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Deletion of myeloid-specific *Orai1* calcium channel does not affect pancreatic tissue damage in experimental acute pancreatitis

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ABSTRACT

Background: Store-operated Ca^{2+} entry (SOCE) mediated by ORAI1 channel plays a crucial role in acute pancreatitis (AP). Macrophage is an important regulator in amplifying pancreatic tissue damage, but little is known about the role of ORAI1 in macrophages. In this study, we examined the effects of macrophage-specific ORAI1 on pancreatic tissue damage in AP.

Method: Myeloid-specific *Orai1* deficient mice was generated by crossing a LysM-Cre mouse line with *Orai1*$^{f/f}$ mice. Bone marrow-derived macrophages (BMDMs) were isolated, cultured, and stimulated to induce M1 or M2 macrophage polarization. Intracellular Ca^{2+} signals were measured by time-lapse confocal microscope imaging, with a Ca^{2+} indicator (Fluo 4). Experimental AP was induced by hourly intraperitoneal injections of caerulein or retrograde biliopancreatic infusion of sodium taurocholate. Pancreatic tissue damage was assessed by histopathological scoring and immunostaining. Sepsis was induced by intraperitoneal injection of lipopolysaccharide; organ damage and serum pro-inflammatory cytokines were measured.

Result: Myeloid-specific *Orai1* deletion exhibited minimal effect on SOCE in M0 macrophages and promoted M2 macrophage polarization *ex vivo*. Myeloid-specific *Orai1* deletion did not affect pancreatic tissue damage, nor neutrophil or macrophage infiltration in two models of AP. Similarly, myeloid-specific *Orai1* deletion did not influence overall survival rate in a model of sepsis, nor lung, kidney, and liver damage; while serum pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1β were higher in *Orai1*$^{\Delta LysM}$ mice, but were largely reduced in mice with Orai1 inhibitor.

Conclusion: Our data suggest that ORAI1 may not be a predominant SOCE channel in macrophages and play a limited role in mediating pancreatic tissue damage in AP.

1. Introduction

Acute pancreatitis (AP) is a common inflammatory disease of the exocrine pancreas, with abdominal pain as the main symptom. Its incidence has been increasing gradually over the years. The mortality rate of severe AP patients can reach 30 %. Currently, there is lack of specific treatment for AP [1–3]. The severity of AP and its

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outcomes are determined by pancreatic local necrosis and persistent distant organ failure. Pancreatitis was initiated within pancreatic acinar and/or ductal cells and sustained by uncontrolled systemic inflammatory responses [4,5]. The degree of pancreatic tissue damage is mediated by initial pancreatic exocrine cell necrosis and exacerbated by activation of infiltrating immune cells. The macrophage is a crucial mediator in amplifying pancreatic tissue damage [6,7]. However, the intrinsic signaling pathway by which macrophages regulate pancreatic tissue damage is not well-understood.

We and others have shown that ORAI1 inhibitors mitigated pancreatitis severity in various mouse models of AP by preventing Ca^{2+}-associated pancreatic acinar cell death and vacuole formation [8–10]. Furthermore, we recently showed that neutrophil-specific deletion of *Orai1* protects against pancreatitis- or sepsis-associated lung injury via inhibiting neutrophil-intrinsic functions, including intracellular Ca^{2+} influx, migration, reactive oxygen species production and neutrophil extracellular trap formation [11]. Limited studies showed that ORAI1 expressed in macrophages and may play some role in the activation of macrophages [12,13]. Given that macrophage is a primary regulator in amplifying pancreatic tissue damage during AP [14,15]. This study aims to examine the effects of ORAI1 in macrophages on pancreatic tissue damage, using two experimental AP models and myeloid cell-specific *Orai1* deficient mice.

Here, we found myeloid cell-specific *Orai1* deletion had minimal effects on Store-operated Ca^{2+} entry (SOCE) in M0 macrophages and promoted M2 macrophage polarization *ex vivo*. Myeloid cell-specific *Orai1* deletion did not affect pancreatic tissue damage in two representative models of AP and in a model of sepsis. Our data suggest that ORAI1 may not be a dominant SOCE channel in macrophages and play limited role in mediating pancreatic tissue damage in AP.

2. Methods

2.1. Regents

Caerulein (CER) (#HY-A0190) and CM4620 (#HY-101942) were obtained from MedChemExpress (Monmouth Junction, NJ). Antibody against Ly6G (#GB11229) and F4/80 (#GB113373) was purchased from Service Biosciences (Wuhan, China). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise specified.

2.2. Animals

C57BL6/J mice weighting 20–22 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The *Orai1*$^{f/f}$ mice was a kind gift from Professor Bo Xiao from the State Key Laboratory of Biotherapy of Sichuan University. LysM-Cre mice purchased from Jackson Laboratories were crossed with *Orai1*$^{f/f}$ mice to generate myeloid-specific *Orai1* deficient mice (*Orai1*$^{\Delta LysM}$). Using a random number table (n = 6–8 for each group), all mice were assigned to each experimental group in a completely randomized manner. Animal Ethics Council of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine approved all research involving animals that were carried out (2019-A019-01, Shanghai, China).

