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A New Role for the Spleen

Aggravation of the Systemic Inflammatory Response in Rats with Severe Acute Pancreatitis

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Little is known about the role of the spleen in mediating systemic inflammatory responses in severe acute pancreatitis (SAP). We investigated the role played by the spleen in rats after SAP induction. Splenectomy was performed at designated time points after SAP induction. Pancreatic tissue and serum samples were collected and subjected to histologic, immunohistochemical, and immunologic analyses. After SAP induction, the splenic immune response was enhanced during SAP progression, as shown by the increased diameter of the splenic periarterial lymphatic sheath and the thickness of the splenic marginal zone. Rats with splenectomy developed acute pancreatitis more slowly than rats without splenectomy. In addition, pancreatic tissues of rats with splenectomy contained lower levels of serum amylase, tumor necrosis factor-α, and IL-6 and exhibited less acinar cell death, leukocyte infiltration, and interstitial edema than those of rats without splenectomy. Compared with splenectomy alone, cotreatment with splenectomy and the administration of splenic cells originating from a rat with SAP 12 hours after induction increased systemic inflammation in SAP rats. Splenic factors exacerbated SAP-associated liver and lung injury and accentuated intestinal mucosal barrier dysfunction. Splenectomy altered the serum cytokine profile in rats with SAP. In a rat model of SAP, the spleen exacerbated the systematic inflammatory responses and injury to multiple organs, indicating a new role for the spleen in SAP. *(Am J Pathol 2019, 189: 2233–2245; https://doi.org/10.1016/j.ajpath.2019.07.008)*

Inflammatory mediators and the subsequent local or systematic inflammatory responses play critical roles in the pathogenesis of pancreatitis.[1] Although the exact pathophysiologic mechanisms that contribute to the inflammatory response in acute pancreatitis (AP) are largely unknown,[2] the progression of inflammation, both local and systemic, during AP require the participation of inflammatory mediators.[3] An overwhelming systemic immune reaction, called the systemic inflammatory response syndrome (SIRS), seems to be responsible for most systemic complications and mortality.[4,5] Acinar cell autodigestion initiates the inflammatory process in the pancreas.[6,7] Along with leukocytes infiltration into the pancreas and epithelial barrier dysfunction, the inflammatory reaction may progress to

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SIRS or multiple organ dysfunction syndrome, which is believed to be the primary cause of mortality.[8–10]

The spleen is the largest lymphoid organ in the body and contains numerous immune cells that play an important role in the immune response.[11,12] The pancreas and spleen are located in such close proximity that splenic involvement may occur in inflammatory diseases of the pancreas.[13]

Moreover, the results of previous studies suggest that the spleen may participate in the progression of pancreatic disease.[14] Local events in AP can induce the up-regulation of tumor necrosis factor (TNF)-α expression in the spleen, suggesting systemic manifestation of AP.[15] Spleen-derived IL-10 may protect against the development of nonalcoholic fatty pancreas disease.[16] In a case-control study in Taiwan, splenectomy was associated with an increased risk of AP,[17] although the underlying mechanism linking splenectomy and AP has not been completely elucidated. However, splenectomy has been reported to decrease inflammatory cytokine production and alleviate severe acute pancreatitis (SAP).[18] The spleen participates in the progression of AP, but the events triggering this immune reaction and the pathophysiologic mechanism that determines disease severity are poorly understood.[14,19] Therefore, this experiment was designed to investigate the role of the spleen in the mechanism underlying the inflammatory response in SAP progression.

Materials and Methods

Animals and Biological Samples

Male Sprague-Dawley (SD) rats (210 to 230 g) were purchased from the Experimental Animal Centre of Xi'an Jiaotong University Health Science Centre (Xi'an, China). Animals were housed at a temperature of 23°C ± 2°C under a 12-hour light/dark cycle with *ad libitum* access to standard laboratory chow and water. For *in vivo* experiments, animals were deprived of food but were allowed access to water from 12 hours before the start of the experiments. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee at Xi'an Jiaotong University.

In the first experiment, SD rats were randomly divided into three groups, including the control (CON), sham operation (SO), and SAP model (SAP) groups ($n = 8$). The CON group did not undergo any operation. In the SO group, incisions were closed immediately after turning over the pancreas. SAP was induced by retrograde injection of 5% sodium taurocholate into the common biliopancreatic duct as described previously.[20] Pancreatic histopathologic score; serum amylase activity; TNF-α, IL-6, and IL-10 levels; splenic periarterial lymphatic sheath (PALS) diameter; and splenic marginal zone (MZ) thickness were observed 15 hours after surgery.

In the second experiment, SD rats were randomly divided into nine groups, including the CON, SO, SP (splenectomy in normal rats), and SAP groups, and five SAP + SP groups [rats subjected to splenectomy at 0 hours (SAP + 0-hour group), 3 hours (SAP + 3-hour group), 6 hours (SAP + 6-hour group), 9 hours (SAP + 9 hour group), and 12 hours (SAP + 12-hour group) after SAP induction] ($n = 8$). The CON group did not undergo any operation. In the SO group, the incisions were closed immediately after turning over the pancreas and spleen. Pancreatic histopathologic score, serum amylase activity, and TNF-α and IL-6 levels were observed 15 hours after surgery.

In the third experiment, SD rats were randomly divided into five groups, including the CON, SAP, SAP + SP (splenectomy immediately after SAP induction), CON + Spl (normal rats with tail vein injection of a single-cell suspension of splenocytes from the spleens of SAP rats 15 hours after SAP induction), and CON + SP + Spl groups (SAP rats with splenectomy and splenocyte injection immediately after SAP induction) ($n = 8$). The CON group did not undergo any operation. Pancreatic histopathologic score, serum amylase activity, and TNF-α and IL-6 levels were observed 15 hours after surgery.

In the fourth experiment, SD rats were randomly divided into five groups, including the CON, SO, SP, SAP, and SAP + SP groups (splenectomy immediately after SAP induction) ($n = 8$). The CON group did not undergo any operation. In the SO group, the incisions were closed immediately after turning over the pancreas and spleen. Pancreatic histopathologic score, serum amylase and lactate dehydrogenase (LDH) activities, and the levels of TNF-α, IL-6, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (r-GT), bilirubin (BIL), hyaluronic acid (HA), intestinal fatty acid binding protein (iFABP), and plasma endotoxin were observed 15 hours after surgery.

Splenectomy

Splenectomy was performed as described by Zierath et al,[21] with slight modifications. Briefly, anesthesia was induced with an i.p. injection of sodium pentobarbital (50 mg/kg, Merck KGaA, Darmstadt, Germany). The splenic vessels and nerves were ligated, and the spleen was removed through an approximately 0.8-cm midline peritoneal incision.

Splenocytes

The spleen was aseptically removed from rats 15 hours after SAP induction, subjected to gentle mechanical dispersion, and then filtered through a cell strainer with a mesh size of 70-μm.[22] After centrifugation, the resulting cell pellet was resuspended in phosphate-buffered saline, lyzed with ammonium-chloride-potassium lysis buffer, and washed twice with phosphate-buffered saline. Isolated splenocytes (1×10^6 cells/mL) were maintained in 0.9% sodium chloride on ice until use. Splenocytes were injected through the caudal vein at a dose of 2 mL/kg body weight in rats in the

CON + Spl and CON + SP + Spl groups. Rats in the CON, SAP, and SAP + SP groups were given the same amount of normal saline. Splenocyte viability was confirmed by trypan blue staining.

Histopathology and Immunohistochemistry

Histopathologic analysis of the pancreas, spleen, liver, lung, and terminal ileum was performed on hematoxylin and eosin (H&E)–stained sections as described,[20,23–26] and hepatocyte (HC) apoptosis was measured with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining. The number of intestinal intraepithelial lymphocytes (iIELs) was evaluated by light microscopy. Two independent, blinded, experienced pathologists scored pancreatic edema, leukocyte infiltration, and necrosis (0 to 3) in ×10 high-power fields per slide per rat. The individual scores were summed[27] (means ± SEM, ≥6 rats per group). TNF-α and IL-6 expression in the spleen, secretory IgA (sIgA) expression in the terminal ileum, and surfactant protein A (SP-A) expression in the lung were measured by immunohistochemistry.

Enzyme Activity and Cytokine Level Measurement or Serum Analysis

The serum amylase, AST, ALT, r-GT, and BIL levels and LDH activity were determined using an automatic biochemical analyzer (Roche, Basel, Switzerland). Serum IL-6, TNF-α, HA, and iFABP levels were measured using enzyme-linked immunosorbent assays (ELISAs) (Neobioscience Biotech, Shenzhen, China; eBioscience, San Diego, CA; and R&D Systems, Minneapolis, MN) according to the manufacturers' protocols. The absorbance at 450 nm was detected by a microplate mode reader (BioTek Inc, Winooski, VT). Plasma endotoxin levels were measured using an end point chromogenic assay (BioEndo, Xiamen, China) according to the manufacturer's protocol.

