RORγt inhibitor SR1001 alleviates acute pancreatitis by suppressing pancreatic IL-17-producing Th17 and γδ-T cells in mice with ceruletide-induced pancreatitis

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Abstract
The management of acute pancreatitis (AP) remains a challenge to clinicians worldwide for limited effective interventions. Retinoid orphan receptor gamma t (RORγt) is a therapeutic target for several diseases; however, it is unclear whether inhibiting RORγt can ameliorate AP. The relative expression of RORγt, IL-17 and IL-23 in the peripheral blood mononuclear cells of AP patients was measured by RT-PCR. An AP mouse model was induced by ceruletide, and SR1001 was injected before ceruletide administration. RORγt+ cells, T helper 17 cells (Th17), regulatory T cells (Tregs) and γδ T cells were assessed in the pancreas and spleen by flow cytometry. Higher RORγt expression in patients indicated the potential role of RORγt in AP progression. Analyses of the IL-17/IL-23 axis confirmed its role. SR1001 significantly alleviated AP histologically in the mouse model. Serum levels of amylase, IL-6, TNFalpha, IL-17 and IL-23 decreased upon SR1001 treatment. SR1001 selectively decreased the number of RORγt+, Th17, Tregs and γδ T cells in the pancreas but not the spleen. Collectively, these results showed that SR1001 exerted therapeutic effects on AP by suppressing IL-17-secreting Th17 and γδ T cells in the pancreas. Thus, SR1001 may be a promising drug for the treatment of AP in the clinic.

KEYWORDS
acute pancreatitis, IL-17, RORγt, SR1001

1 | INTRODUCTION AND BACKGROUND

Acute pancreatitis (AP) is a common acute gastrointestinal disease that affects nearly 34/100000 individuals globally every year, especially middle-aged and older adults.
The elevated incidence and substantial sequelae of AP, such as post-pancreatitis diabetes mellitus and exocrine pancreatic dysfunction, impose a huge economic burden.\(^1\) However, therapeutic agents are limited to octreotide and preventative antibiotics.\(^2\) Thus, to improve the prognosis of AP, the development of novel effective drugs is promptly needed.

Studies have attempted to elucidate the molecular mechanisms underlying AP. The crucial role of the activated immune system, initiated by pancreatic injury, in amplifying the inflammatory response and worsening tissue damage in AP has garnered increasing attention.\(^3\) Recent studies have shown T helper cell 1 (Th1)/Th17 differentiation in the pancreatic tissue of a ceruletid-induced mouse model of AP\(^4\) and improvement of an L-arginine-induced AP model by oxymatrine via the reduction of Th1/Th17 cells secreting pro-inflammatory cytokines.\(^5\) However, it remains unclear if the selective inhibition of IL-17-secreting T cells exerts protective effects on AP.

Retinoid orphan receptor gamma t (ROR\(\gamma\)t) is a master transcription factor for Th17-producing interleukin 17 (IL-17) cells.\(^6\) ROR\(\gamma\)t inhibitors exert therapeutic efficacy in autoimmune diseases by inhibiting the differentiation of naive T cells to Th17 cells.\(^7,8\) In addition, enhanced ROR\(\gamma\)t expression in Th17 cells exacerbates experimental autoimmune encephalomyelitis.\(^9\) ROR\(\gamma\)t is generally recognized as a promising pharmacological target in some autoimmune diseases characterized by the high production of IL-17 from Th17 cells. Rats with L-arginine-induced AP exhibit overexpression of ROR\(\gamma\)t protein;\(^5\) however, whether ROR\(\gamma\)t is upregulated in AP patients and its role in the pathogenesis and progression of AP remains unclear.

In these contexts, a series of novel compounds targeting ROR\(\gamma\)t have been continuously synthesized, and their potential feasibility in treating Th17-mediated diseases has been assessed.\(^1^0\) SR1001, which specifically targets ROR\(\gamma\)t, functions as a prominent Th17 inhibitor, and its therapeutic efficacy has been widely evaluated in many autoimmune disorders such as multiple sclerosis\(^3^1\) and pathological retinal angiogenesis.\(^3^2\) Whether SR1001 contributes to the amelioration of AP by inhibiting IL-17-secreting T cells is unknown.