2.3. Confirmation of Orai1 deletion

Total RNA was collected from bone marrow-derived macrophages (BMDMs) to confirm the deletion of *Orai1*. The relative expression of *Orai1* was assessed using reverse-transcription quantitative polymerase chain reaction (RT-qPCR) with specific *Orai1* primers: Forward: 5′-GCTGCTCTGCTGGGTCAAGTTC-3′ and Reverse: 5′-GCTGCTGTCGCTGTGGTTGG-3'. Samples were expressed as fold changes compared to controls after being normalized to β-actin.

2.4. Isolation of mouse bone marrow-derived macrophages

Mice were sacrificed and bone marrow was extracted from tibia and femur bones following removal of surrounding muscle. To do so, joints were cut and the exposed bone marrow was flushed out using a needle and a 1 ml syringe filled with phosphate buffer saline (PBS), then cells were filtered through a 70 μm cell strainer. The cell suspension was centrifuged at 600 g for 5 min. The supernatant was discarded and cells were resuspended in PBS. BMDMs can be carried out using density gradients of Percoll: cell samples were loaded on 62 % (v/v) and 81 % (v/v) Percoll medium, separated by centrifugation at 1500*g* for 20 min. After centrifugation, BMDMs form a band in the upper layer of the medium. BMDMs were aspirated with a Pasteur pipette and centrifuged at 600 g for 5 min. After removal of red blood cells, BMDMs were re-suspended in Dulbecco's modified Eagle's medium/Ham (DMEM) F-12 medium with 10 % fetal bovine serum (FBS) and recombinant mouse macrophage colony-stimulating factor (20 ng/mL, rmM-CSF.) On day 3, nonadherent cells were removed and medium was replaced. $CD11b^{+}F4/80^{+}$ cells were confirmed by flow cytometry on day 6. Lipopolysaccharide (LPS, 100 ng/mL) and interferon gamma (IFN-γ, 10 ng/mL) were used to induce M1 macrophages, and interleukin-4 (IL-4, 50 ng/mL) was used to induce M2 macrophages on day 6.

2.5. Calcium measurement

In order to evaluate cytoplasmic Ca^{2+} signaling, the time-source photography of fluorescence confocal microscope was used to collection image every 10 s, as previously described [8]. Briefly, freshly isolated and cultured BMDMs was resuspended in N′-a-hydroxythylpiperazine-N′-ethanesulfanic acid (HEPES) buffer containing $CaCl_2$ (1.2 mmol/L) and staining for 30 min with a calcium sensitive fluorescent dye Fluo-4 (4 μM. Exit: 488 nm and emission: 515 nm). The data recorded at first 60 s were the baseline, followed by thapsigargin infusion through the perfusion system to empty endoplasmic reticulum calcium and continued recording for 600 s. Subsequently, 5 mM Ca^{2+} was added at 600 s, continuously recording until 1320 s. After the image collection was completed, the region of interest (ROI) was select using ImageJ to calculate the fluorescence intensity. Each cell's fluorescence intensity (F) was converted to a ratio of F/F0 after being normalized to the background fluorescence (F_0).

2.6. Measurement of macrophage polarization ex vivo by RT-qPCR

The mRNA transcripts in isolated bone marrow-derived macrophages were analyzed by qRT-PCR. Primers are shown as follows. Fold changes for each mRNA were calculated using the comparative CT ($2^{-\Delta\Delta CT}$) method. To control for unwanted sources of variation, the mRNA levels were normalized to β-actin. The mean values of the control group were set to 1, and the values of other groups were normalized to control group values, presented as fold mean of the controls. Each target gene was analyzed in triplicate in each experiment. Primers were shown below in Table 1.

2.7. Induction of experimental models of pancreatitis and sepsis

Mice with caerulein-induced acute pancreatitis were received ten hourly injected intraperitoneally caerulein (100 μg/kg). Saline

Table 1
Primer sequences used for measuring macrophage polarization.

Gene name	Forward primers (5′-3′)	Reverse primers (3′-5′)
Actb	GTCCCTCACCCTCCCAAAAG	GCTCCCTCAACACCTCAACCC
Tnf	TCTCTTCAAGGGACAAGGCTG	ATAGCAAATCGGCTGACGGT
Nos	AGGGAATCTTGGAGCGAGTT	GCAGCCTCTTGTCTTTGACC
Il1b	TTGACGGACCCCAAAAGAT	GAAGCTGGATGCTCTCATCTG
Il6	CACGGCCTTCCCTACTTC	TTTCCACGATTTCCCAGA
Pparg	AGCCCATCGAGGACATCCAA	CTTGGCGAACAGCTGAGAGG
Chil3	GATGGCCTCAACCTGGACTG	GAGTAGCAGCCTTGGAATGTCT
Retnla	AGGATGCCAACTTTGAATAGGATG	CAGTGGAGGGATAGTTAGCTGG
Mrc1	GGAGGCTGATTACGAGCAGT	CATAGGAAACGGGAGAACCA