Arterial Partial Pressure of Oxygen Measurement

The arterial partial pressure of oxygen (Pao_2) was determined using an automatic blood gas analyzer (AVL Omni, Basel, Switzerland).

Ultrastructural Observation

The liver, lung, and terminal ileum were fixed using 2.5% prechilled glutaraldehyde at 4°C for 24 hours and then immersed in 1% osmium tetroxide at room temperature for 2 hours, followed by immersion in uranyl acetate for 2 hours. The samples were dehydrated through serial solutions of ethanol and acetone before being embedded in epoxy resin. For examination by electron microscopy, 50-nm ultrathin sections were stained with lead citrate and mounted on 200-mesh copper grids. The ultrastructure of the liver, lung, and terminal ileum was observed under a Hitachi H-600 transmission electron microscope (Hitachi, Tokyo, Japan).

Bacterial Translocation Assay

Bacterial cultures of pancreas, liver, kidney, and mesenteric lymph node homogenates were performed to assess bacterial translocation.

Intestinal Mucosal Barrier Dysfunction Evaluation

iFABP served as a marker of intestinal mucosal barrier injury in the early stage of SAP. iIELs, sIgA-positive cells, and bacterial translocation were used to evaluate intestinal mucosal barrier dysfunction.

Biochip Array and ELISA

A rat cytokine array and ELISA were used to investigate the cytokine profiles in the serum and spleen of SAP rats before and after splenectomy.

Statistical Analysis

The results are presented as the means ± SEM of three or more independent experiments. Statistical analysis was performed using a *t*-test or analysis of variance in SPSS statistical software version 19.0 (SPSS Inc., Chicago, IL), and $P < 0.05$ was considered significant.

Results

Enhancement of Splenic Immune Response during SAP Progression

The changes in pancreatic and splenic histopathologic findings, serum amylase activity, and expression of TNF-α, IL-6, and IL-10 in the serum and spleen were determined 15 hours after surgery. The acinar architecture of the SO group was entirely normal. However, SAP group animals exhibited pancreatic injury characterized by gross tissue edema, sublobular hemorrhage, cell lysis associated with obvious neutrophil infiltration, and parenchymal necrosis (Figure 1, A and B). Serum amylase activity and the levels of IL-6, TNF-α, and IL-10 in the SAP group were significantly increased compared with those in the SO group (Figure 1B).

Compared with SO group animals, SAP group animals exhibited significant morphologic changes: an increased area of white pulp, an increased splenic PALS diameter, and an increased splenic MZ thickness were observed in SAP rats 15 hours after SAP induction (Figure 1, C and F).

In addition to investigating histologic changes, splenic TNF-α and IL-6 expression was also assessed by immunohistochemistry. The expression levels of TNF-α and IL-6 in the white pulp area of the spleen were higher in SAP rats

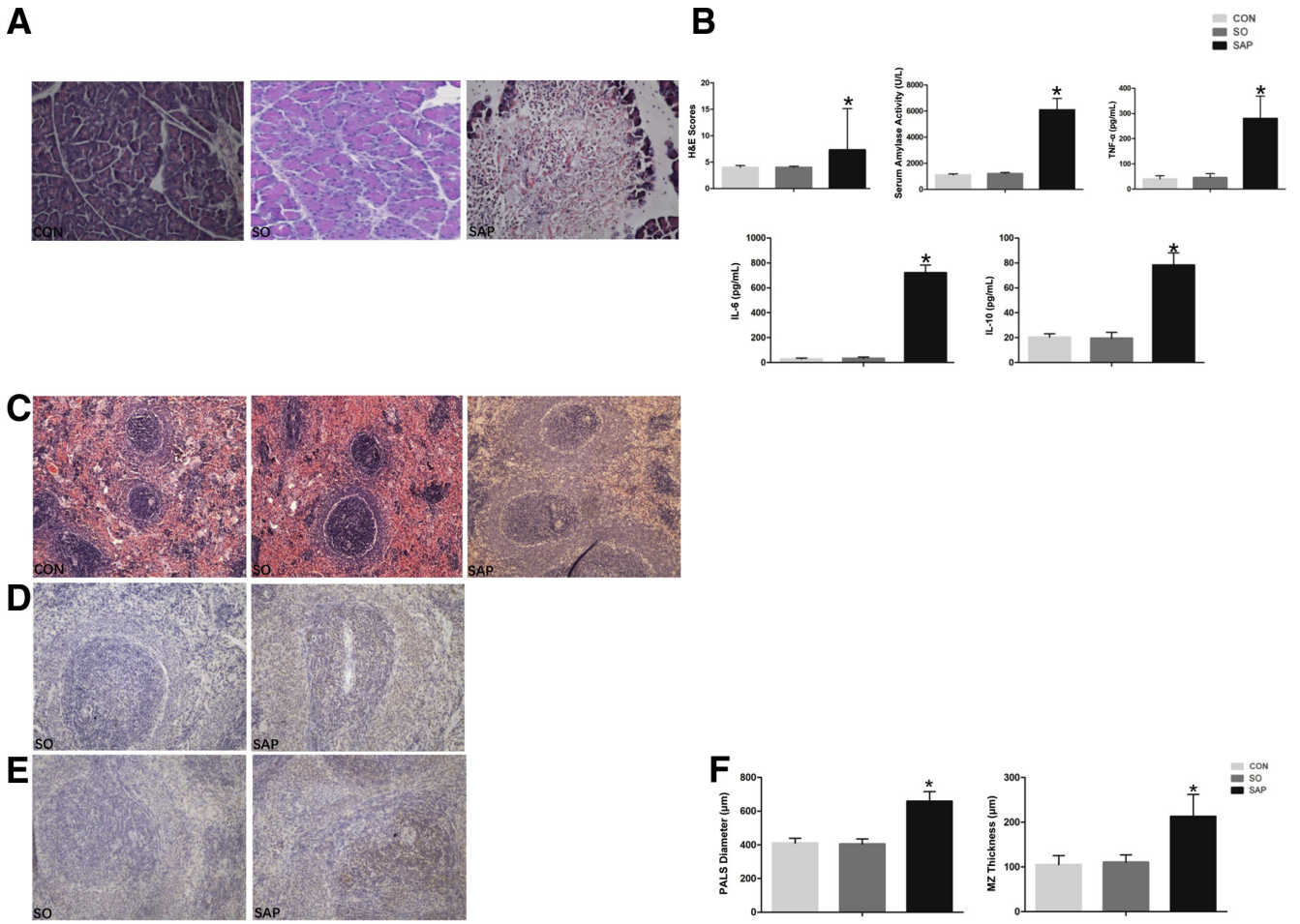


Figure 1 The splenic immune response is enhanced during the progression of severe acute pancreatitis (SAP). **A:** Histopathologic changes in the pancreas in SAP rats [hematoxylin and eosin (H&E)]. Pancreatic tissues from the control (CON) and sham operation (SO) groups show a normal structure. Pancreatic tissues from SAP rats show extensive parenchymal necrosis and focal hemorrhage. **B:** Histopathologic score, serum amylase activity, and the levels of tumor necrosis factor (TNF)-α, IL-6, and IL-10 are significantly increased in SAP rats compared with SO rats. **C:** Histopathologic changes in the spleen in SAP rats (H&E). Splenic tissues from the CON and SO groups show a normal structure. Splenic tissues from SAP rats show an increased area of white pulp. **D:** TNF-α expression in the pancreas is significantly increased in SAP rats compared with SO rats. **E:** IL-6 expression in the pancreas is significantly higher in SAP rats than in SO rats. **F:** Periarterial lymphatic sheath (PALS) diameter and marginal zone (MZ) thickness are significantly increased in SAP rats compared with SO rats. Data are expressed as means ± SEM. *$P < 0.05$ versus the SAP group. Original magnification, ×200 (**A** and **C**–**E**).

than in SO group rats. These findings suggested a significant modification of splenic structures along with SAP progression; the role of the spleen in SAP was therefore investigated (Figure 1, D and E).

