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Mesenteric adipose tissue B lymphocytes promote intestinal injury in severe acute pancreatitis by mediating enteric pyroptosis

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ABSTRACT

Background: Visceral adipose tissue (VAT) has been linked to the severe acute pancreatitis (SAP) prognosis, although the underlying mechanism remains unclear. It has been reported that pyroptosis worsens SAP. The present study aimed to verify whether mesenteric adipose tissue (MAT, a component of VAT) can cause secondary intestinal injury through the pyroptotic pathway.

Methods: Thirty-six male Sprague Dawley (SD) rats were divided into six different groups. Twelve rats were randomly divided into the SAP and control groups. We monitored the changes of MAT and B lymphocytes infiltration in MAT of SAP rats. Twelve SAP rats were injected with MAT B lymphocytes or phosphate buffer solution (PBS). The remaining twelve SAP rats were first injected with MAT B lymphocytes, and then with MCC950 (NLRP3 inhibitor) or PBS. We collected blood and tissue samples from pancreas, gut and MAT for analysis.

Results: Compared to the control rats, the SAP group showed inflammation in MAT, including higher expression of tumor necrosis factor (TNF-α) and interleukin-6 (IL-6), lower expression of IL-10, and histological changes. Flow cytometry analysis revealed B lymphocytes infiltration in MAT but not T lymphocytes and macrophages. The SAP rats also exhibited intestinal injury, characterized by lower expression of zonula occludens-1 (ZO-1) and occludin, higher levels of lipopolysaccharide and diamine oxidase, and pathological changes. The expression of NLRP3 and n-GSDMD, which are responsible for pyroptosis, was increased in the intestine of SAP rats. The injection of MAT B lymphocytes into SAP rats exacerbated the inflammation in MAT. The upregulation of pyroptosis reduced tight junction in the intestine, which contributed to the SAP progression, including higher inflammatory indicators and worse histological changes. The administration of MCC950 to SAP + MAT B rats downregulated pyroptosis, which subsequently improved the intestinal barrier and ameliorated inflammatory response of SAP.

Conclusions: In SAP, MAT B lymphocytes aggravated local inflammation, and promoted the injury to the intestine through the enteric pyroptotic pathway.

Introduction

Although previous study has demonstrated a correlation between visceral adipose tissue (VAT) and the prognosis of severe acute pancreatitis (SAP) [1], which component of VAT that plays the most significant role remains unclear. Mesenteric adipose tissue (MAT) is a part of VAT that lies between the pancreas and the gut, wrapping around blood vessels, lymphatic vessels and nerves. The location of MAT makes it possible to affect the development of SAP and the subsequent intestinal injury by secreting inflammatory cytokines, adipocytokines, and other factors [2]. Gea-Sorlí et al. [3] discovered that among VAT, only MAT induced a robust inflammatory response in SAP rats. Intestinal injury is the most frequent systemic complication in SAP. Secondary bacterial translocation often ensues, which can lead to a worse prognosis [4,5]. MAT was reported to have some effects on the intestinal function, contributing to the pathogenesis of Crohn's disease [6], and the intestinal barrier integrity in mice with nonalcoholic fatty liver disease (NAFLD) [7]. However, the role of MAT in intestinal injury of SAP is not clear.

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MAT comprises different types of cells, including adipocytes, fibrocytes and nearly all types of lymphocytes [8]. Wu et al. [9] demonstrated that B lymphocytes infiltrated MAT early, contributing to local inflammation. B lymphocytes can contribute to tissue inflammation by producing cytokines, secreting immunoglobulin, and interacting with T lymphocytes and macrophages [10]. The occurrence of immune responses was related to the pathogenesis of acute pancreatitis (AP). The circulation of B lymphocytes in SAP patients was significantly lower than that of healthy controls [11]. Furthermore, in SAP patients who experienced secondary intestinal infection, the reduction in the percentage of peripheral B lymphocytes was even more pronounced [12]. The depletion of circulating lymphocytes may be caused by their migration to the inflammatory site [13], such as the pancreas, MAT and gut.

Researchers have become increasingly interested in the role of pyroptosis in the pathogenesis of SAP [14]. Pyroptosis is a newly designated term for programmed cell death occurring in various cell types [15]. The signaling pathways associated with pyroptosis are commonly activated by initiating pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP). These patterns trigger inflammasome components, including NOD-like receptor thermal protein domain associated protein 3 (NLRP3), etc. The activated inflammasomes induce the cleavage of the pro-caspase1 to generate a mature form (c-caspase1). C-caspase1 activates the maturation of IL-1β and IL-18, cleaves gasdermin D (GSDMD), and initiates an oligomerization process of the 31kDa amino-terminal portion (n-GSDMD) to create membrane pores. These pores facilitate the massive secretion of inflammatory factors and the infiltration of immune cells [16]. A previous study has reported that pyroptosis-regulated acinar cell death drives the progression of SAP [14]. Furthermore, Lin et al. [17] demonstrated that the downregulation of GSDMD could reduce the severity of AP and the related intestinal damage. In the present study, we investigated whether the MAT inflammation would affect the SAP related intestinal damage through the pyroptosis pathway.

The current study investigated the inflammatory responses of MAT and lymphocytes infiltration during SAP. We explored whether B lymphocytes promoted MAT inflammation, potentially exacerbating secondary intestinal injury.

Methods

Animals and SAP models

Male Sprague Dawley (SD) rats, weighing approximately 250 g and aged 4–6 weeks, were supplied by Beijing Vital River Laboratory Animal Technology (Beijing, China). The rats were raised in a specific pathogen-free facility with constant temperature and humidity. After 5 days of adaptive feeding, the rats were allowed access to water but fasted for approximately 12 h before the experiment.

Rats were divided into 6 groups: (1) sham control, (2) SAP, (3) SAP + PBS, (4) SAP + MAT B, (5) SAP + MAT B + PBS, and (6) SAP + MAT B + MCC950; six rats in each group. Group 2 was used to test the success of SAP model. SAP model was created as following, rats were anesthetized with isoflurane and had their abdomen exposed along the midline. They were administered retrograde pumping of 5% sodium taurocholate (0.1 mL/100 g) into the pancreaticobiliary duct, and then returned to their original position. Sham was with the same procedure with no injection of sodium taurocholate.

