Tregs in all subjects. Over 2 weeks, the frequency of Tregs continued to increase. Tregs can actively suppress the activation of the immune system and prevent pathological
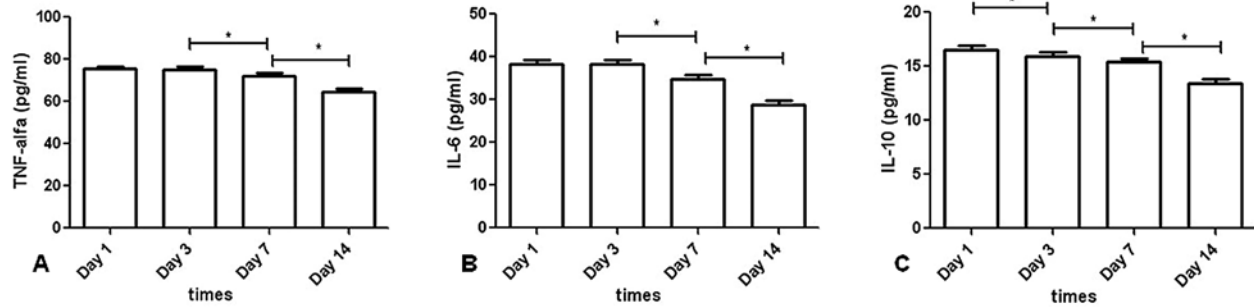
Figure 5. The levels of TNF-α, IL-6 and IL-10. (A) TNF-α levels were high on Day 1 and Day 3 but decreased on Day 7; on Day 14, the TNF-α level was significantly decreased compared with Day 7. (B) The level of IL-6 was increased on Day 1 and Day 3 compared with Day 7; on Day 14, the IL-6 level continued to decrease. (C) The level of IL-10 was higher on Day 1 than on Day 3; during the course of SAP, the IL-10 level continued to decrease on Day 7 and Day 14. * P<0.01.
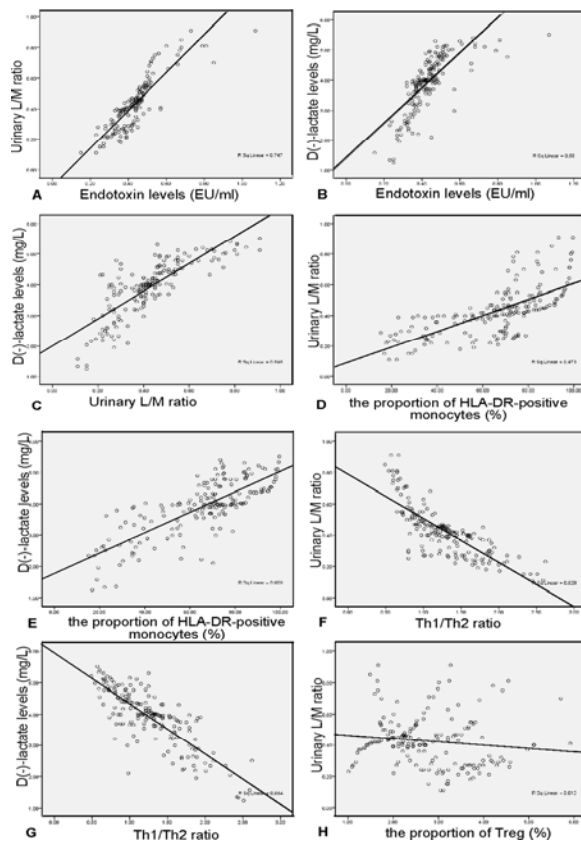

Figure 6. Correlations. When all of the subjects were considered over 14 days, correlations were found between (A) endotoxin levels and the L/M ratio, (B) endotoxin levels and D(-)-lactate levels, (C) the urinary L/M ratio and D(-)-lactate levels, (D) the proportion of HLA-DR-positive monocytes and urinary L/M ratio, (E) the proportion of HLA-DR-positive monocytes and D(-)-lactate levels, (F) the Th1/Th2 ratio and urinary L/M ratio, (G) the Th1/Th2 ratio and D(-)-lactate concentration, and (H) the proportion of Tregs and urinary L/M ratio. The data indicate positive associations in (A), (B), (C), (D) and (E) and a negative association in (F), (G) and (H). The data were analyzed by Spearman's rank correlation coefficient.