Aggravation of SAP Severity by the Spleen

Next, the role of the spleen was studied during SAP progression by evaluating the pancreatic histopathologic score, serum amylase activity, and serum cytokine levels in SAP rats with splenectomy at 0 hours, 3 hours, 6 hours, 9 hours, and 12 hours after SAP induction. SAP rats without splenectomy exhibited worsening pancreatic injury and inflammatory reactions (Figure 2). Intriguingly, in contrast to SAP rats without splenectomy, SAP rats with splenectomy at 0 hours, 3 hours, or 6 hours after SAP induction exhibited decreased pancreatic histopathologic scores, amylase activity, and serum TNF-α and IL-6 levels at 15 hours. Nevertheless, compared with SAP rats that did not undergo splenectomy, SAP rats that underwent splenectomy at 9 and 12 hours after SAP induction did not have significantly decreased pancreatic histopathologic scores, amylase activity, and serum TNF-α and IL-6 levels. To further confirm the role of the spleen in the progression of SAP, we evaluated whether this phenomenon could be duplicated via the administration of single-cell suspensions of the spleen (splenocytes that originated from a rat with SAP 12 hours after induction) to normal rats and SAP rats with splenectomy. Compared with splenectomy alone (SAP + SP), cotreatment with splenectomy and splenocyte administration via the caudal vein (SAP + SP + Spl) increased pancreatic histopathologic scores, amylase activity, and systemic inflammation at 15 hours (Figure 2). Moreover, normal rats administered splenocytes (CON + Spl) had more severe pancreatic injury and higher serum TNF-α levels than rats in the CON group.

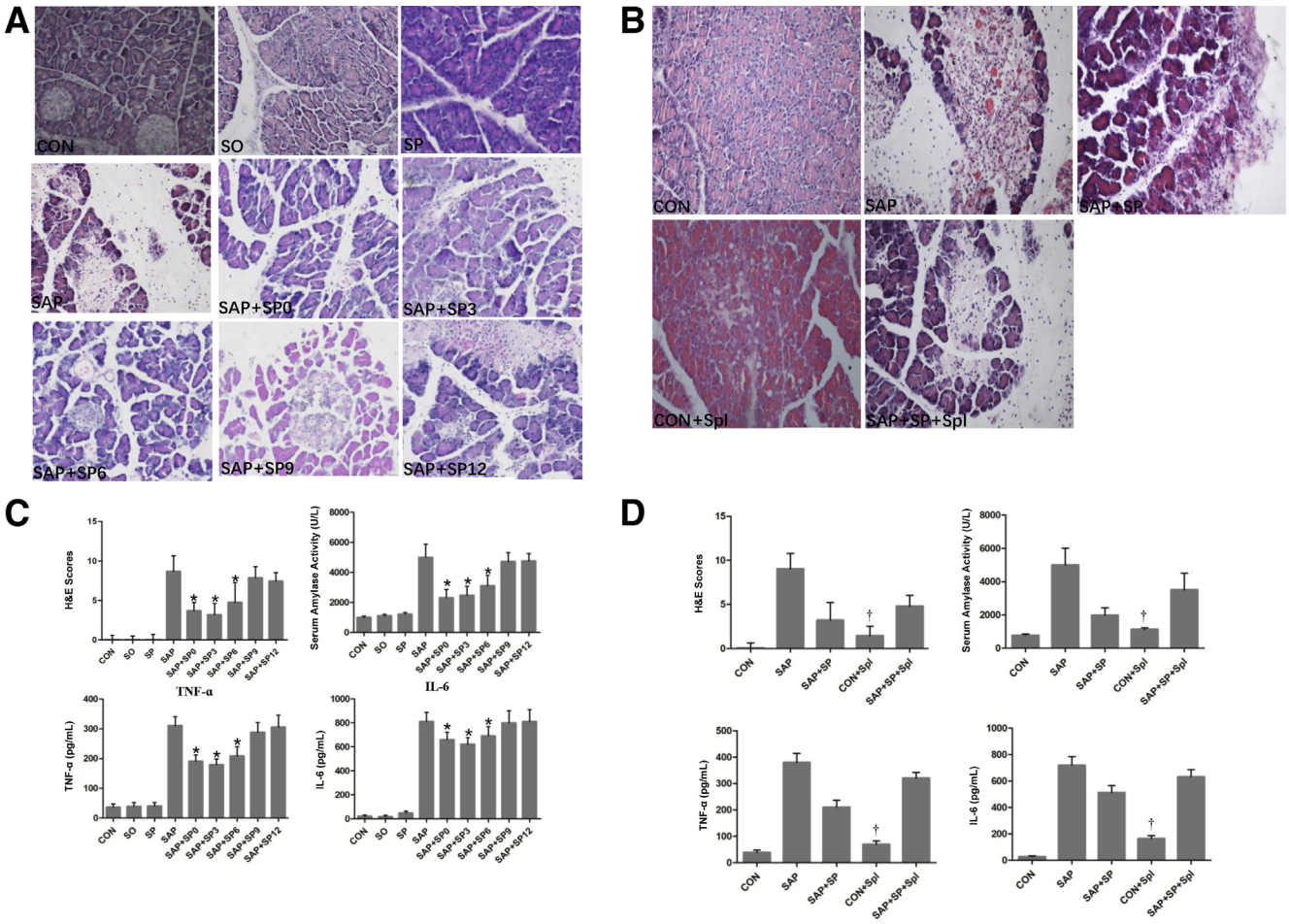


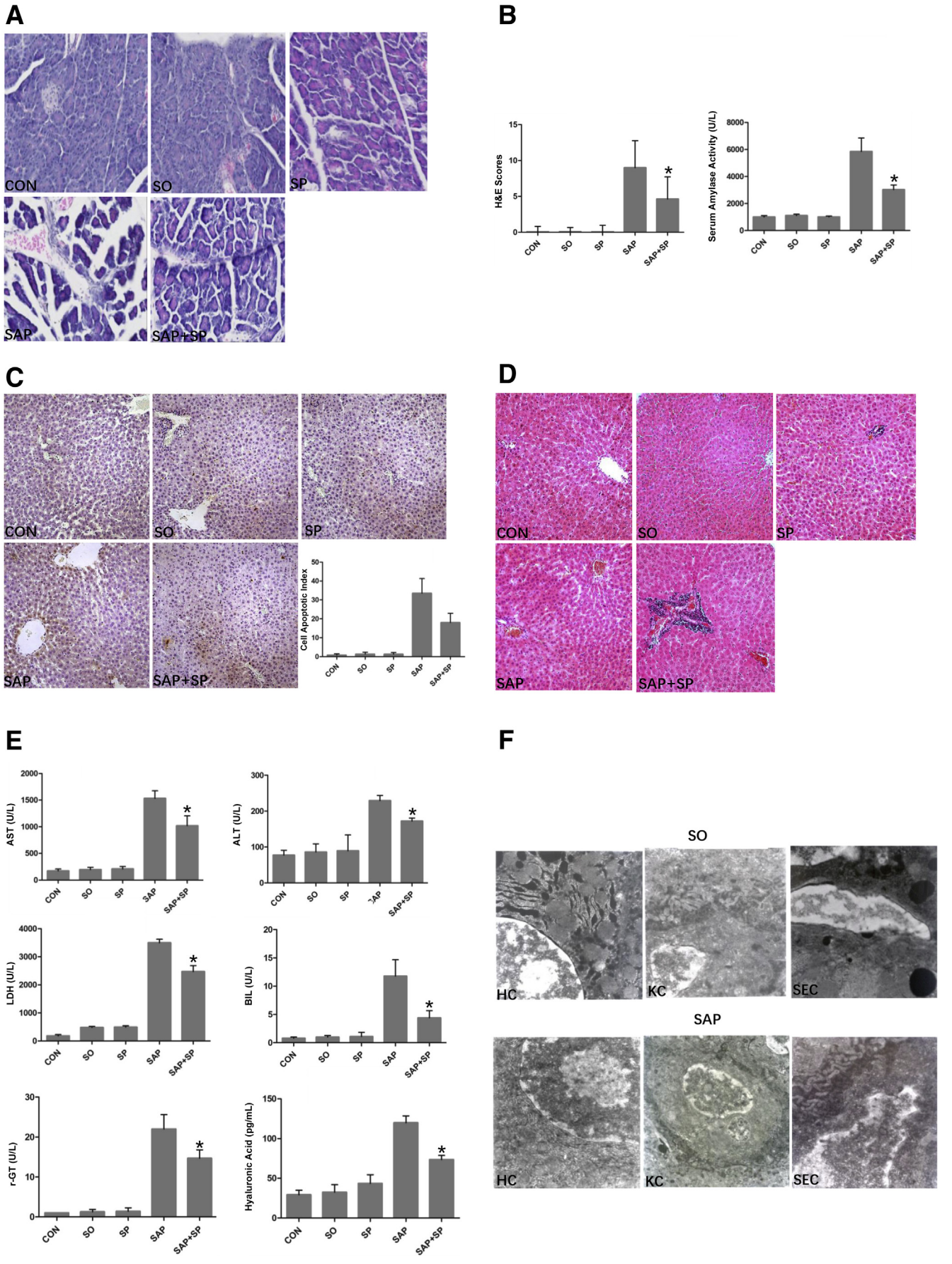
Figure 2 The spleen aggravates the severity of severe acute pancreatitis (SAP). **A:** Histopathologic changes in the pancreas in SAP rats [hematoxylin and eosin (H&E)]. Pancreatic tissues from rats in the control (CON), shame operation (SO), and splenectomy (SP) groups show a normal structure. Pancreatic tissues from SAP rats show extensive parenchymal necrosis and focal hemorrhage. Pancreatic tissues from SAP rats with SP at 0 hours (SP0), 3 hours (SP3), 6 hours (SP6), 9 hours (P9), and 12 hours (SP12) after SAP induction show an alleviation of injury compared with the SAP group. **B:** Histopathologic changes in the pancreas in SAP rats (H&E). Pancreatic tissues from the CON group show a normal structure. Pancreatic tissues from SAP rats show extensive parenchymal necrosis and focal hemorrhage. Pancreatic tissues from the SAP + SP + splenocyte (Spl) group show more pathological damage than those from the SAP + SP group. Pancreatic tissues from the CON + Spl group show more single acinar cell necrosis than those from the CON group. **C:** Histopathologic score, serum amylase activity, and the levels of tumor necrosis factor (TNF)-α and IL-6 are significantly lower in the SAP + SP group than in the SAP group. **D:** Histopathologic score, serum amylase activity, and the levels of TNF-α and IL-6 are significantly higher in the SAP + SP + Spl and CON + Spl groups than in the SAP + SP and CON groups, respectively. Data are expressed as means ± SEM. $^{*}P < 0.05$ versus the SAP group; $^{\dagger}P < 0.05$ versus the CON group. Original magnification, ×200 (**A** and **B**).