Rats in the SAP + PBS group and SAP + MAT B group were injected with 1 mL of PBS or MAT B lymphocytes suspension (10^7/mL) via the tail vein immediately after SAP modeling. The rats in the SAP + MAT B + PBS group and SAP + MAT B + MCC950 group were injected with a 1 mL suspension of MAT B lymphocytes (10^7/mL) through the tail vein immediately after SAP modeling. Afterward, they received either 1 mL of PBS or MCC950 (0.25 mg/mL, an inhibitor of NLRP3), through intraperitoneal injection 2 h after SAP modeling. After 24 h of SAP modeling, all rats were euthanized. Serum and several tissues were collected, including pancreas, gut and MAT.

Table 1
PCR primer sequences of target genes in rats.

Primers	Forward 5'→ 3'	Reverse 5' → 3'
ZO-1	GCCGCTAAGAGCACAGCAA	GCCCTCCTTTTAACACATCAGA
Occludin	GCTTATCTTGGGAGCCTGGACA	GTCATTGCTTGGTGCATAATGATTG
TNF-α	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
IL-10	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG

ZO-1: zonula occludens-1; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; IL-10: interleukin-10.

Histological analysis

Samples of pancreas, gut and MAT were sliced into 4-μm cross-sections and stained with hematoxylin-eosin (HE) for histological analysis. The sections were observed under a light microscope. Two pathologists used a double-blinded method to evaluate the inflammatory status of tissues in the experiment. The pancreatic pathology was assessed according to the scoring criteria proposed by Schmidt [18]. The intestinal pathology was scored according to the criteria described by Chiu [19]. The histological assessment of MAT was to count the number of "crown-like structure" (CLS), which was defined as one adipocyte surrounded by at least five macrophages.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted using an RNA extraction kit (Qiangen, Hilden, Germany) according to the manufacturer's protocol. cDNA was synthesized using an cDNA Synthesis kit (Thermo Fisher Scientific, Vilnius, Lithuania) according to the manufacture's protocol. Realtime-PCR was carried out with a StepOne Realtime-PCR System (Applied Biosystems, Waltham, MA, USA) using SYBR premix EX Taq II kit (Takara, Tokyo, Japan). The sequences of primers are listed in Table 1.

Flow cytometry

Immune cells were labelled with CD45-PE, CD19-APC, CD3-FITC and F4/80-PerCP/Cy5.5 antibodies (Biolegend, San Diego, CA, USA) and measured using a FACS Calibur flow cytometer (BD Immunocytometry Systems, Frnklin Lakes, NJ, USA). Data were analyzed using Flowjo 10 (Flow Cytometry Software Inc., OR, USA).

Western blot analysis

Protein was extracted from intestinal tissue and the protein concentrations were measured. A total of 60 μg protein was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter membrane. The protein was then incubated at 4 °C overnight in primary antibody on a shaking table. The primary antibodies were as follows: anti-occludin, anti-zonula occludens-1 (ZO-1), anti-NLRP3, anti-GSDMD, anti-n-GSDMD, anti-c-caspase1, anti-IL-1β and anti-IL-18 (Abcam,

Cambridge, UK). After extensive washing, the second antibody was used. Glyceraldehyde phosphate dehydrogenase (GAPDH) was used as an internal control and the protein bands were scanned by Image-Pro Plus 6.0 software.

Enzyme-linked immunosorbent assay (ELISA)

Levels of C-reactive protein (CRP), lipopolysaccharide (LPS) and diamine oxidase (DAO) in serum were detected using ELISA kits as per manufacturer's instruction (Minneapolis, MN, USA).

Blood routine and biochemistry detection

The blood cell count was detected using an automatic whole blood counter. The concentrations of amylase (AMY) and lipase were detected using an automatic biochemical detector-Labospect 008 (Hitachi Limited, Tokyo, Japan).

Statistical analysis

Data were presented as mean ± standard deviation. A two-tailed Student's *t* test was used to compare values between groups. All the analyses were conducted using SPSS version 26 software (IBM, Amonk, NY, USA). GraphPad Prism 8.3 (GraphPad Software Inc., La Jolla, CA, USA) was used to plot graphs. A P value < 0.05 was considered statistically significant.

Results

MAT appears inflammatory status and B lymphocytes infiltration in SAP rats

Firstly, we confirmed the success of SAP modeling by comparing the SAP group to the control group. We found statistically higher counts of white blood cells (WBC) ($P < 0.01$), a higher percentage of neutrophils ($P < 0.01$), higher serum levels of CRP ($P < 0.01$) and AMY ($P < 0.01$) in the SAP group (Fig. 1A). Additionally, the pancreas tissue in the SAP group showed interstitial edema and leukocyte infiltration, resulting in a significantly higher pathological score than that in the control group ($P < 0.01$) (Fig. 1B).

To clarify the changes in MAT within SAP, we investigated the inflammatory status and immune cell infiltration. Our analysis found that the mRNA expression of inflammatory cytokines [tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)] in MAT was significantly higher in the SAP group compared to the control group (both $P < 0.01$). In comparison, the mRNA expression of IL-10 in MAT was significantly lower ($P < 0.01$) in the SAP group (Fig. 1C). Histological assessment of MAT revealed that the number of CLSs was significantly higher in the SAP group ($P < 0.01$) (Fig. 1D). The gating strategy for flow cytometry analysis of MAT is depicted in Fig. 1E, where CD45+CD19+ lymphocytes were identified as B lymphocytes, CD45+CD3+ lymphocytes were considered T lymphocytes, and CD45+F4/80+ lymphocytes were regarded as macrophages. Compared to the control group, B lymphocytes in MAT were significantly higher in the SAP group ($P < 0.01$), while T lymphocytes were significantly lower ($P < 0.01$). However, the number of macrophages showed no significant difference between the SAP and control groups ($P > 0.05$).

SAP rats present intestinal barrier damage and enteric pyroptosis

To evaluate the intestinal barrier permeability, we detected the expression of intestinal tight junction proteins (ZO-1 and occludin) as well as the levels of serum endotoxin (LPS and DAO). Compared to the control group, the mRNA and protein expression of ZO-1 (both $P < 0.01$) and occludin (both $P < 0.01$) were significantly decreased in the SAP group (Fig. 2A). Additionally, the serum levels of LPS ($P < 0.01$) and DAO ($P < 0.01$) were significantly higher in the SAP group (Fig. 2B). Furthermore, histological assessment of the gut showed structural damage in the SAP group compared to the control group, with significantly higher pathological scores ($P < 0.01$) (Fig. 2C). To explore the activation of pyroptosis in the intestine, we detected the expression of critical proteins, specifically NLRP3 and n-GSDMD. The protein expression of NLRP3 ($P < 0.01$) and n-GSDMD ($P < 0.01$) was significantly increased in the SAP group compared to that in the control group (Fig. 2D).