self-reactivity, playing anti-inflammatory and immunomodulatory roles (40, 41). Because immunosuppression had been observed in these subjects, as mentioned above, these increased Tregs may play a role in immunosuppression through inhibiting immune responses against inflammation in SAP patients. The increased expression of Tregs in the periphery may be a compensatory response to systemic inflammation as a protective response of the body. However, the network of this immune response involving Th1, Th2 and Tregs is complex, and further studies are needed to investigate its precise mechanism, which would provide information for inflammation therapy in SAP.

In this study, the levels of TNF-α, IL-6 and IL-10 were all initially high during the first week of SAP and then decreased. The levels of these cytokines were positively correlated with HLA-DR. Thus, the profound reduction of these inflammatory cytokines may reflect immunosuppression in these SAP patients. IL-10, the most potent anti-inflammatory cytokine, could be responsible for the decreased monocyte HLA-DR expression in these subjects (42). The high level of the anti-inflammatory cytokine IL-10 may follow the increase in the pro-inflammatory factors TNF-α and IL-6, which might be a part of the CARS (43-45).

The data from this study showed that immunosuppression, the shift of the Th1/Th2 balance toward a Th2 response, increased Tregs, and related inflammatory cytokines are involved in the complex process of inflammation and infection caused by gut mucosal barrier dysfunction in patients with early SAP. The network of immune cells and various cytokines participating in the regulation of the inflammatory processes is complex, and the precise mechanism underlying this process should be the focus of further study in the future.

6. ACKNOWLEDGMENTS

7. REFERENCES

1. SS Vege, ST Chari. Severe acute pancreatitis: impact of organ failure on mortality. *Indian Journal of Gastroenterology*, (2006)

2. Abu-Zidan FM, Bonham MJ, Windsor JA. Severity of acute pancreatitis: a multivariate analysis of oxidative stress markers and modified Glasgow criteria. *Br J Surg* 87, 1019-1023 (2000)

3. Isaji S, Takada T, Kawarada Y, Hirata K, Mayumi T, Yoshida M, Sekimoto M, Hirota M, Kimura Y, Takeda K, Koizumi M, Otsuki M, Matsuno S. JPN Guidelines for the management of acute pancreatitis: surgical management. *J Hepatobiliary Pancreat Surg* 13, 48-55 (2006)

4. Triester SL, Kowdley KV. Prognostic factors in acute pancreatitis. *J Clin Gastroenterol* 34, 167-176 (2002)

5. de Beaux AC, Palmer KR, Carter DC. Factors influencing morbidity and mortality in acute pancreatitis; an analysis of 279 cases. *Gut* 37, 121-126 (1995)

6. Tenner S, Sica G, Hughes M, Noordhoek E, Feng S, Zinner M, Banks PA. Relationship of necrosis to organ failure in severe acute pancreatitis. *Gastroenterology* 113, 899-903 (1997)

7. Erstad BL. Enteral nutrition support in acute pancreatitis. *Ann Pharmacother* 34, 514-521 (2000)

8. Papapietro K,MarinM, Diaz E,Watkins G, Berger Z, Rappoport J. Digestive refeeding in acute pancreatitis. When and how? *Rev Med Chil* 129, 391-396 (2001)

9. Fang J, DiSario JA. Nutritionalmanagement of acute pancreatitis. *Curr Gastroenterol Rep* 4, 120-127 (2002)

10. Abou-Assi S, Craig K, O'Keefe SJ. Hypocaloric jejunal feeding is better than total parenteral nutrition in acute pancreatitis: results of a randomized comparative study. *Am J Gastroenterol* 97, 2255-2262 (2002)