Exacerbation of SAP-Associated Liver Injury by the Spleen

The effect of the spleen on SAP-associated liver injury was evaluated in SAP rats subjected to splenectomy immediately after SAP induction (SAP rats with splenectomy at 0 hours after SAP induction). SAP rats without splenectomy exhibited substantial liver morphologic damage (ie, HC edema, hepatic sinusoid hyperemia, and leukocyte infiltration) accompanied by aggravated pancreatic morphologic abnormalities and elevated serum amylase activity, as expected, at 15 hours (Figure 3). In addition, hepatic function, as measured by serum AST, ALT, r-GT, BIL levels, and LDH activity, was decreased.

SAP rats exhibited a marked increase in hepatic tissue ultrastructural injury characterized by HC edema, mitochondrial swelling, endoplasmic reticulum dilatation and degranulation in HCs, cell necrosis, lysosome increase in Kupffer cells, typical secondary lysosome formation, sinus endothelial cell disfiguration, nucleolar pyknosis, and cell apoptosis at 15 hours, as measured by transmission electron microscopy. Immunohistochemistry assays revealed a significant increase in HC apoptosis at 15 hours in the SAP group compared with the SO group.

However, compared with the SAP rats without splenectomy, SAP rats with splenectomy exhibited ameliorated SAP-associated hepatic injury and improved hepatic function at 15 hours. Similarly, ultrastructural injury and HC


A
CON
SO
SP
SAP
SAP+SP
B
H&E Scores
Serum Amylase Activity (U/L)
CON
SO
SP
SAP
SAP+SP
C
CON
SO
SP
SAP
SAP+SP
Cell Apoptotic Index
D
CON
SO
SP
SAP
SAP+SP
E
AST (U/L)
ALT (U/L)
LDH (U/L)
BIL (U/L)
r-GT (U/L)
Hyaluronic Acid (pg/mL)
F
SO
HC
KC
SEC
SAP
HC
KC
SEC

apoptosis at 15 hours were significantly lower in these rats than in rats with SAP but without splenectomy. The ELISA results revealed an increase in serum HA levels at 15 hours in rats with splenectomy relative to these levels in SAP rats without splenectomy.

Aggravation of SAP-Associated Lung Injury by the Spleen

The extent of SAP-associated lung injury was assessed in SAP rats with or without splenectomy. In the SAP-alone group, edema, alveolar-capillary membrane thickening, hyperemia, and neutrophil infiltration increased significantly 15 hours after SAP induction. Interestingly, splenectomy resulted in a decrease in lung injury during SAP relative to that in SAP rats without splenectomy. In addition, the ultrastructural changes in lung tissue were studied via transmission electron microscopy. The SAP group had capillary hyperemia and thrombosis, neutrophil aggregation, pulmonary capillary endothelial edema, cell nexus widening, and mitochondrial vacuole formation. However, rats that received splenectomy after SAP induction exhibited marked attenuation of lung tissue ultrastructural injuries.

SP-A is often overexpressed in acute lung injury. Thus, the expression level of SP-A was used to follow the effect of splenectomy on lung injury in SAP rats. Lung SP-A expression increased in SAP rats but was significantly decreased in SAP rats with splenectomy. This finding suggests that the existence of the spleen leads to the aggravation of SAP-associated lung injury and that splenectomy leads to the alleviation of SAP-associated lung injury. In addition, the Pa_{O_2} level and the ratio of lung wet weight/lung dry weight was measured. Compared with SAP rats without splenectomy, SAP rats with splenectomy had increased Pa_{O_2} levels and lung wet weight/lung dry weight ratios (Figure 4). These results again suggest that the existence of the spleen leads to accentuation of lung injury in SAP.

Accentuation of SAP-Associated Intestinal Mucosal Barrier Dysfunction by the Spleen

To assess the degree of intestinal mucosal injury, the serum iFABP level was evaluated by ELISA.[28,29] The SAP group exhibited an increase in the iFABP level at 15 hours (Figure 5). However, SAP rats with splenectomy exhibited a significant decrease in iFABP levels.

Because intestinal mucosal barrier dysfunction promotes systemic injury during SAP, whether the presence of the spleen further augmented intestinal mucosal barrier dysfunction and its consequences was evaluated. The SAP group exhibited an increase in the partial disintegration and hemorrhage of the terminal ileum lamina propria and irregularity, collapse, or damage of terminal ileum villi at 15 hours (Figure 5). To examine the ultrastructural changes in the terminal ileum, tissues were examined from SAP rats with or without splenectomy via transmission electron microscopy. The swelling of absorptive cells, loss of microvilli, disordered arrangement and establishment of intestinal microvilli, weakening of cell junctions, increased vacuolization in goblet cells, degranulation of the endoplasmic reticulum, and apoptosis of cells were observed in SAP rats. Intriguingly, compared with SAP rats without splenectomy, rats with SAP that were treated with splenectomy had significantly less severe pathologic and ultrastructural changes in the terminal ileum.

Next, the changes in the chemical and immunologic barrier functions of the intestinal mucosa were evaluated by assaying the numbers of sIgA-positive cells and iIELs in SAP rats with and without splenectomy.[30] Splenectomy increased the number of sIgA-positive cells and iIELs at 15 hours in SAP rats relative to the numbers of these cells in SAP rats without splenectomy (Figure 5).

Bacterial dissemination to various organs was evaluated at the 15-hour time point by culturing organ homogenates. Cultures of homogenates from the liver, kidney, and mesenteric lymph nodes revealed significantly fewer colony-forming units in the SAP group with splenectomy than in the SAP group without splenectomy. These data clearly indicate a significant decrease in bacterial translocation at 15 hours after SAP induction in the SAP model (Table 1). Furthermore, compared with no splenectomy, SAP rats with splenectomy had a statistically significant decrease in endotoxin (lipopolysaccharide) levels in blood plasma (Figure 5). These results suggest a significant decrease in the biological barrier function of the intestinal mucosa in the presence of the spleen in SAP rats.

Alteration of Cytokine Profiles in SAP by the Spleen

To assess differences in cytokine profiles in SAP rats with or without splenectomy, broad profiles of cytokines in the

Figure 3 The spleen exacerbates severe acute pancreatitis (SAP)—associated liver injury. **A:** Histopathologic changes in the pancreas in SAP rats [hematoxylin and eosin (H&E)]. Pancreatic tissues from the control (CON), sham operation (SO), and splenectomy (SP) groups show a normal structure. Pancreatic tissues from the SAP group show extensive parenchymal necrosis and focal hemorrhage. Pancreatic tissues from the SAP + SP group show alleviation of damage compared to the SAP group. **B:** Histopathologic score and serum amylase are significantly lower in the SAP + SP group than in the SAP group. **C:** Cellular apoptosis in the liver shows a significant decrease in the SAP + SP group compared with the SAP group. **D:** Histopathologic changes in the liver in SAP rats (H&E). Hepatic tissues from the CON, SO, and SP groups show normal structure. Liver tissues from SAP rats show hepatocyte (HC) edema, hepatic sinusoid hyperemia, and leukocyte infiltration. Hepatic tissues from the SAP + SP group show an alleviation of injury compared with the SAP group. **E:** Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), bilirubin (BIL), γ-glutamyl transferase (r-GT), and hyaluronic acid (HA) levels are significantly lower in the SAP + SP group than in the SAP group. **F:** Ultrastructure of hepatic tissue. Hepatic tissues in the SO group show a normal ultrastructure of HCs, Kupffer cells (KCs), and sinus endothelial cells (SECs). In the SAP group, HCs show cell edema and necrosis, mitochondrial swelling, reduced glycogen levels, and endoplasmic reticulum dilatation and degranulation; KCs show increased lysosomes and typical secondary lysosome formation; and SECs show cell deformation and nucleolar pyknosis. Data are expressed as means ± SEM. $*P < 0.05$ versus the SAP group. Original magnification: ×200 (**A** and **D**); ×200 (**C**); ×15,000 (**F**).