MAT B lymphocytes aggravate MAT inflammation, damage intestinal barrier, enhance enteric pyroptosis, and exacerbate progression of pancreatitis

Based on the previous results, B lymphocytes within MAT were increased significantly in the SAP rats. To investigate whether the infiltration of these MAT B lymphocytes would aggravate local inflammation in SAP, we injected MAT B lymphocytes into SAP rats via the tail vein. Firstly, we found that the number of B lymphocytes in MAT was increased significantly in the SAP + MAT B group ($P < 0.05$) compared to the SAP + PBS group. In contrast, there were no statistical changes in the number of T lymphocytes and macrophages in MAT between the SAP + MAT B and the SAP + PBS groups (Fig. 3A). Secondly, the mRNA expression of TNF-α and IL-6 in MAT was significantly increased in the SAP + MAT B group (both $P < 0.01$), while the mRNA expression of IL-10 in MAT was decreased significantly ($P < 0.01$) (Fig. 3B). Furthermore, the pathological analysis of MAT showed a more pronounced inflammatory response and significantly higher scores ($P < 0.05$) in the SAP + MAT B group than in the SAP + PBS group (Fig. 3C).

To determine whether the exacerbation of MAT inflammation would contribute to intestinal injury in SAP, we examined the changes in the intestinal barrier and pancreatitis. The SAP + MAT B group showed a significant decrease in the mRNA and protein expression of ZO-1 ($P < 0.05$ and $P < 0.01$, respectively) and occludin (both $P < 0.01$) compared to the SAP + PBS group (Fig. 4A). The serum levels of LPS ($P < 0.01$) and DAO ($P < 0.01$) were significantly higher in the SAP + MAT B group (Fig. 4B). Additionally, the intestinal tissue showed obvious inflammatory response with higher pathological scores ($P < 0.01$) in the SAP + MAT B group (Fig. 4C). Considering pyroptosis in the intestine, the expression of critical proteins, including NLRP3 ($P < 0.05$), c-caspase1 ($P < 0.01$), IL-1β ($P < 0.05$), IL-18 ($P < 0.05$) and n-GSDMD ($P < 0.05$), were all increased in the SAP + MAT B group compared to the SAP + PBS group (Fig. 4D). Consequently, the counts of WBC ($P < 0.01$), the ratio of neutrophil ($P < 0.05$), and the serum levels of CRP ($P < 0.01$), AMY ($P < 0.01$) and lipase ($P < 0.01$) were all significantly higher in the SAP + MAT B group than those in the SAP + PBS group (Fig. 4E). The pancreas tissue revealed more severe inflammation with higher histological scores ($P < 0.01$) in the SAP + MAT B group than in the SAP + PBS group (Fig. 4F).

B lymphocytes in the MAT accelerate damage to the intestinal barrier through enteric pyroptosis, and aggravate pancreatitis

As previously mentioned, the accumulation of MAT B lymphocytes may contribute to local inflammation and aggravate intestinal barrier damage in SAP. During this process, an upregulation of pyroptosis was observed in the intestine. To investigate whether the MAT inflammation provoked intestinal damage through the pyroptotic pathway in SAP, we injected MAT B lymphocytes and MCC950 (an NLRP3 inhibitor) into SAP rats and compared the inflammatory status of the gut, MAT and pancreas between the two groups. In