Here, we determined the expression of ROR\(\gamma\)t and IL-17 in the peripheral blood mononuclear cells (PBMCs) of AP patients and established a ceruletid-induced mouse model of AP pretreated with SR1001. Our findings demonstrated the therapeutic efficacy of SR1001 in AP, providing some evidence for the potential promising clinical application of SR1001 in patients with this condition.

2  |  MATERIALS AND METHODS

2.1  |  Human samples

According to the 2012 Revised Atlanta Classification criteria of typical abdominal pain, at least triply elevated serum amylase and/or lipase, and significant imaging findings (abdominal computed tomography [CT] or magnetic resonance imaging [MRI]),\(^1^3\) patients diagnosed with AP and confirmed by three surgeons separately were recruited in this study. Blood samples were collected from 17 healthy controls (HCs) and 22 AP patients within 24 h after admission to our ward. Demographic and clinical characteristics including the results of routine blood, liver function, renal function, C-reactive protein (CRP) and amylase tests were recorded (Table 1). Serum was extracted after coagulation and stored at \(-80^\circ\)C until use. All procedures were approved by the Ethics Committee of Minhang Hospital, Fudan University (2021-Approval-035-01K, Shanghai, China), and written informed consent was obtained from the volunteers. This study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology Policy for Experimental and Clinical Studies.\(^1^4\)

2.2  |  PBMC isolation

PBMCs were isolated from fresh anticoagulant blood within 6 h according to the manufacturer’s protocols. Briefly, blood samples were diluted with sterile phosphate-buffered saline (PBS), followed by the gentle addition of Lymphocyte Separation Medium (Biosharp Life Sciences, Beijing, China). After centrifugation, the PBMC layer was extracted and washed twice, resuspended in PBS, snap-frozen with liquid nitrogen and stored at \(-80^\circ\)C until use.

2.3  |  Reagents

Ceruletid and SR1001 were purchased from Abmole Bioscience Inc. (Houston, TX, USA). A Mouse Th17 Staining Kit and Mouse Treg Staining Kit were purchased from MultiScience (Hangzhou, China), together with human and mouse IL-6, IL-10, tumour necrosis factor alpha (TNF-\(\alpha\)), IL-17 and IL-23 enzyme-linked immunoaassay (ELISA) kits. FITC Mouse Anti-Mouse CD45.1, PE Hamster Anti-Mouse \(\gamma\delta\) T-cell receptor, APC Hamster Anti-Mouse CD3e and BV421 Mouse Anti-Mouse ROR\(\gamma\)t Clone Q31–378 (RUO) were purchased from BD Pharmingen™ (San Jose, CA, USA). An RNA Purification Kit was purchased from ES
Science (Shanghai, China), PrimeScript™ RT Master Mix was purchased from TaKaRa Bio Inc. (Beijing, China), and PowerUp™ SYBR™ Green Master Mix was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.4 | Mice

Eight-week-old male C57BL/6 mice were used in our study. All specific pathogen-free mice were purchased from JieSiJie Laboratory Animals Co., Ltd. (Shanghai, China), maintained under ambient temperature (24°C) and humidity and kept at a 12-h light/dark cycle. All mice had access to standard rodent chow (Shore Biotechnology Co., Ltd., Shanghai, China) and sterile water ad libitum. All animal experiments were approved by the Ethics Committee of Minhang Hospital, Fudan University (2021-Approval-035-01K, Shanghai, China).

2.5 | Mouse model of AP

After acclimation for 1 week, 20 mice were equally divided into four groups: control group received dimethyl sulfoxide (DMSO), AP model group was intraperitoneally administered 50 μg/kg ceruletide every hour for 7 consecutive hours, and SR1001 25 mg/kg and SR1001 50 mg/kg groups received 25 and 50 mg/kg SR1001 for 2 days by intraperitoneal injection twice daily, which started 1 day before AP model construction, respectively. Mice were anaesthetized and killed at 24 h after the last injection of ceruletide, and then serum and other tissues were collected. All animal experiments were repeated three times independently.