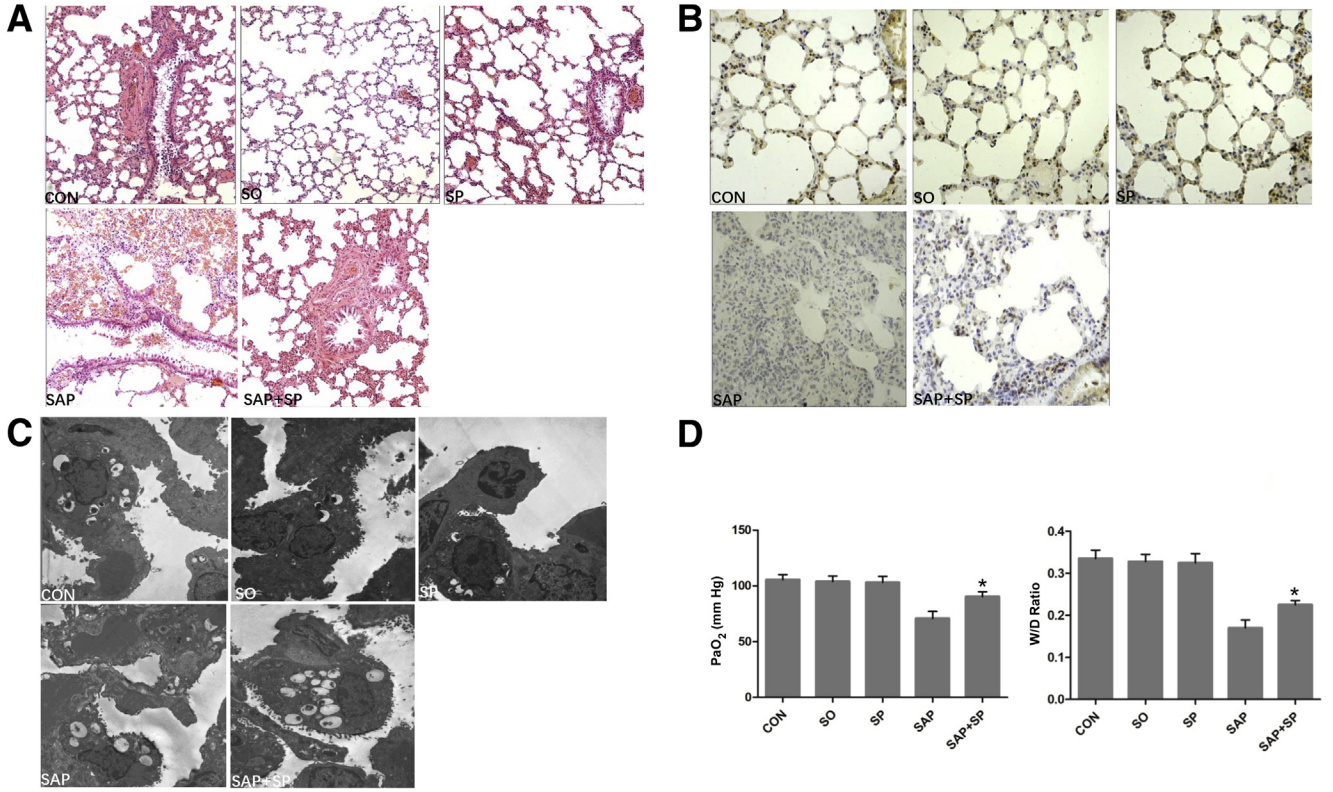


Figure 4 The spleen aggravates severe acute pancreatitis (SAP)—associated lung injury. **A:** Histopathologic changes in the lungs in SAP rats (hematoxylin and eosin). Lung tissues from the control (CON), sham operation (SO), and splenectomy (SP) groups show a normal structure. Lung tissues from SAP rats show vascular congestion, perivascular edema, dense inflammatory infiltration, hemorrhage, and complete destruction of pulmonary architecture. Lung tissues from SAP + SP rats show mild vascular congestion and mild perivascular inflammatory infiltration. **B:** Lung surfactant protein A (SP-A) expression in SAP rats. Lung SP-A expression is higher in the CON, SO, and SP groups than in the SAP and SAP + SP groups. Lung SP-A levels are higher in the SAP + SP group than in the SAP group. **C:** Lung tissue ultrastructure. Lung tissues from the CON, SO, and SP groups show a normal ultrastructure. Lung tissues from the SAP group show damage to type II epithelial cells and the extracellular matrix and emptied lamellar bodies. Lung tissues from the SAP + SP group show an alleviation of injury compared to the SAP group. **D:** Arterial partial pressure of oxygen (Pa_{O_2}) level and lung wet weight/lung dry weight (W/D) ratio are higher in the SAP + SP group than in the SAP group. Data are expressed as means ± SEM. *$P < 0.05$ versus the SAP group. Original magnification: ×200 (**A** and **B**); ×5000 (**C**).

serum and spleen were measured via a biochip array. Compared with the normal group, the SAP group exhibited significant elevations in the levels of C-terminal Src kinase, fractalkine, matrix metalloproteinase 8, matrix metalloproteinase 13, muscle-specific kinase, angiopoietin-1 receptor, Toll-like receptor 4 (TLR4), and ubiquitin and significant decreases in the levels of activin A, FasL/tumor necrosis factor ligand superfamily member 6 (TNFSF6), IL-4, IL-10, and IL-13 in both the serum and spleen. However, in the serum of SAP rats subjected to splenectomy concurrent with SAP induction, the levels of activin A, FasL/TNFSF6, IL-4, IL-10, and IL-13 were increased. Furthermore, in the serum of SAP rats subjected to splenectomy 6 hours after SAP induction, the up-regulation and down-regulation of the abovementioned cytokines were inverted from the pattern observed in SAP rats without splenectomy (Table 2, Figure 6). To gain insight into the probable biological functions of the differentially expressed cytokines, these cytokines were classified by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. GO analysis indicated that 14 differentially expressed cytokines were enriched in the following terms: inflammatory response, positive regulation of major histocompatibility complex class II biosynthetic process, positive regulation of B-cell proliferation, and immune response (Figure 6). KEGG pathway analysis found that the differentially expressed cytokines were mainly involved in cytokine-cytokine receptor interactions, TNF signaling, and the Jak-STAT signaling pathway.

Discussion

The spleen contains numerous immune cells that participate in the course of systemic inflammatory responses.[31] Previous studies have suggested that $CD4^+$ T cells decrease and $CD8^+$ T cells increase in the spleen 12 hours after AP induction in rats, indicating a detrimental immune function of the spleen in SAP.[32] In this study, we first report splenic histologic changes in the course of SAP. The PALS consists of T cells, and the MZ is populated by B cells and macrophages, with functions of antigen capture, antigen recognition, and immune response induction. The areas of the PALS and MZ and the splenic expression of inflammatory