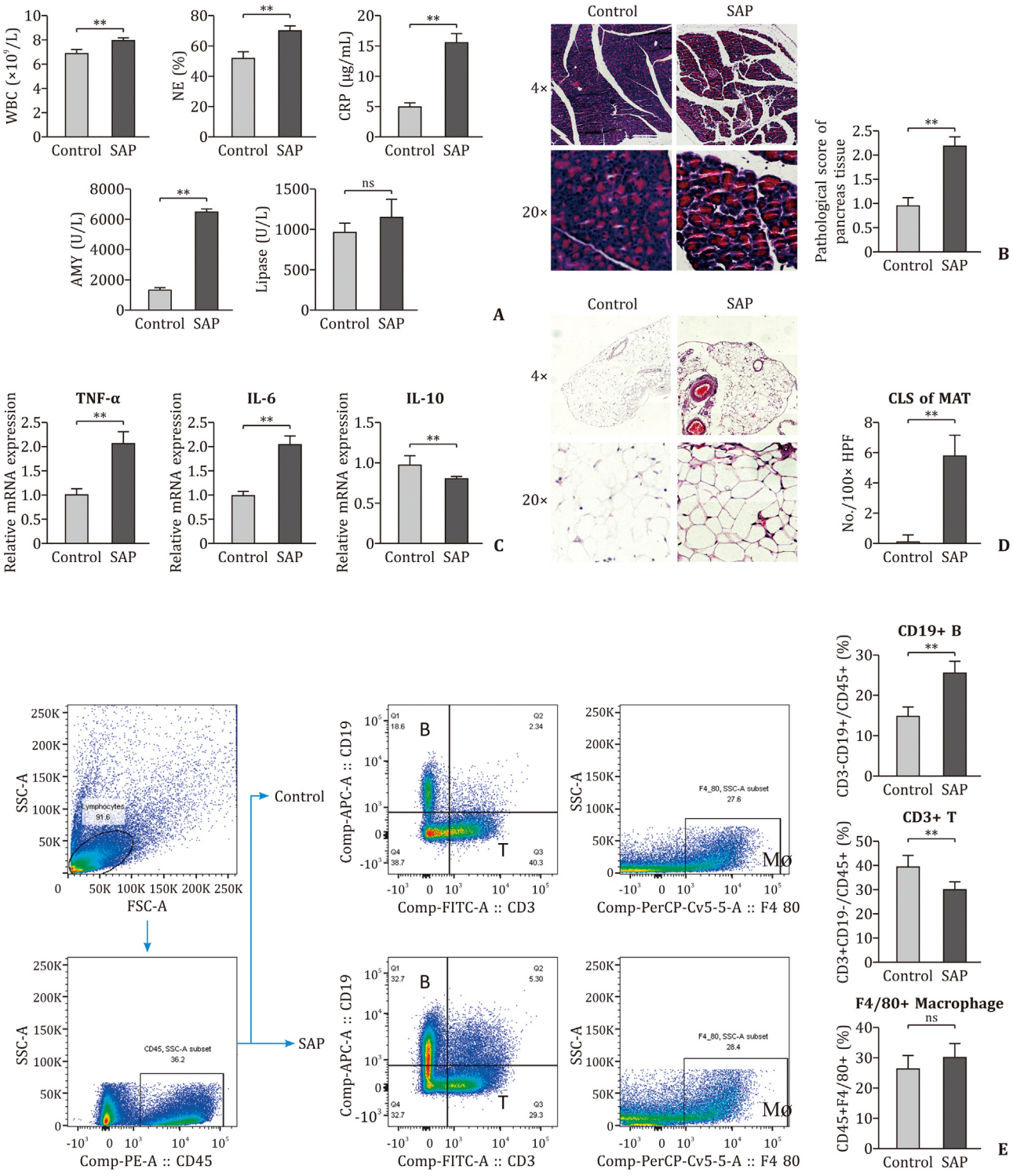

Fig. 1. MAT appeared inflammation and B lymphocytes infiltration in SAP rats compared with the control group. **A:** Inflammatory indicators, specifically, the counts of WBC, the ratio of neutrophil and serum levels of CRP, AMY and lipase; **B:** representative HE staining of pancreas and the pathological scores of pancreas tissues; **C:** mRNA expression of cytokines responsible for MAT inflammation, specifically, TNF-α, IL-6 and IL-10; **D:** representative HE staining of MAT and the numbers of CLS in MAT; **E:** the gating strategy of flow cytometry for the MAT. The proportion of CD45+CD19+ B lymphocytes, CD45+CD3+ T lymphocytes and CD45+F4/80+ macrophages in the MAT. Values represent means ± standard deviation. ** $P < 0.01$; ns: no significance; SAP: severe acute pancreatitis; WBC: white blood cell; NE: neutrophil; CRP: C-reactive protein; AMY: amylase; HE: hematoxylin-eosin; MAT: mesenteric adipose tissue; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; IL-10: interleukin-10; CLS: crown-like structure; HPF: high power field.

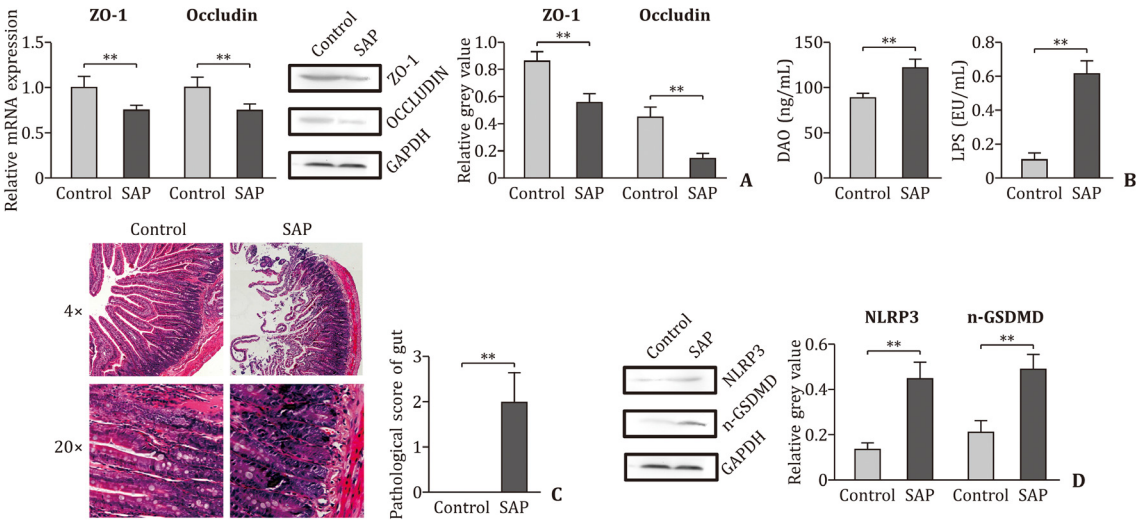

Fig. 2. SAP rats presented intestinal barrier injury with activation of enteric pyroptosis in the SAP group compared with the control group. **A:** Expression of mRNA and proteins responsible for intestinal tight junction, specifically, ZO-1 and occludin; **B:** levels of serum endotoxins DAO and LPS; **C:** representative HE staining of intestine and the pathological scores of intestinal tissues; **D:** expression of pyroptotic proteins in intestine, specifically, NLRP3 and n-GSDMD. Values represent means ± standard deviation. ** $P < 0.01$; SAP: severe acute pancreatitis; ZO-1: zonula occludens-1; GAPDH: glyceraldehyde phosphate dehydrogenase; DAO: diamine oxidase; LPS: lipopolysaccharide; NLRP3: NOD-like receptor thermal protein domain associated protein 3; GSDMD: gasdermin D.