2.6 | Histological analyses

Haematoxylin and eosin (H&E) staining was conducted to evaluate the severity of AP, and mouse pancreatic
tissues were processed as previously described. Multiple sections of the same slide were assessed by two pathologists in a blinded manner, and AP severity was scored according to the following criteria: oedema, inflammatory cell infiltration, haemorrhage and fat necrosis and acinar necrosis; each item was scored 0–3, as previously described.

### 2.7 Measurement of serum alanine aminotransferase and creatinine

Serum levels of alanine aminotransferase (ALT) and creatinine in all mice were measured with the corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions. The absorbance of ALT and creatinine was measured at 510 and 546 nm, respectively, in a plate reader (Cytation 5; Shanghai BioGene Biotech Co., Ltd., Shanghai, China).

### 2.8 Flow cytometry

The pancreas and spleen were harvested from the mice, and a single-cell suspension was prepared by incubating with collagenase IV and trypsin solution. After washing twice and resuspending in 10% foetal bovine serum to reach a concentration of $1 \times 10^5$/ml, cells were stained with CD3ε-FITC, CD4-PerCP-Cy5.5 and IL-17A-PE for Th17 (CAT#KTH217, LOT#A00613) and Mouse Anti-Mouse CD45.1-FITC, γδ T-Cell Receptor-PE (331210, A014586) and CD3ε-APC for γδ T cells. CD4-FITC and CD25-APC antibodies were used to measure the regulatory T cells (Tregs), after which the cells were fixed, permeabilized and stained with Foxp3+PE antibody (KTR201, A01115). RORγt was stained with RORγt + BV421 antibody (25-6981-82, RO73620). Finally, $1 \times 10^5$ cells were measured using the Beckman Coulter Cyan ADP Analyser (Beckman Coulter Inc., Sykesville, MD, USA) and analysed by FlowJo software v.10 (TreeStar Inc., Ashland, OR, USA).

### 2.9 ELISA

According to the manufacturer’s instructions, the concentrations of pro-inflammatory and anti-inflammatory cytokines including IL-6 (CAT#EK106, LOT#A10610535 for mouse cytokine, EK206, A20610435 for human), IL-10 (EK110, A11001144, EK210, A21000714), TNF-α (EK182, A18201042, EK217, A21710141), IL-17 (EK117, A11700641, EK223, A22310354) and IL-23 (EK123, A12301234, EK282, A28210345) in all mouse and human blood samples and serum amylase (ab102523; Abcam) in mice were measured with the corresponding ELISA kits.

### 2.10 Real-time PCR

RNA in PBMCs was isolated using the RNA Purification Kit, and 500 ng RNA was reverse-transcribed into cDNA with PrimeScript™ RT Master Mix, after which SYBR™ Green Master Mix was added to the RT-PCR reaction. The relative expression of IL-17, IL-23 and RORγt was calculated by $\Delta\Delta$CT, and β-actin was chosen as the reference gene. The primer sequences of each target gene are shown in Table 2.

### 2.11 Statistical analyses

One-way analysis of variance was used to compare data among groups and the Mann–Whitney U test was used for data comparisons between two groups. Data are expressed as the mean ± standard error of the mean. GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA) was applied for all statistical analyses. $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered highly significant.