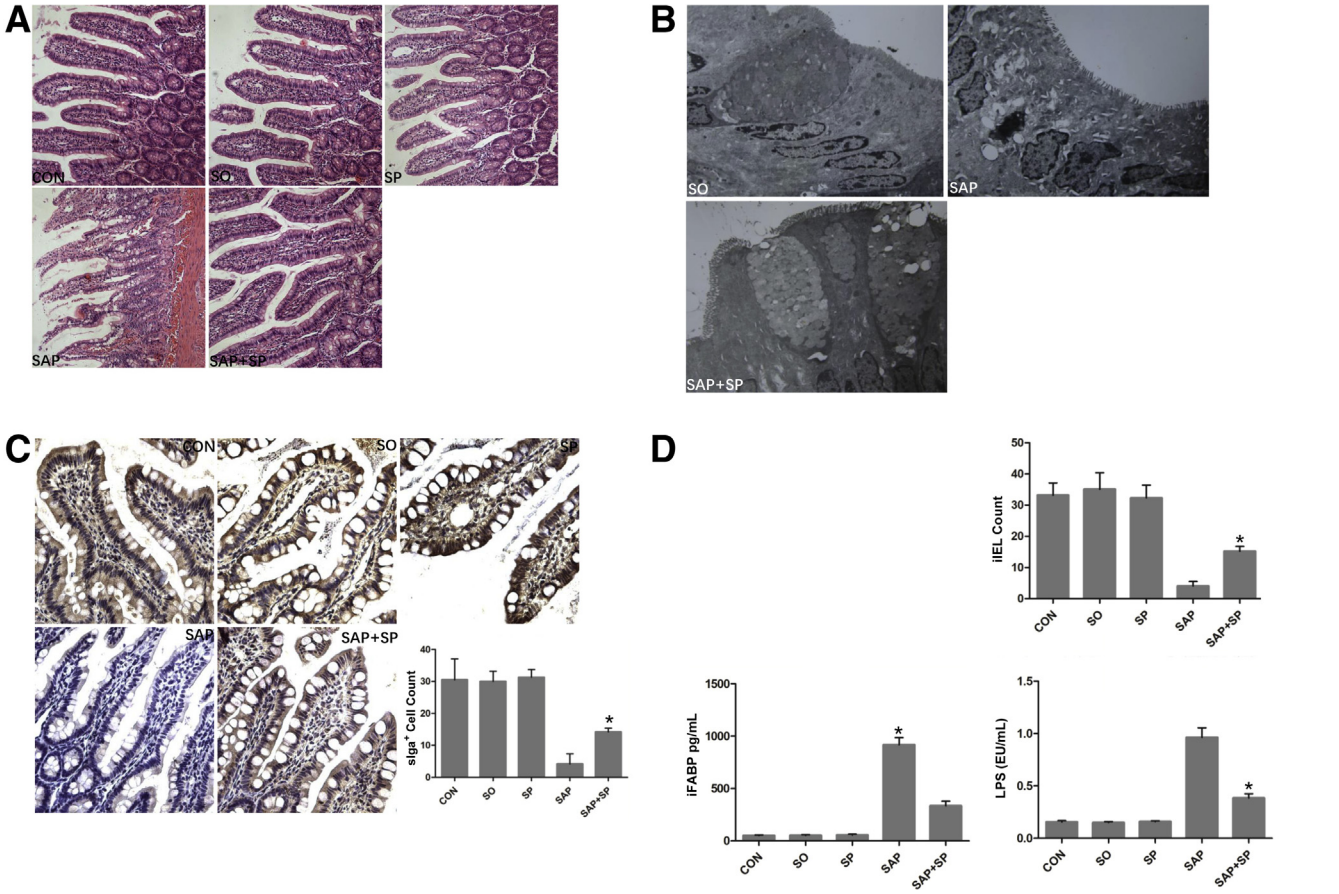


Figure 5 The spleen accentuates severe acute pancreatitis (SAP)—associated intestinal mucosal barrier dysfunction. **A:** Histopathologic changes in the terminal ileum in SAP rats (hematoxylin and eosin). Terminal ileum tissues from the control (CON), sham operation (SO), and splenectomy (SP) groups show a normal structure. Terminal ileum tissues from SAP rats show partial disintegration and hemorrhage and villi collapse or damage in the terminal ileum. Terminal ileum tissues from the SAP + SP group show an alleviation of injury compared to the SAP group. **B:** Ultrastructure of the terminal ileum. The terminal ileum in the SO group shows a normal ultrastructure of ileal epithelial cells and goblet cells. Terminal ileum tissues from SAP rats show absorptive cell swelling, cell junction weakening, and increased goblet cell vacuolization. The terminal ileum of the SAP + SP group shows an alleviation of injury compared with the SAP group. **C:** Secretory IgA (sIgA) expression and number of $sIgA^+$ cells in the terminal ileum. sIgA expression in terminal ileum tissue is higher in the CON, SO, and SP groups than in the SAP and SAP + SP groups. sIgA expression in the terminal ileum is significantly higher in the SAP + SP group than in the SAP group. **D:** Intestinal intraepithelial lymphocytes (iIEL) number, serum intestinal fatty acid binding protein (iFABP), and lipopolysaccharide (LPS) are improved in the SAP + SP group compared to the SAP group. Data are expressed as means ± SEM. $*P < 0.05$ versus the SAP group. Original magnification: ×200 (**A** and **C**); ×3500 (**B**).

cytokines increased in SAP rats, indicating that the immune responses in the spleen are enhanced during the progression of SAP.

The histologic structure of the spleen was altered under pathologic conditions in the pancreas. However, whether the function of the spleen changed in response to these histologic structural changes is unknown, and the role of the spleen in SAP is also undetermined.

Evidence indicates that the spleen participates in the progression of AP. Splenectomy before AP induction significantly decreased inflammatory cytokine production and protected the intestinal mucosal barrier in rats.[33] However, the effect of splenic contributions to SAP pathogenesis after SAP onset is unclear. The findings of this study supported the results of the abovementioned report. In addition to a group subjected to splenectomy immediately after SAP induction (SAP + 0-hour group), four groups of animals subjected to splenectomy at 3 hours, 6 hours, 9 hours, and 12 hours after SAP induction were added. The results were intriguing; compared with SAP rats without splenectomy, SAP rats subjected to splenectomy at 0 hours, 3 hours, and 6 hours after SAP induction exhibited a decrease in the severity of SAP. Moreover, first single-cell suspensions obtained from the spleen of SAP rats 15 hours after SAP induction were used as a splenic supplement to observe the role of the spleen in the progression of SAP. Compared with splenectomy alone, the administration of exogenous splenocytes with splenectomy aggravated the severity of SAP in rats. Furthermore, the administration of splenocytes to normal rats induced SAP-like injuries. These results implied that the spleen serves as a contributing factor in SAP progression. Although the splenocytes produced an effect similar to that of other interventions in the animal experiments in this study, the splenocyte is not

Table 1 Bacterial Translocation Occurring within the Mesenteric Lymph Nodes and Remote Organs (100%)

	Bacterial translocation, % (no./total no.)				
Location	CON	SO	SP	SAP	SAP + SP
Mesenteric lymph nodes	0 (0/6)	16.7 (1/6)	16.7 (1/6)	100 (6/6)	50 (3/6)*
Remote organs	0 (0/6)	0 (0/6)	0 (0/6)	100 (6/6)	33.3 (2/6)*

*$P < 0.05$ versus the SAP group.

CON, control; SAP, severe acute pancreatitis; SO, sham operation; SP, splenectomy.

representative of the spleen; rather, it is only one of the cellular elements in the spleen. Further studies need to be performed to understand the precise role of the spleen in the pathogenesis of SAP.

SAP is a severe acute inflammatory disorder of the pancreas that can be complicated by the involvement of other remote organs, including the liver, lung, kidney, brain, and intestines.[34] The spleen has been reported to participate in the progression of SAP and SAP-associated intestinal mucosal barrier dysfunction, but splenectomy before SAP onset could ameliorate pancreatic injuries and SAP-associated intestinal mucosal barrier dysfunction.[33] However, the role of the spleen in the development of SAP-associated liver and lung injury is unknown. Thus, this study was performed to determine the role of the spleen in SAP and remote organ damage. The results indicate that the spleen plays a crucial role in promoting the progression of SAP. Splenectomy was performed at 0 hours, 3 hours, and 6 hours after SAP induction and led to decreases in the systemic inflammatory response; SAP-associated pancreatic, liver, and lung injury; and intestinal mucosa barrier dysfunction compared with these parameters in SAP rats without splenectomy. These findings indicate that the spleen is the main player in aggravating SAP and SAP-associated remote organ damage, a role that we hypothesized was related to the crucial role of the spleen in the inflammatory cytokine cascade of the inflammatory response.[19,35] However, inflammatory factors that can mediate SAP have not been completely defined.

In this study, various inflammatory mediators were investigated in the serum and spleen to compare cytokine profile differences in SAP rats with or without splenectomy. Splenectomy altered the cytokine profiles in rats with SAP; 13 cytokines, including some known to be SAP related,

Table 2 Differentially Expressed Cytokines in Serum and Spleen between SAP Rats with or without Splenectomy

Cytokine name	Fold change (serum of 15-hour SAP vs serum of normal)	Fold change (spleen of 15-hour SAP vs spleen of normal)	Fold change (serum of 15-hour SAP with splenectomy concurrent with SAP induction vs serum of 15-hour SAP)	Fold change (serum of 15-hour SAP with splenectomy after 6 hours of the SAP induction vs serum of 15-hour SAP)
Down-regulated cytokines after splenectomy				
CSK	1.89	1.88	/	0.68
Fractalkine	20.91	2.09	/	0.76
MMP-8	1.43	6.16	/	0.47
MMP-13	1.74	2.32	/	0.10
MuSK	1.96	11.89	/	0.75
TIE-2	19.53	1.73	/	0.38
TLR4	1.66	1.36	/	0.73
Ubiquitin	2.89	2.87	/	0.57
Up-regulated cytokines after splenectomy				
Activin A	0.60	0.2	3.87	1.97
FasL/TNFSF6	0.63	0.65	1.65	1.86
IL-4	0.75	0.67	1.57	1.73
IL-10	0.73	0.21	2.08	2.34
IL-13	0.75	0.71	1.42	1.59

A list of significantly dysregulated cytokines in serum and spleen of SAP rats compared with SAP rats with splenectomy is shown with the fold change. Fold change >1.3 and ≤ 0.77 and >0; $P < 0.05$.