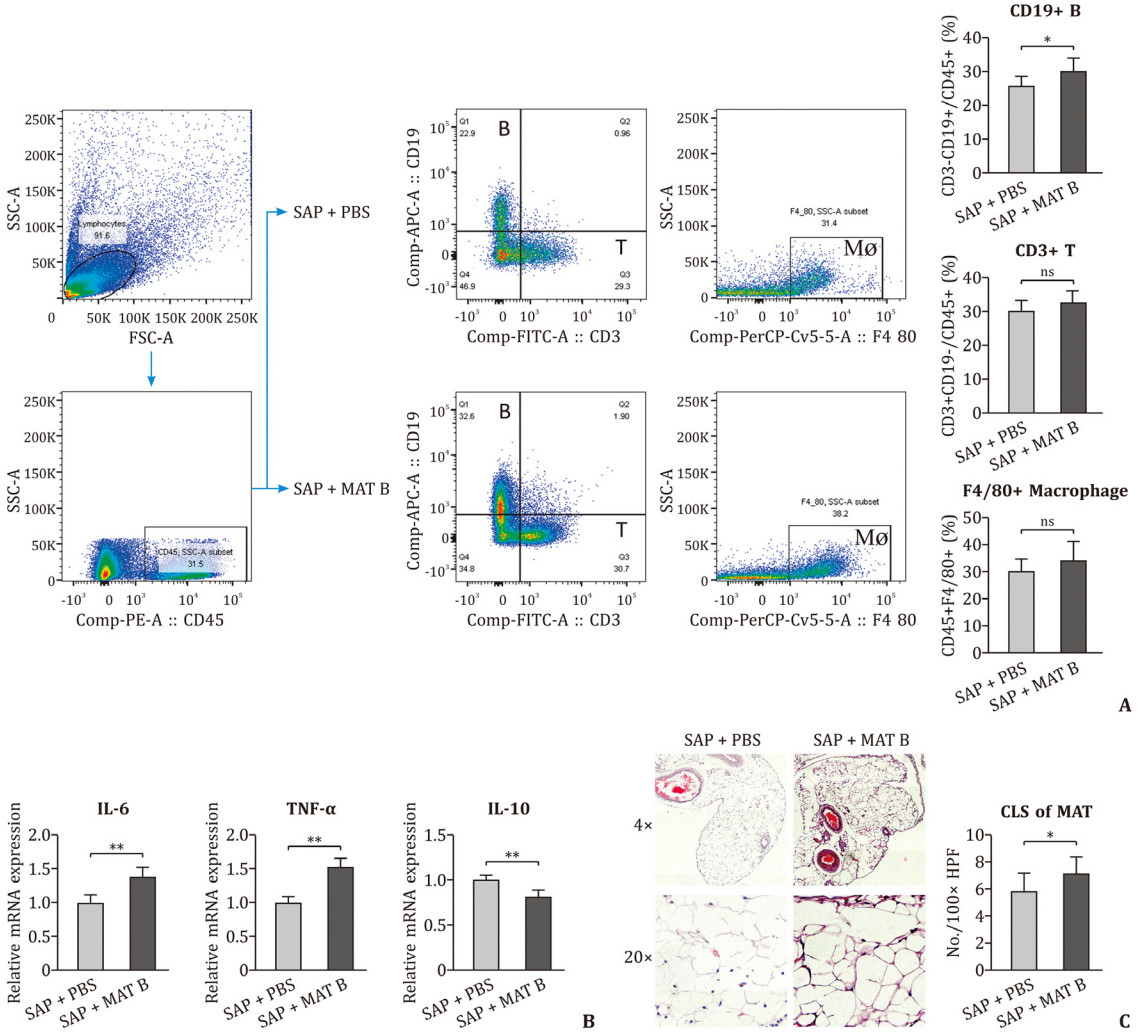

Fig. 3. MAT B lymphocytes promoted MAT local inflammation. **A:** The gating strategy of flow cytometry for the MAT. The proportion of CD45+CD19+ B lymphocytes, CD45+CD3+ T lymphocytes and CD45+F4/80+ macrophages in the MAT; **B:** expression of mRNA responsible for MAT inflammation; **C:** representative HE staining of MAT and the numbers of CLS in MAT. Values represent means ± standard deviation. * $P < 0.05$; ** $P < 0.01$; ns: no significance. SAP: severe acute pancreatitis; PBS: phosphate buffer solution; MAT: mesenteric adipose tissue; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; CLS: crown-like structure; HE: hematoxylin-eosin; HPF: high power field.

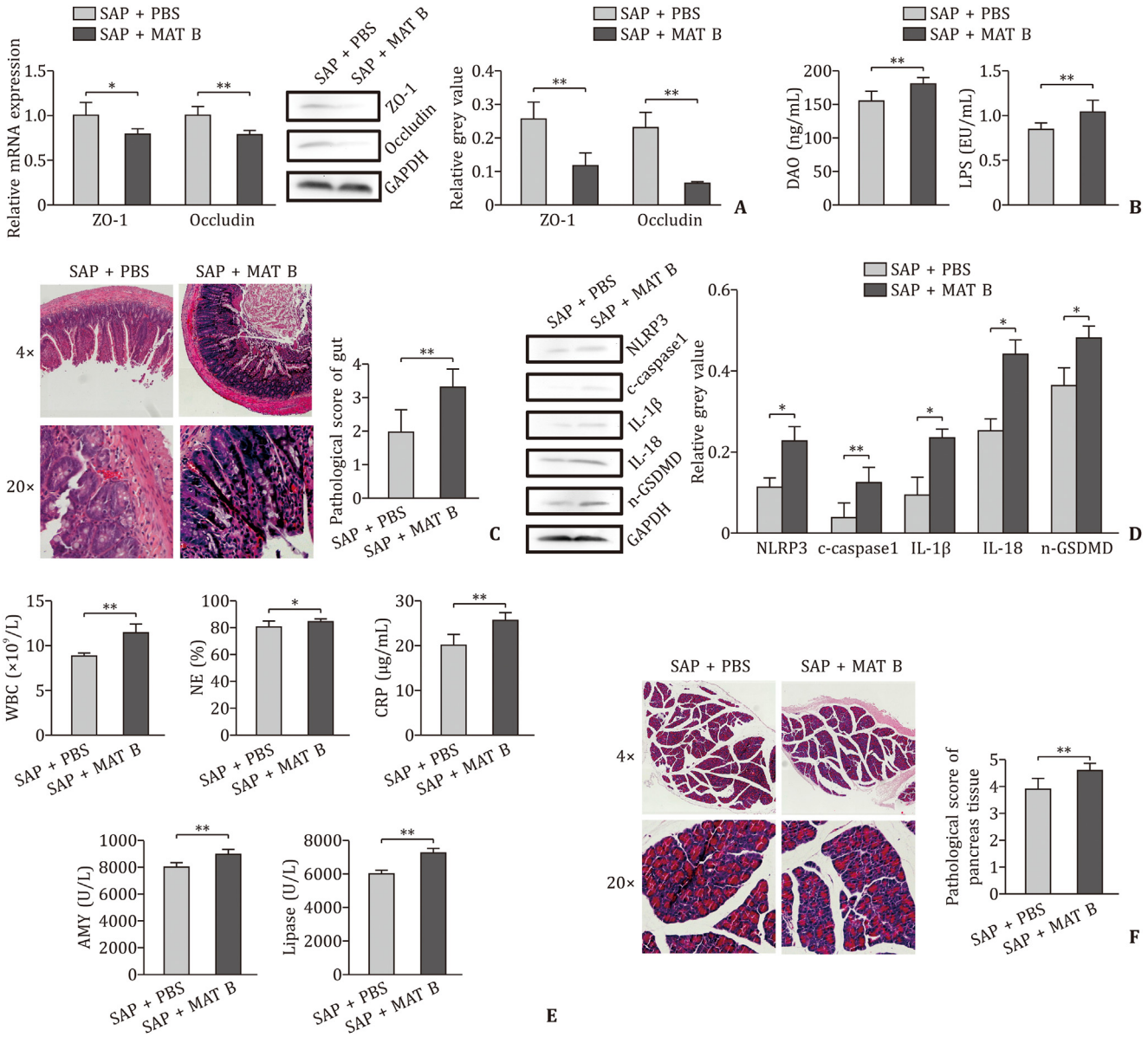

Fig. 4. MAT B lymphocytes aggravated intestinal barrier injury and enteric pyroptosis, resulting in pancreatitis progression. **A:** Expression of mRNA and proteins responsible for intestinal tight junction; **B:** levels of serum endotoxins DAO and LPS; **C:** representative HE staining of intestine and the pathological scores of intestinal tissues; **D:** expression of pyroptotic proteins in intestine, specifically, NLRP3, c-caspase1, IL-1β, IL-18 and n-GSDMD; **E:** inflammatory indicators and pancreatin in rats; **F:** representative HE staining of pancreas and the pathological scores of pancreas tissues. Values represent means ± standard deviation. * $P < 0.05$; ** $P < 0.01$. SAP: Severe acute pancreatitis; MAT: mesenteric adipose tissue; WBC: white blood cells; NE: neutrophil; CRP: C-reactive protein; AMY: amylase; DAO: diamine oxidase; LPS: lipopolysaccharide; IL-1β: interleukin-1β; IL-18: interleukin-18.