**Table 2** Primer sequences for RT-qPCR in this study

| Gene name | Primer sequence | NCBI accession number |
|-----------|-----------------|-----------------------|
| IL-17     | F: 5’-TACAACCGATCCACCTCACC-3’<br>R: 5’-AGTGGTCCTTCCAGGTGTTGA-3’ | NG_033021.1 |
| IL-23     | F: 5’-CAGGTATGAAGTAGGGGCGTG-3’<br>R: 5’-GGGACTGAGGCTTGGAATCT-3’ | NC_000012.12 |
| RORγt     | F: 5’-GTGGGGGACAAAGTTGCTCGG-3’<br>R: 5’-GGACTGAGGCTTGGATATTTG-3’ | NM_001001523.2 |
| β-Actin   | F: 5’-GAGACCTCCAACACCCACGC-3’<br>R: 5’-ATGTCACGCACAGATTTCCCC-3’ | NM_001083538.3 |
3 | RESULTS

3.1 | RORγt is upregulated and IL-17 is overexpressed in AP patients

In this study, 39 participants were recruited including 17 HCs and 22 patients with AP. The demographic and clinical characteristics of the AP patients and HCs including age; sex; body mass index; white blood cell count and classification; and serum levels of ALT, aspartate transaminase (AST), amylase, CRP, creatinine and blood urea nitrogen (BUN) were also recorded. As indicated in Table 1, AP patients exhibited significantly elevated CRP and amylase levels. PBMCs and serum were separated from the blood samples. RT-PCR was conducted to detect the relative expression of RORγt and IL-17, and ELISA was used to measure the IL-17 level. As shown in Figure 1A, RORγt was upregulated by approximately 4.8-fold in AP patients compared to HCs. Highly expressed IL-17 at the transcriptional and translational levels (Figure 1B,C) was detected as well. These results demonstrated the overexpression of RORγt and IL-17 in AP patients.

3.2 | SR1001 effectively attenuates AP in a ceruletide-induced mouse model of AP

To investigate whether AP can be alleviated by an RORγt inhibitor, a mouse model of AP model was constructed by consecutive intraperitoneal injection of ceruletide,15 and SR1001 (25 and 50 mg/kg), a traditional RORγt inhibitor, was administered before AP induction. The experimental design is shown in Figure 2A. Histological analysis of the pancreas by H&E staining (Figure 2B) showed that acinar cell oedema and inflammatory cell infiltration were significantly improved in SR1001-treated pancreatic tissues compared with the AP group. The pathological score (Figure 2C) showed alleviated pancreatic inflammation upon SR1001 treatment. The serum levels of amylase, ALT and creatinine were increased by more than sixfold in AP mice compared to control mice, whereas the level decreased by at least one-half in SR1001-treated mice (Figure 2D–F). These results demonstrated that RORγt inhibition by SR1001 had marked therapeutic efficacy in mice with ceruletide-induced AP.

IL-6 is an early detector and biomarker for evaluating the severity of AP.18 We found that the serum level of IL-6 was greatly increased in the AP group and largely decreased in the SR1001-treated group (Figure 2G), indicating the substantial alleviation of AP. As evidenced by the reduced pro-inflammatory cytokine TNF-α (Figure 2H) and elevated anti-inflammatory cytokine IL-10 (Figure 2I), there was great amelioration of the inflammatory response following SR1001 treatment.

3.3 | SR1001 selectively inhibits RORγt in the pancreas rather than spleen of mice with ceruletide-induced AP

Because SR1001 is a traditional RORγt inhibitor with high affinity, it specifically suppresses the transcription of IL-17 and reduces the differentiation of Th17.11 Overexpression of RORγt in sepsis is associated with pancreatic injury in a mouse model,19 L-arginine-induced AP in a rat model5 and elevated serum IL-17 in AP patients.18 It remains unknown whether RORγt is upregulated in a ceruletide-induced AP mouse model. In this study, the percentage of RORγt+ cells in the pancreas of ceruletide-induced AP mice was analysed by flow cytometry. As shown in Figure 3A,B, the proportion of pancreatic RORγt+ was increased by approximately threefold in AP mice compared to control mice, while SR1001 significantly reduced the elevated RORγt+ cells in SR1001-treated mice. To investigate whether SR1001 functions systemically, splenic RORγt+ cells were detected as well. Figure 3C,D shows that the percentage of RORγt+ cells was comparable among the three groups. RORγt plays a fundamental role in the transcription of IL-17; therefore, we investigated whether the level of IL-17 was affected by SR1001 administration. As shown in Figure 3E, serum IL-17 was markedly elevated by 18-fold in mice with ceruletide-induced AP compared to the controls and was greatly declined in the 25 and 50 mg/kg SR1001-treated groups. Furthermore, IL-23 was also measured and found to be altered, similar to IL-17.