CSK, C-terminal Src kinase; MMP, matrix metalloproteinase; MuSK, muscle-specific kinase; SAP, severe acute pancreatitis; TIE-2, angiopoietin-1 receptor; TLR4, Toll-like receptor 4; TNFSF6, tumor necrosis factor ligand superfamily member 6.

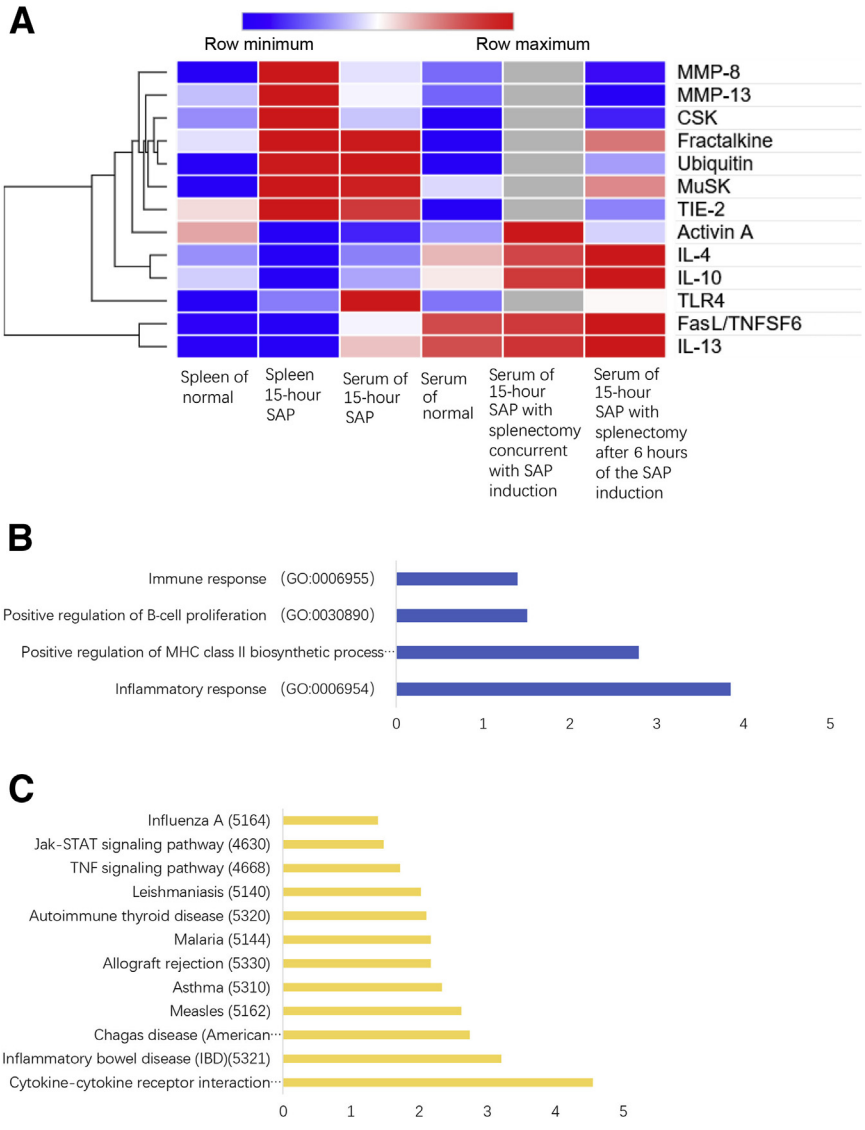


Figure 6 The spleen alters the cytokine profile in severe acute pancreatitis (SAP). **A:** Microarray analysis of cytokine expression was performed on total protein extracted from the serum and spleen of rats with or without splenectomy. The heat map shows the hierarchical clustering of differentially expressed cytokines. Each row and column represents a cytokine and a condition, respectively. The closer the color is to bright blue, the lower the expression; the closer to bright red, the higher the expression. In the serum of SAP rats subjected to splenectomy 6 hours after SAP induction, the up-regulation and down-regulation of the cytokines were inverted from the pattern observed in SAP rats without splenectomy, which suggested that the spleen altered the cytokine profile in SAP. **B:** Representative Gene Ontology (GO) analysis indicates that differentially expressed cytokines are enriched in the following terms: inflammatory response, positive regulation of major histocompatibility complex (MHC) class II biosynthetic process, positive regulation of B-cell proliferation, and immune response. **C:** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis shows that the differentially expressed cytokines are mainly involved in cytokine-cytokine receptor interactions, tumor necrosis factor (TNF) signaling, and the Jak-STAT signaling pathway. The false discovery rate value is <0.01 for all significant GO terms and pathways. CSK, C-terminal Src kinase; MMP, matrix metalloproteinase; MuSK, muscle-specific kinase; TIE-2, angiopoietin-1 receptor; TLR4, Toll-like receptor 4; TNFSF6, tumor necrosis factor ligand superfamily member 6.

were significantly differentially expressed between SAP rats with and without splenectomy. For example, fractalkine, a cytokine with chemokine activity and an adhesion function, was highly expressed in both the serum and pancreas of SAP rats.[36] TLR4 has been reported to be highly expressed in SAP rats and positively associated with chemokine production, neutrophil recruitment, and tissue damage in SAP.[37] Interestingly, both fractalkine and TLR4 were highly expressed in the serum and spleen of SAP rats and could be down-regulated by splenectomy. As anti-inflammatory mediators, IL-4 and IL-10 have been suggested to be valuable treatment strategies for SAP.[38,39] However, the expression of IL-4 and IL-10 was down-regulated in the serum and spleen of SAP rats, but this pattern could be inverted by splenectomy. In addition, the 13 differentially expressed cytokines were significantly enriched in the GO terms inflammatory response, positive regulation of major histocompatibility complex class II biosynthetic process, positive regulation of B-cell proliferation and immune response, and the KEGG pathways cytokine-cytokine receptor interaction, TNF signaling, and JAK-STAT signaling. Thus, our results were consistent with previous reports on the mechanism underlying SAP.[20] The cytokine expression profiles and bioinformatic analysis indicate that the influence of the spleen on the modulation of serum cytokine expression may be an important mechanism in SAP.

In summary, we conclude that splenic contributions to SAP mainly occur through the promotion of inflammatory cytokine production and amylase activation. The spleen participates in the progression of SAP, and it was characterized as promoting the aggravation of SAP and SAP-associated remote organ damage. Splenectomy before SAP onset could reduce the production and release of SAP-

related proinflammatory cytokines and prevent SAP progression. Furthermore, this study is the first systematic study to explore the cytokine expression profiles in SAP rats with and without splenectomy. Splenectomy performed at different times after SAP induction affected serum cytokine expression in SAP rats, indicating that splenectomy performed in the early stage of SAP could downregulate proinflammatory cytokine production. This article provides guidance and a foundation for revealing the role of the spleen, which influences cytokine production in SAP. Characterization of the spleen in the context of SAP will be important for the future management and treatment of SAP, and this characterization may enable the development of new therapeutic strategies for splenic modulation during the management and treatment of SAP. Overall, a comprehensive understanding of the molecular and cellular pathways controlling splenic homeostasis and pathophysiology is important for the development of new therapies for SAP.