the SAP + MAT B + MCC950 group, the mRNA and protein expression of ZO-1 (both $P < 0.01$) and occludin ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 5A) were increased, and the serum levels of LPS ($P < 0.01$) and DAO ($P < 0.01$) were decreased (Fig. 5B). Histological analysis revealed that the inflammatory response of the intestinal tissue was reduced with significantly lower pathological scores ($P < 0.05$) in the SAP + MAT B + MCC950 group (Fig. 5C). Regarding pyroptosis in the intestine, the critical proteins expression of NLRP3 ($P < 0.01$), c-caspase1 ($P < 0.01$), IL-1β ($P < 0.05$), IL-18 ($P < 0.05$), GSDMD ($P < 0.05$) and n-GSDMD ($P < 0.05$) were all decreased significantly in the SAP + MAT B + MCC950 group (Fig. 5D). As a result, the counts of WBC ($P < 0.01$), the percentage of neutrophil ($P < 0.05$), the serum levels of CRP ($P < 0.01$), AMY ($P < 0.01$) and lipase ($P < 0.01$) were significantly lower in the SAP + MAT B + MCC950 group compared to those in the SAP + MAT B + PBS group (Fig. 5E). The levels of pancreatic inflammation were lower, with significantly lower histological scores ($P < 0.05$) in the SAP + MAT B + MCC950 group (Fig. 5F).

NLRP3 inhibitor ameliorates B lymphocyte associated MAT local inflammation

Flow cytometry results indicated no significant difference in the infiltration of B lymphocytes, T lymphocytes and macrophages in MAT between the SAP + MAT B + PBS group and the SAP + MAT B + MCC950 group (Fig. 6A). However, compared to the SAP + MAT B + PBS group, the SAP + MAT B + MCC950 group showed a significant decrease in mRNA expression of TNF-α and IL-6 in MAT ($P < 0.05$ and $P < 0.01$, respectively) and a significant increase in mRNA expression of IL-10 ($P < 0.05$) (Fig. 6B). In addition, the histological assessment revealed a significant reduction in the inflammatory response of MAT tissue, as indicated