11. Olah A, Pardavi G, Belagyi T, Nagy A, Issekutz A, Mohamed GE. Early nasojejunal feeding in acute pancreatitis is associated with a lower complication rate. *Nutrition* 18, 259-262 (2002)

12. Dejong CH, Greve JW. Nutrition in patients with acute pancreatitis. *Curr Opin Crit Care* 7, 251-256 (2001)

13. Ditschkowski M, Kreuzfelder E, Rebmann V, Ferencik S, Majetschak M, Schmid E, et al. HLA-DR expression and soluble HLA-DR levels in septic patients after trauma. *Ann Surg* 229, 246–54. (1999)

14. Volk H-D, Reinke P, Krausch D, Zuckermann H, Asadullah K, Mulle JM, et al. Monocyte deactivation— rationale for a new therapeuti c strategy in sepsis. *Int Care Med* 22 Suppl 4, S474–81 (1996)

15. Heumann D, Glause MP, Calandra T. Monocyte deactivation in septic shock. *Curr Opin Infect Dis* 11, 27 9-83. (1998)

16. Do¨cke W, Randow F, Syrbe U, Krausch D, Zuckermann H, Asadullah K, et al. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. *Nature Med* 3, 678–81. (1997)

17. Kylanpaa ML, Repo H, Puolakkainen PA: Inflammation and immunosuppression in severe acute pancreatitis. *World J Gastroenterol* 16, 2867-2872. (2010)

18. Pezzilli R, Billi P, Gullo L, Beltrandi E, Maldini M, Mancini R, Incorvaia L, Miglioli M: Behavior of serum soluble interleukin-2 receptor, soluble CD8 and soluble CD4 in the early phases of acute pancreatitis. *Digestion* 55, 268-273. (1994)

19. Ueda T, Takeyama Y, Yasuda T, Takase K, Nishikawa J, Kuroda Y: Functional alterations of splenocytes in severe acute pancreatitis. *J Surg Res* 102, 161-168. (2002)

20. Pietruczuk M, Dabrowska MI, Wereszczynska-Siemiatkowska U, Dabrowski A: Alteration of peripheral blood lymphocyte subsets in acute pancreatitis. *World J Gastroenterol* 12, 5344-5351. (2006)

21. Miyoshi H, Uchida K, Taniguchi T, Yazumi S, Matsushita M, Takaoka M, et al. Circulating naive and CD4CD25high regulatory T cells in patients with autoimmune pancreatitis. *Pancreas* 36, 133–40. (2008)

22. RONG Zhong-hou, WU He-shui, YANG Zhi-yong, WANG Chun-you. Dynamic changes of CD4 + CD25 high regulatory T cells in peripheral blood of severe acute pancreatitis patients and its significance. *Chin J Gen Surg*. 25(12) , 992-994. (2010)

23. Davies MG, Hagen PO: Systemic inflammatory response syndrome. *Br J Surg* 84, 920-935. (1997)

24. Garcia-Sabrido JL, Valdecantos E, Bastida E, Tellado JM: The anergic state as a predictor of pancreatic sepsis. *Zentralbl Chir* 114, 114-120. (1989)

25. Richter A, Nebe T, Wendl K, Schuster K, Klaebisch G, Quintel M, Lorenz D, Post S, Trede M: HLA-DR expression in acute pancreatitis. *Eur J Surg* 165, 947-951. (1999)

26. Kylanpaa-Back ML, Takala A, Kemppainen E, Puolakkainen P, Kautiainen H, Jansson SE, Haapiainen R, Repo H: Cellular markers of systemic inflammation and immune suppression in patients with organ failure due to severe acute pancreatitis. *Scand J Gastroenterol* 36, 1100-1107. (2001)

27. Mentula P, Kylanpaa ML, Kemppainen E, Jansson SE, Sarna S, Puolakkainen P, Haapiainen R, Repo H: Early

prediction of organ failure by combined markers in patients with acute pancreatitis. *Br J Surg* 92, 68-75. (2005)