**P < 0.001, ***P < 0.005
These findings suggest that SR1001 targets pancreatic ROR\(\gamma_t\) cells, possibly through suppressing the IL-17/IL-23 immune pathway, and this effect was independent of immune cells in the spleen.

### 3.4 Inhibition of ROR\(\gamma_t\) by SR1001 significantly reduces Th17 differentiation in the pancreas of mice with AP

Mounting evidence has shown that IL-17 is a distinctive cytokine generated by Th17 cells, which in turn works with IL-23 to stimulate Th17.\(^{20}\) During AP progression, IL-17 + Th17 cells increase at all time points after ceruletide induction.\(^{17}\) Similar results were obtained in our study by flow cytometry. The percentage of pancreatic CD4+/IL-17 + Th17 cells was increased by nearly twofold in mice with ceruletide-induced AP; however, it declined after SR1001 injection (Figure 4A,B). Tregs are vital for maintaining immune homeostasis. We detected the significant reduction of CD25+/Foxp3 + Tregs in the pancreas of mice with AP, which decreased further in the SR1001-treated group (Figure 4C,D). Figure 4I shows that the pancreatic Th17/Treg ratio in AP mice was increased by more than sixfold compared to the control, but was significantly decreased in the SR1001-treated group, indicating that the immune imbalance in AP mice was significantly reversed upon SR1001 treatment. Splenic Th17 and Tregs were also measured, and no differences in their levels were found (Figure 4E–HJ). Together, these results demonstrated that targeted pancreatic ROR\(\gamma_t\) inhibition by SR1001 suppressed Th17 and Treg differentiation, thereby reversing the immune imbalance in AP.
γδ T cells are considered an important early first line of immune defence of acute infection and initial IL-17 producer. RORγt antagonism has obvious inhibitory effects on IL-17-secreting γδ T cells (also named Tγδ17) in patients with spondyloarthritis. Therefore, to evaluate the function of γδ T cells in AP progression, we measured the percentage of γδ T cells in the pancreas and spleen. The flow cytometry results revealed that pancreatic CD3ε+γδ-TCR+γδ T cells increased from 0.875% in control mice to 5.112% in mice with ceruletide-induced AP but decreased to 1.884% in SR1001-treated mice (Figure 5A, B). However, similar results were not observed in the spleen (Figure 5C,D). These findings suggest the crucial role of Tγδ17 in promoting the development of AP, while reducing Tγδ17 by the RORγt inhibitor SR1001 may contribute to a good prognosis of AP.

Given that levels of IL-17 and IL-23 were increased in mice with AP and significantly decreased upon SR1001 treatment (Figure 3D,E), we investigated whether the IL-17/IL-23 immune axis is activated in AP patients. ELISA results showed that pro-inflammatory cytokines IL-6 and TNF-α were increased by about 200-fold (from 0.39 ± 0.15 to 82.45 ± 54.16 pg/ml) and 4-fold (from 334.23 ± 149.55 to 1227.03 ± 250.67 pg/ml), respectively, and the anti-inflammatory cytokine IL-10 was increased by 18-fold in AP patients. Furthermore, the RT-PCR assay confirmed activation of the IL-17/IL-23 immune pathway in AP patients, as indicated by the upregulated expression of IL-17 and IL-23 and corresponding protein concentrations in serum (Figures 1B,C and 6D,E). These results demonstrated that the IL-17/IL-23 immune axis was considerably activated in AP patients.