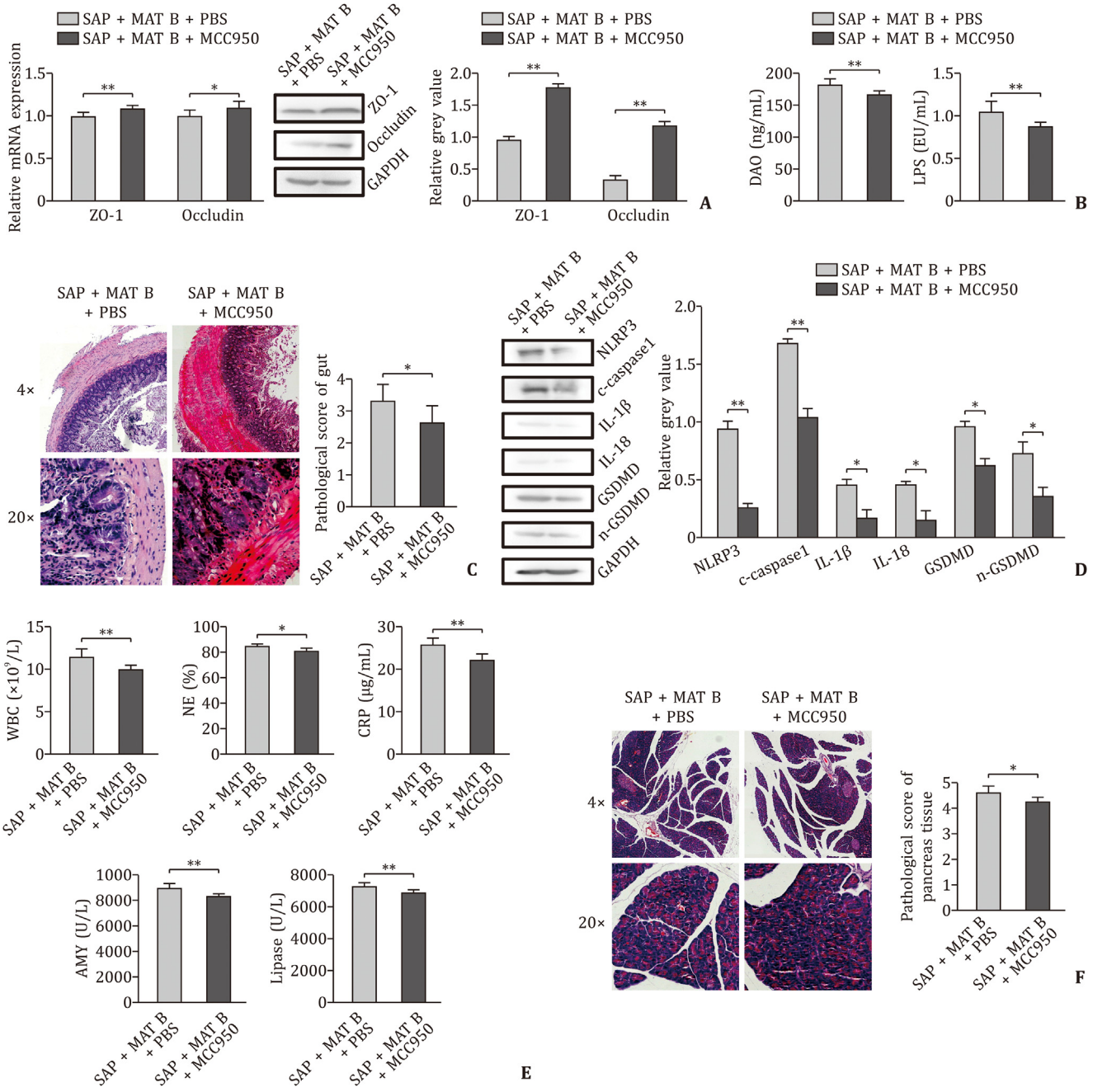

Fig. 5. MAT B lymphocytes accelerated intestinal barrier damage via enteric pyroptosis, resulting in pancreatitis deterioration. **A:** Expression of mRNA and proteins responsible for intestinal tight junction; **B:** levels of serum endotoxins DAO and LPS; **C:** representative HE staining of intestine and the pathological scores of intestinal tissues; **D:** expression of pyroptotic proteins in intestine, specifically, NLRP3, c-caspase1, IL-1β, IL-18, GSDMD and n-GSDMD; **E:** inflammatory indicators and pancreatin in rats; **F:** representative HE staining of pancreas and the pathological scores of pancreas tissues. Values represent means ± standard deviation. * $P < 0.05$; ** $P < 0.01$. SAP: severe acute pancreatitis; MAT: mesenteric adipose tissue; PBS: phosphate buffer solution; WBC: white blood cells; NE: neutrophil; CRP: C-reactive protein; AMY: amylase; DAO: diamine oxidase; LPS: lipopolysaccharide; HE: hematoxylin-eosin.

by fewer CLSs ($P < 0.05$), in the SAP + MAT B + MCC950 group (Fig. 6C).

Discussion

The volume of VAT is a crucial prognostic indicator for the severity of SAP [20–22]. Compared to healthy individuals, AP patients had thicker VAT, regardless of their body mass index (BMI) [23]. Although obesity has a significant impact on the progression of AP [24], Yoon et al. [25] revealed that VAT had a more substantial effect on the severity of the condition than body weight or BMI. However, it remains unclear which part of VAT plays the most pivotal role. VAT predominantly comprises mesenteric, perirenal, retroperitoneal, epididymal and omental adipose tissue. These adipose tissues reside in a distinct microenvironment and serve various functions that require individual assessment [26]. The MAT, a VAT component surrounding blood vessels, lymphatic vessels and nerves between the pancreas and gut, may contribute to the developing AP and secondary intestinal injury. Our study on MAT tissue in rats with SAP revealed an increase in inflammatory factors and histological inflammation, suggesting that MAT may play a potential role in the progress of SAP.

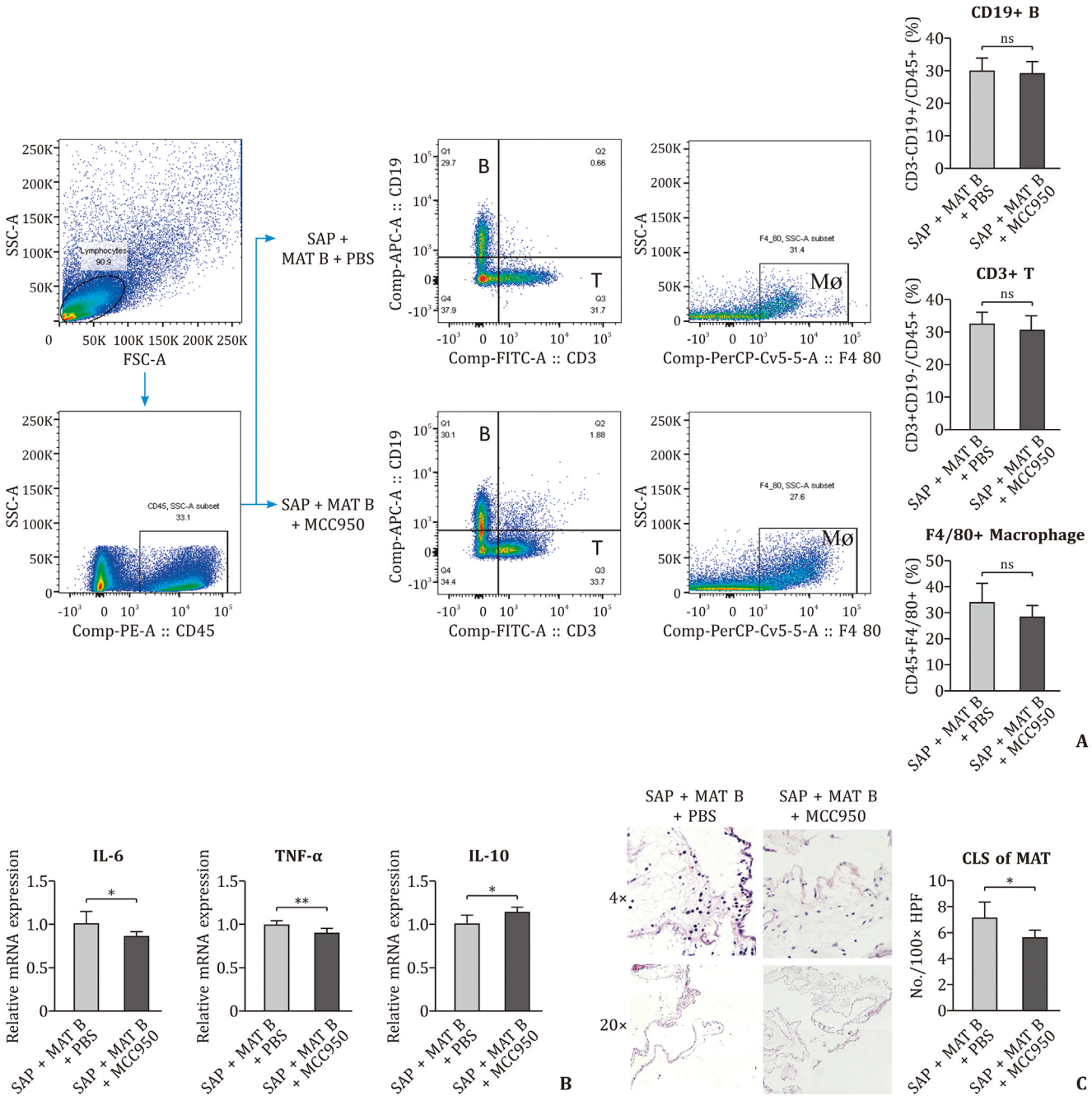

Fig. 6. NLRP3 inhibitor ameliorated B lymphocytes associated MAT local inflammatory response. **A:** The gating strategy of flow cytometry for the MAT. The proportion of CD45+CD19+ B lymphocytes, CD45+CD3+ T lymphocytes and CD45+F4/80+ macrophages in the MAT; **B:** expression of mRNA responsible for MAT inflammation; **C:** representative HE staining of MAT and the numbers of CLS in MAT. Values represent means ± standard deviation. * $P < 0.05$; ** $P < 0.01$; ns: no significance. SAP: severe acute pancreatitis; PBS: phosphate buffer solution; MAT: mesenteric adipose tissue; TNF-α: tumor necrosis factor-α; IL: interleukin; CLS: crown-like structure; HE: hematoxylin-eosin; HPF: high power field.