28. Banks PA, Freeman ML. Practice guidelines in acute pancreatitis. *Am J Gastroenterol* 101, 2379-2400. (2006)

29. Barboza JM, Silva TM, Guerrant RL, Lima AA. Measurement of intestinal permeability using mannitol and lactulose in children with diarrheal diseases. *Brazilian J Med Biolog Res* 32, 1499-504. (1999)

30. Brandt RB, Siegel SA, Waters MG, Bloch MH. Spectrophotometric assay for D(-)-lactate in plasma. *Anal Biochem*, 102, 39-46 (1980)

31. Kylanpaa-Back ML, Takala A, Kemppainen E, Puolakkainen P, Kautiainen H, Jansson SE, Haapiainen R, Repo H: Cellular markers of systemic inflammation and immune suppression in patients with organ failure due to severe acute pancreatitis. *Scand J Gastroenterol* 36, 1100-1107. (2001)

32. Nagpal K, Minocha VR, Agrawal V, Kapur S. Evaluation of intestinal mucosal permeability function in patients with acute pancreatitis. *Am J Surg* 192, 24-28. (2006)

33. Magnotti LJ, Deitch EA. Burns, bacterial translocation, gut barrier function, and failure. *J Burn Care Rehabil* 26:383-391. (2005)

34. Penalva JC, Martínez J, Laveda R, Esteban A, Muñoz C, Sáez J, et al. A study of intestinal permeability in relation to the inflammatory response and plasma endocab IgM levels in patients with acute pancreatitis. *J Clin Gastroenterol* 38, 512-517. (2004)

35. Liu H, Li W, Wang X, Li J, Yu W. Early gut mucosal dysfunction in patients with acute pancreatitis. *Pancreas* 36, 192-196. (2008)

36. Berg RD, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in the gnotobiotic mouse model. *Infect Immun* 23, 403-411. (1979)

37. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. *Crit Care Med* 24, 1125-1128 (1996)

38. Mentula P, Kylänpää ML, Kemppainen E, Jansson SE, Sarna S, Puolakkainen P, Haapiainen R, Repo H. Plasma antiinflammatory cytokines and monocyte human leucocyte antigen-DR expression in patients with acute pancreatitis. *Scand J Gastroenterol* 39,178-187 (2004)

39. Richter A, Nebe T, Wendl K, Schuster K, Klaebish G, Quintel M, et al. HLA-DR expression in acute pancreatitis . *Eur J Surg* 165, 947-51 (1999)

40. Bluestone JA, Tang Q. How do CD4$^+$CD25$^+$ regulatory T cells control autoimmunity? *Curr Opin Immunol* 17, 638–642. (2005)

41. Jiang H, Chess L. Regulation of immune responses by T cells. *N Engl J Med* 354, 1166–1176. (2006)

42. Fumeaux T, Pugin J. Role of interleukin-10 in the intracel 36 Fumeaux T, Pugin J. Role of interleukin-10 in the intracellular sequestration of human leukocyte antigen-DR in monocytes during septic shock. *Am J Respir Crit Care Med* 166, 1475-1482 (2002)

43. Ni Choileain N, Redmond HP. The immunological consequences of injury. *Surgeon* 4, 23-31(2006)

44. Pietruczuk M, Dabrowska MI, Wereszczynska-Siemiatkowska U, Dabrowski A. Alteration of peripheral blood lymphocyte subsets in acute pancreatitis. *World J Gastroenterol* 12, 5344-5351 (2006)

45. Smith JW, Gamelli RL, Jones SB, Shankar R. Immunologic responses to critical injury and sepsis. *J Intensive Care Med* 21, 160-172 (2006)

Abbreviations: SAP: severe acute pancreatitis; L/M lactulose/mannitol; MOF: multiple organ failure; MODS: multiple organ failure syndrome; Th: T-helper; Tregs: regulatory T-cells; ICU: intensive care unit; HPLC: high-performance liquid chromatography; PBS: phosphate-buffered saline; CARS: compensatory anti-inflammatory response syndrome

Key Words: severe acute pancreatitis, regulatory T-cells, compensatory anti-inflammatory response syndrome

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