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References

1. Simsek O, Kocael A, Kocael P, Orhan A, Cengiz M, Balci H, Ulualp K, Uzun H: Inflammatory mediators in the diagnosis and treatment of acute pancreatitis: pentraxin-3, procalcitonin and myeloperoxidase. Arch Med Sci 2018, 14:288−296
2. Singh P, Garg PK: Pathophysiological mechanisms in acute pancreatitis: current understanding. Indian J Gastroenterol 2016, 35:153−166
3. Sendler M, Weiss FU, Golchert J, Homuth G, van den Brandt C, Mahajan UM, Partecke LI, Doring P, Gukovsky I, Gukovskaya AS, Wagh PR, Lerch MM, Mayerle J: Cathepsin B-mediated activation of trypsinogen in endocytosing macrophages increases severity of pancreatitis in mice. Gastroenterology 2018, 154:704−718.e10
4. Dambrauskas Z, Giese N, Gulbinas A, Giese T, Berberat PO, Pundzius J, Barauskas G, Friess H: Different profiles of cytokine expression during mild and severe acute pancreatitis. World J Gastroenterol 2010, 16:1845−1853
5. Akinosoglou K, Gogos C: Immune-modulating therapy in acute pancreatitis: fact or fiction. World J Gastroenterol 2014, 20: 15200−15215
6. Leach SD, Modlin IM, Scheele GA, Gorelick FS: Intracellular activation of digestive zymogens in rat pancreatic acini. Stimulation by high doses of cholecystokinin. J Clin Invest 1991, 87:362−366
7. Chvanov M, De Faveri F, Moore D, Sherwood MW, Awais M, Voronina S, Sutton R, Criddle DN, Haynes L, Tepikin AV: Intracellular rupture, exocytosis and actin interaction of endocytic vacuoles in pancreatic acinar cells: initiating events in acute pancreatitis. J Physiol 2018, 596:2547−2564
8. Xiang H, Tao X, Xia S, Qu J, Song H, Liu J, Shang D: Targeting microRNA function in acute pancreatitis. Front Physiol 2017, 8:726
9. Portelli M, Jones CD: Severe acute pancreatitis: pathogenesis, diagnosis and surgical management. Hepatobiliary Pancreat Dis Int 2017, 16:155−159
10. Wang GJ, Wang Y, Teng YS, Sun FL, Xiang H, Liu JJ, Xia SL, Zhang GX, Chen HL, Shang D: Protective effects of emodin-induced neutrophil apoptosis via the Ca(2+)-caspase 12 pathway against SIRS in rats with severe acute pancreatitis. Biomed Res Int 2016, 2016:1736024
11. Li L, Duan M, Chen W, Jiang A, Li X, Yang J, Li Z: The spleen in liver cirrhosis: revisiting an old enemy with novel targets. J Transl Med 2017, 15:111
12. Zhang Y, Ding Y, Lu T, Zhang Y, Xu N, McBride DW, Tang J, Zhang JH: Biliverdin reductase-A attenuated GMH-induced inflammatory response in the spleen by inhibiting toll-like receptor-4 through eNOS/NO pathway. J Neuroinflammation 2018, 15:118
13. Lankisch PG: The spleen in inflammatory pancreatic disease. Gastroenterology 1990, 98:509−516
14. Li ZF, Ren S, Zhang S: New progress in basic research of spleen. Chin DJ Expo Surge 2012, 29:361−364
15. Hughes CB, Henry J, Kotb M, Lobaschevsky A, Sabek O, Gaber AO: Up-regulation of TNF alpha mRNA in the rat spleen following induction of acute pancreatitis. J Surg Res 1995, 59:687−693
16. Gotoh K, Inoue M, Shiraishi K, Masaki T, Chiba S, Mitsutomi K, Shimasaki T, Ando H, Fujiwara K, Katsuragi I, Kakuma T, Seike M, Sakata T, Yoshimatsu H: Spleen-derived interleukin-10 downregulates the severity of high-fat diet-induced non-alcoholic fatty pancreas disease. PLoS One 2012, 7:e53154
17. Lai SW, Lin CL, Liao KF: Splenectomy correlates with increased risk of acute pancreatitis: a case-control study in Taiwan. J Epidemiol 2016, 26:488−492
18. Yan JS, Cheng Y, Luo JF, Yu L: An experimental study on role of spleen in acute pancreatitis. Chin J Hepatobiliary Surg 2005, 11: 335−337
19. Xia XM, Li YD, Li ZF: Spleen and acute pancreatic. Int J Surg 2007, 34:388−391
20. Li J, Wu Y, Zhang S, Zhang J, Ji F, Bo W, Guo X, Li Z: Baicalein protect pancreatic injury in rats with severe acute pancreatitis by inhibiting pro-inflammatory cytokines expression. Biochem Biophys Res Commun 2015, 466:664−669
21. Zierath D, Shen A, Stults A, Olmstead T, Becker KJ: Splenectomy does not improve long-term outcome after stroke. Stroke 2017, 48: 497−500
22. Lee HY, Park YM, Lee YH, Kang YG, Lee HM, Park DS, Yang HJ, Kim MJ, Lee YR: Immunostimulatory effect of zanthoxylum schinifolium-based complex oil prepared by supercritical fluid extraction in splenocytes and cyclophosphamide-induced immunosuppressed rats. Evid Based Complement Alternat Med 2018, 2018: 8107326
23. Li Y, Jiang W, Li ZZ, Zhang C, Huang C, Yang J, Kong GY, Li ZF: Repetitive restraint stress changes spleen immune cell subsets through glucocorticoid receptor or beta-adrenergic receptor in a stage dependent manner. Biochem Biophys Res Commun 2018, 495: 1108−1114
24. Li L, Wei W, Li Z, Chen H, Li Y, Jiang W, Chen W, Kong G, Yang J, Li Z: The spleen promotes the secretion of CCL2 and supports an M1 dominant phenotype in hepatic macrophages during liver fibrosis. Cell Physiol Biochem 2018, 51:557−574
25. Li ZF, Wang J, Huang C, Zhang S, Yang J, Jiang A, Zhou R, Pan D: Gas chromatography/time-of-flight mass spectrometry-based metabonomics of hepatocarcinoma in rats with lung metastasis: elucidation of the metabolic characteristics of hepatocarcinoma at formation and metastasis. Rapid Commun Mass Spectrom 2010, 24: 2765−2775
26. Chen X, Zhao HX, Bai C, Zhou XY: Blockade of high-mobility group box 1 attenuates intestinal mucosal barrier dysfunction in experimental acute pancreatitis. Sci Rep 2017, 7:6799

27. Spormann H, Sokolowski A, Letko G: Effect of temporary ischemia upon development and histological patterns of acute pancreatitis in the rat. Pathol Res Pract 1989, 184:507−513
28. Goswami P, Sonika U, Moka P, Sreenivas V, Saraya A: Intestinal fatty acid binding protein and citrulline as markers of gut injury and prognosis in patients with acute pancreatitis. Pancreas 2017, 46: 1275−1280
29. Besselink MG, van Santvoort HC, Renooij W, de Smet MB, Boermeester MA, Fischer K, Timmerman HM, Ahmed Ali U, Cirkel GA, Bollen TL, van Ramshorst B, Schaapherder AF, Witteman BJ, Ploeg RJ, van Goor H, van Laarhoven CJ, Tan AC, Brink MA, van der Harst E, Wahab PJ, van Eijck CH, Dejong CH, van Erpecum KJ, Akkermans LM, Gooszen HG; Dutch Acute Pancreatitis Study Group: Intestinal barrier dysfunction in a randomized trial of a specific probiotic composition in acute pancreatitis. Ann Surg 2009, 250:712−719
30. Qiao SF, Lu TJ, Sun JB, Li F: Alterations of intestinal immune function and regulatory effects of L-arginine in experimental severe acute pancreatitis rats. World J Gastroenterol 2005, 11: 6216−6218
31. Herath K, Cho J, Kim A, Kim HS, Han EJ, Kim HJ, Kim MS, Ahn G, Jeon YJ, Jee Y: Differential modulation of immune response and cytokine profiles of Sargassum horneri ethanol extract in murine spleen with or without concanavalin A stimulation. Biomed Pharmacother 2018, 110:930−942
32. Yang SJ, Sun Y, Cheng Y: Changes of splenic T-lymphocyte subsets in rats with acute pancreatitis. Chin J Curr Adv Gen Surg 2004, 7:356−357
33. Lu Z, Zhu YL, He CL, Liu JX: Effect of the spleen on intestinal barrier function in rats with acute pancreatitis. Chin J Gen Surg 2005, 14:327−330
34. Ateyya H, Wagih HM, El-Sherbeeny NA: Effect of tiron on remote organ injury in rats with severe acute pancreatitis induced by L-arginine. Naunyn Schmiedebergs Arch Pharmacol 2016, 389:873−885
35. Zhang S, Li ZF: Questions and thoughts on the study of the spleen. Chin J Exp Surg 2015, 32:230−231
36. Huang LY, Chen P, Xu LX, Zhou YF, Li WG, Yuan YZ: Fractalkine as a marker for assessment of severe acute pancreatitis. J Dig Dis 2012, 13:225−231
37. Awla D, Abdulla A, Regner S, Thorlacius H: TLR4 but not TLR2 regulates inflammation and tissue damage in acute pancreatitis induced by retrograde infusion of taurocholate. Inflamm Res 2011, 60:1093−1098
38. Zhang C, Ge CL, Guo RX, He SG: Effect of IL-4 on altered expression of complement activation regulators in rat pancreatic cells during severe acute pancreatitis. World J Gastroenterol 2005, 11: 6770−6774
39. Li J, Zhou R, Bie BB, Huang N, Guo Y, Chen HY, Shi MJ, Yang J, Zhang J, Li ZF: Emodin and baicalein inhibit sodium taurocholate-induced vacuole formation in pancreatic acinar cells. World J Gastroenterol 2018, 24:35−45