Given that MAT is adjacent to the intestine, we hypothesized that it could impact SAP-induced intestinal injury, a common occurrence in most AP patients. Such injury often leads to acute gastrointestinal dysfunction and damages the intestinal barrier, allowing for secondary bacterial translocation to the bloodstream and other tissues, leading to a poorer prognosis. As a potent producer of cytokines, adipokines and fatty acids, MAT is thought to play a significant role in regulating intestinal immune and inflammatory. Furthermore, studies have shown that MAT is closely linked to gut changes, such as muscular hypertrophy, fibrosis and stricture formation in Crohn's disease [27–29]. It contributes to maintaining intestinal barrier integrity and preventing NAFLD in mice [7]. However, despite this knowledge, research on the effects of MAT on intestinal injury still needs to be conducted. Our study found that SAP rats presented with MAT inflammation and suffered from intestinal barrier damage. Interestingly, when we aggravated the MAT inflammation through intervention, we observed a corresponding worsening of the intestinal barrier injury, ultimately leading to the progression of pancreatitis. These findings suggest that MAT inflammation promotes intestinal injury in SAP.

However, the factors that contribute to MAT inflammation remain unclear. The present study found that B lymphocytes, rather

than T lymphocytes and macrophages, infiltrated within MAT in SAP rats. B lymphocytes promote local inflammation by producing cytokines and secreting immunoglobulin [8]. A previous study showed that B lymphocytes infiltrated MAT and promoted local inflammation in NAFLD [9]. Accumulated data suggested that innate and adaptive immune systems play critical roles in AP progression. The peripheral neutrophil-lymphocyte ratio has been proposed to have a moderately high diagnostic value in predicting the severity of AP [30], and total circulating lymphocyte counts are markedly lower in SAP patients than in healthy individuals [31]. Additionally, the counts of B lymphocytes were decreased more obviously and persistently than T lymphocytes in SAP [32]. Shi et al. [33] reported that B lymphocytes could predict organ failure during the early phase of SAP. Circulating B lymphocytes may migrate to inflammatory sites, leading to sustained inflammatory responses and local damage [34]. In our study, when we injected MAT B lymphocytes into SAP rats, we observed a worsening of the inflammatory responses in MAT, indicating that B lymphocytes may promote MAT inflammation.

In our study, we observed intestinal barrier damage and the activation of pyroptosis in the intestine of SAP rats. Furthermore, upon injecting MAT B lymphocytes into SAP rats to induce MAT inflammation, we observed a deteriorating effect on intestinal injury and upregulation of enteric pyroptosis. This evidence suggests that the MAT inflammation may mediate pyroptosis in the intestine and affect intestinal barrier function in SAP. The contribution of pyroptosis to the pathogenesis of AP has been demonstrated in recent years. The canonical inflammasome pathway is initiated by PAMPs or DAMPs, which trigger NLRP3, etc. Activated NLRP3 binds with pro-caspase1 and facilitates its self-cleavage to generate c-caspase1 which identifies and incorporates IL-1β and IL-18 precursors, resulting in their maturation. Additionally, c-caspase1 also activates GSDMD to its mature form, n-GSDMD, which creates cell membrane pores and induces pyroptotic cell death with inflammatory factor secretion and immune cell infiltration. The process of pyroptosis promotes local and systemic inflammatory reactions [16].

In this study, we utilized MCC950, an NLRP3 inhibitor, to explore whether MAT-related inflammation aggravated by B lymphocytes leads to intestinal injury through the pyroptotic pathway in SAP rats. The results of this experiment are consistent with our initial hypothesis, and the SAP rats appeared better intestinal barrier integrity with the downregulation of enteric pyroptosis after MCC950 injection. Emerging evidence suggests that systemic inflammation reaction and intestinal injury in SAP may be associated with the elevated expression levels of pyroptotic proteins. Gao et al. [35] reported that the activation of NLRP3 and GSDMD facilitated pyroptosis and systemic inflammation in AP. Similarly, Zhang et al. [36] demonstrated that the *caspase1* gene restriction mitigated inflammation in AP rats. In the experiments, caspase1 activated pro-IL-1β, released outside the cells, triggering immune cells to produce inflammatory mediators. Recently, research conducted by Wu et al. [37] showed that the excessive activation of NLRP3 and subsequent pyroptosis in alveolar macrophages could promote lung injury in SAP. The serum levels of TNF-α, IL-1β and IL-6 were reduced, and lung injury was alleviated in *NLRP3*-gene knockout mice with SAP [38]. Furthermore, the downregulation of GSDMD reduced the serum levels of IL-1β and IL-8, and improved intestinal damage in SAP [17]. Xu et al. [39] also demonstrated the involvement of NLRP3 in SAP-associated intestinal injury via the caspase1 pathway. In our study, the intervention of NLRP3 inhibitor improved the intestinal barrier function and attenuated the B lymphocyte-associated inflammation of MAT. This may be due to better intestinal barrier integrity, reducing inflammation and bacterial translocation. Further studies are required to elucidate the role of B lymphocytes in the communication between MAT and the intestine in AP pathogenesis, which may lead to new strategies for the management of SAP.

In conclusion, we demonstrated that the infiltration of B lymphocytes aggravated local inflammation in MAT, promoting the injury to the intestinal barrier through the enteric pyroptotic pathway in SAP.

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CRediT authorship contribution statement

Qing Huang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Writing – original draft. **Jia-Wen Liu:** Methodology, Validation, Visualization. **Hai-Bin Dong:** Methodology, Validation. **Zheng-Jie Wei:** Validation. **Jin-Zhe Liu:** Validation. **Yu-Tang Ren:** Data curation. **Xuan Jiang:** Conceptualization, Data curation, Supervision, Writing – review & editing. **Bo Jiang:** Conceptualization, Supervision, Writing – review & editing.

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Ethical approval

This study was approved by the Ethics Committee of Beijing Tsinghua Changgung Hospital (22271-4-01).

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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