**Figure 3** SR1001 targets RORγt in the pancreas rather than spleen of mice with ceruletide-induced AP. Flow cytometry analyses of RORγt in the pancreas and spleen of mice with ceruletide-induced AP and control mice. (A) Representative images of RORγt in the pancreas of mice with ceruletide-induced AP (left) and a fluorescence histogram of RORγt+ cells (right). (B) Quantitative percentage of RORγt+ cells in the pancreas. (C) Representative images of RORγt in the spleen of mice with ceruletide-induced AP (left) and fluorescence histogram of RORγt+ cells (right). (D) Quantitative percentage of RORγt+ cells in the spleen. (E,F) Quantitation of serum levels of IL-17 (E) and IL-23 (F) by ELISA in mice with ceruletide-induced AP and control mice. Data are presented as the mean ± SEM; n = 5. *P < 0.05, **P < 0.01, ***P < 0.005. ns, not significant
SR1001 selectively inhibits Th17 differentiation in pancreatic rather than splenic tissues. Flow cytometry was conducted to determine the proportion of CD4+/IL-17A + Th17 cells in the pancreas (A,B) and spleen (C,D) of mice with AP. (A) Representative images of cytometry results (left) profiling pancreatic CD4+/IL-17A + Th17, fluorescence histogram of Th17 cells (right) and (B) quantified analyses of Th17 cells in pancreatic tissues. (C) Representative images of cytometry results (left) profiling splenic Th17, fluorescence histogram of Th17 (right) and (D) quantitative analyses of Th17 in spleen. The proportion of CD25+/Foxp3+ Treg cells in the pancreas (E,F) and spleen (G,H) of mice with AP was assessed by flow cytometry. (E) Representative images of cytometry results (left) showing the percentage of Tregs, fluorescence histogram of Tregs (right) and (F) quantitative analyses of Treg in pancreatic tissues. (G) Representative images of cytometry results (left), fluorescence histogram of Tregs (right) and (H) quantitative analyses of Tregs in the spleen. (I,J) the ratio of Th17 to Tregs in the pancreas (I) and spleen (J) of mice with ceruletide-induced AP. Data are expressed as the mean ± SEM; n = 5. *P < 0.05, **P < 0.005. ns, not significant.
Here, we report RORγt is highly expressed in the PBMCs of AP patients, making it a potential therapeutic target for AP. RORγt was discovered as a novel transcriptional factor for IL-17, which plays a key role in maintaining Th17 differentiation.22 Th17, characterized by IL-17 secretion, is a newly discovered subset differentiated from naïve T cells, which normally contributes to anti-inflammatory immune homeostasis.22,23 However, its pathogenicity in eliciting various autoimmune or inflammatory diseases has been widely reported.24 Similar to Th17, IL-17-producing γδ T cells are another main source of innate IL-17 in the early stage of infectious or autoimmune diseases, which are also regulated by the expression of RORγt.25 AP is a local and systemic inflammatory disease, in which complex immunological reactions occur.3 In this study, we found that Th17 and γδ T cells were significantly increased in the pancreas of an AP mouse model, accompanied by the elevated secretion of IL-17. These
results indicate the possible function of IL-17-producing Th17 cells and γδ T cells in the pathogenesis of AP.

AP, mainly caused by gallstones, alcohol abuse or hypertriglyceridaemia, is becoming one of the most common emergent GI diseases. Nearly 80% of AP patients exhibit a mild course, and the remaining 20% progress to severe disease, in which systemic inflammatory response syndrome or fatal complications might occur.26,27 Thanks to the updated 2012 Revised Atlanta Classification for AP diagnosis and grading13 and multiple Pancreatitis Activity Scoring System for severity prediction,28 AP patients in the early stage can be identified and undergo initial therapies. Accordingly, the American Gastroenterological Association recommends initial management paradigms in the early stage of AP, such as goal-directed fluid management.29 Despite these advancements in clinical guidelines and interventions, the mortality rate of AP remains 1.16/100000 person-years annually.1 Therefore, to achieve the goal of alleviating acute inflammatory responses and improving outcomes of AP, more emphasis is suggested to be attached to effective drug development.

Since RORγt is specific for IL-17 and acts as an important mediator for type 17 T cells, it has been universally recognized as an attractive target for many autoimmune diseases.6 RORγt protein level was proved to be associated with AP in animal model7; furthermore, our results detected RORγt upregulation in transcriptional level of AP patients and overexpression in translational level in pancreas of ceruletide-induced AP mice. A recent study underlines that RORγt inhibitors show great pharmacological potentials in ameliorating aberrant ‘type 17 responses’ in patients with spondyloarthritis17 and enhanced RORγt expression in Th17 cells would accelerate the progression of experimental autoimmune encephalomyelitis.5 Therefore, a series of small molecular RORγt inhibitors is constantly developed, and their therapeutic applications are continuously elucidated in several autoimmune disorders.10 SR1001 is a traditional RORγt inhibitor, and it could conspicuously alleviate multiple sclerosis by inhibiting Th17 differentiation and IL-17 secretion. Its inhibitory effects on oxygen-induced retinopathy and spontaneous subretinal neovascularization mouse model have been demonstrated.12 Therefore, SR1001 was administered to ceruletide-induced AP mice in this study, and our results demonstrate a remarkable therapeutical efficacy on AP. Furthermore, pancreatic Th17 and γδ T cells decline upon SR1001 treatment in an AP mice model, which means that SR1001 exerts an inhibitory effect on Th17 and γδ T cells in pancreas. Upregulated RORγt and IL-17 in PBMC of AP patients provide evidence for the potential application of SR1001 in clinic.

Type 17 cells, comprising Th17, γδ-T, natural killer T and innate lymphoid cells, bear the capability of IL-17 production and function as sentinels when tissue injury or acute infection occurs.30 Increased proportion of Th17 in pancreas, together with reduced Tregs, has been demonstrated in Xu’s study,4 which is in accordance with our results; ulteriorly, we found a significant augment of pancreatic γδ-T cells as well. Intriguingly, upon administration of SR1001, pancreatic Th17 and γδ-T cells simultaneously decreased and reduced Th17/Treg ratio help maintain immune tolerance. Th17 differentiation is mainly dependent on IL-17 expression. The transcription of IL-17 is induced by RORγt, which then triggers early-stage Th17 differentiation.22 During the AP progression, over-secreting IL-17 contributes to the imbalance of Th17 homeostasis. However, SR1001 administration can reverse the excessive expression of Th17 by inhibiting RORγt transcription and reverse IL-17 secretion in the pancreas. Even though similar findings are not presented in spleen of mice with AP, it still means that SR1001 functions in local pancreas rather than peripheral immune organs in the early stage of AP. These negative results from the spleen may be attributed to the gaps between ceruletide-induced AP mouse model and pancreatitis in the clinic. Th17 cells bear pathogenic and non-pathogenic capacities. IL-6-STAT3 pathway, a prerequisite for the expression of RORγt, jointly with IL-17-IL-23 axis and IL-1β functions as inducing factor for pathogenic Th17.20,31 In our study, IL-6, IL-17 and IL-23 are collectively increased notably in AP patients and ceruletide-induced AP model, which hints inductive pathogenic Th17 in pancreas. Tregs plays a fundamental role in regulating immune balance between Th1/Th2 differentiation and immune suppression, and Tregs presents absent during AP.5 Here, Th17/Tregs ratio was reversed, yet Tregs decreased upon SR1001 treatment. We speculated that this result was caused by SR1001 administration because of the complexity of Tregs.32 Effects of SR1001 on Tregs and the complicated interrelationship between RORγt and Tregs require more investigations.

5 | CONCLUSION

We first demonstrated that inhibiting RORγt by SR1001 exerted significant therapeutic effects on ceruletide-induced AP mice, which was dependent on the selective inhibition of pancreatic IL-17-producing Th17 and γδ T cells. The overexpression of RORγt was observed in the PBMCs of AP patients. The IL-17/IL-23 immune pathway was activated during AP with SR1001 treatment, leading to downregulation of this axis. These findings indicate
that SR1001 may have therapeutic efficacy in the early stage of AP.

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CONFLICT OF INTEREST
These authors declare that they have no conflicts of interest.

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