injections of the same volume were given to the littermate controls, which were sacrificed 12 h later from the induction. Mice with biliary acute pancreatitis were injected with 2 % sodium taurocholate (Inject with infusion pump at the rate of 5 μL/min for over 10 min) by retrograde biliopancreatic ductal injection. Humane killing was 24 h later. The sham group received laparotomy only. Mice with chronic pancreatitis were received six hourly injected intraperitoneally caerulein (100 μg/kg) three times a week for a total of 4 weeks. Mice were injected a lethal dose of LPS (40 mg/kg) intraperitoneally to induce sepsis. The Orai1 inhibitor, CM4620 at the dose of 20 mg/kg was given before the induction of sepsis. Humane killing was 24 h later to assess the severity of tissue damage and the survival rate was monitored up to 80 h.

2.8. Measurement of serum amylase and lipase

To obtain serum, blood samples from mice with different treatment were centrifuged at 400 g for 20 min at 4 °C. Serum were collected and measured by Roche Analyzer for amylase and lipase (Roche Diagnostics, Basel, Switzerland).

2.9. Histology and immunohistochemistry

Pancreatic tissues were prepared for H&E or immunohistochemistry staining after being fixed in 4 % formalin and paraffin. Two blinded independent researchers score edema, inflammatory infiltration, and necrosis of the pancreas on 10 randomly selected fields (× 200) and calculate means ± standard error of the mean (SEM, using 6 mice per group). Paraffin-embedded pancreatic tissue slices were subjected to immunohistochemistry for Ly6G and F4/80. Rabbit anti-mouse Ly6G and F4/80 primary antibodies (1:1000) were employed and incubated overnight at 4 °C. The Horseradish peroxidase-labeled secondary antibodies were then incubated for 1 h at room temperature. Then visualized by using diaminobenzidine chromogenic reaction system (Vector Laboratories, Burlingame, CA).

2.10. Statistical analysis

Data were showed as mean ± SEM and analyzed by GraphPad Prism 7.0 software. Comparisons between groups were performed by using analysis of variance. A P value < 0.05 was considered statistically significant.

3. Result

3.1. Myeloid cell-specific Orai1 has minimal effects on store-operated calcium entry in mouse bone marrow-derived macrophages

To investigate the role of ORAI1 in macrophages, we generated myeloid-specific *Orai1* deficient mice by crossing a LysM-Cre mouse

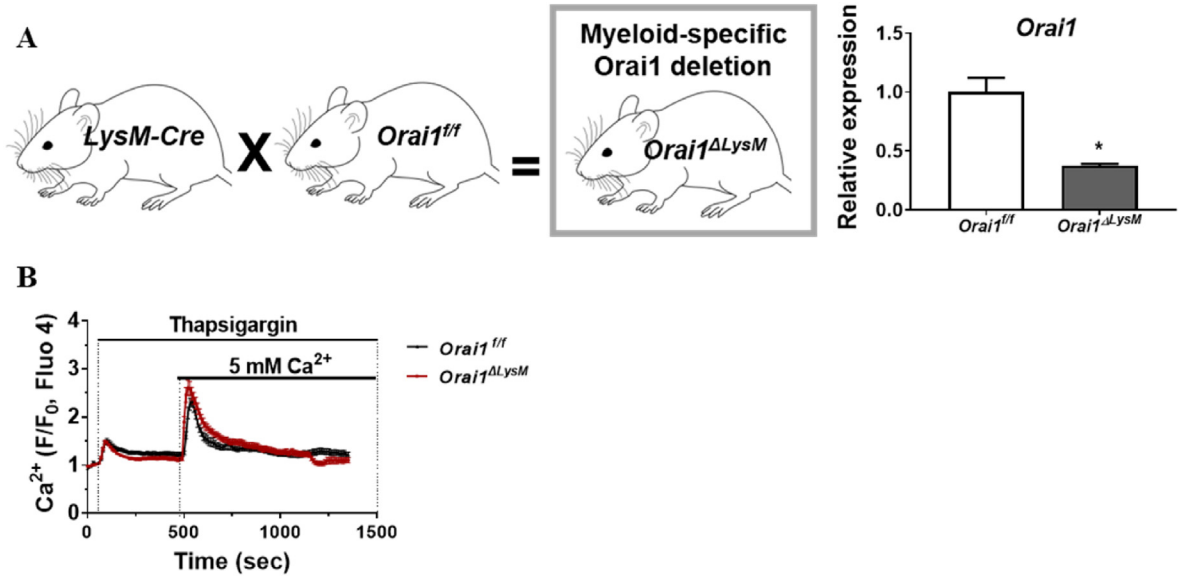

Fig. 1. Myeloid-specific *Orai1* deletion had minimal role in mediating Ca^{2+} influx in macrophages. (A) Schematic diagram for generation of myeloid-specific Orai1 deficient mice and Orai1 expression in mouse bone marrow-derived macrophages (BMDMs) from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice. (B) Changes of cytosolic Ca^{2+} in M0 macrophages from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice. *p < 0.05, compared to *Orai1*$^{f/f}$.

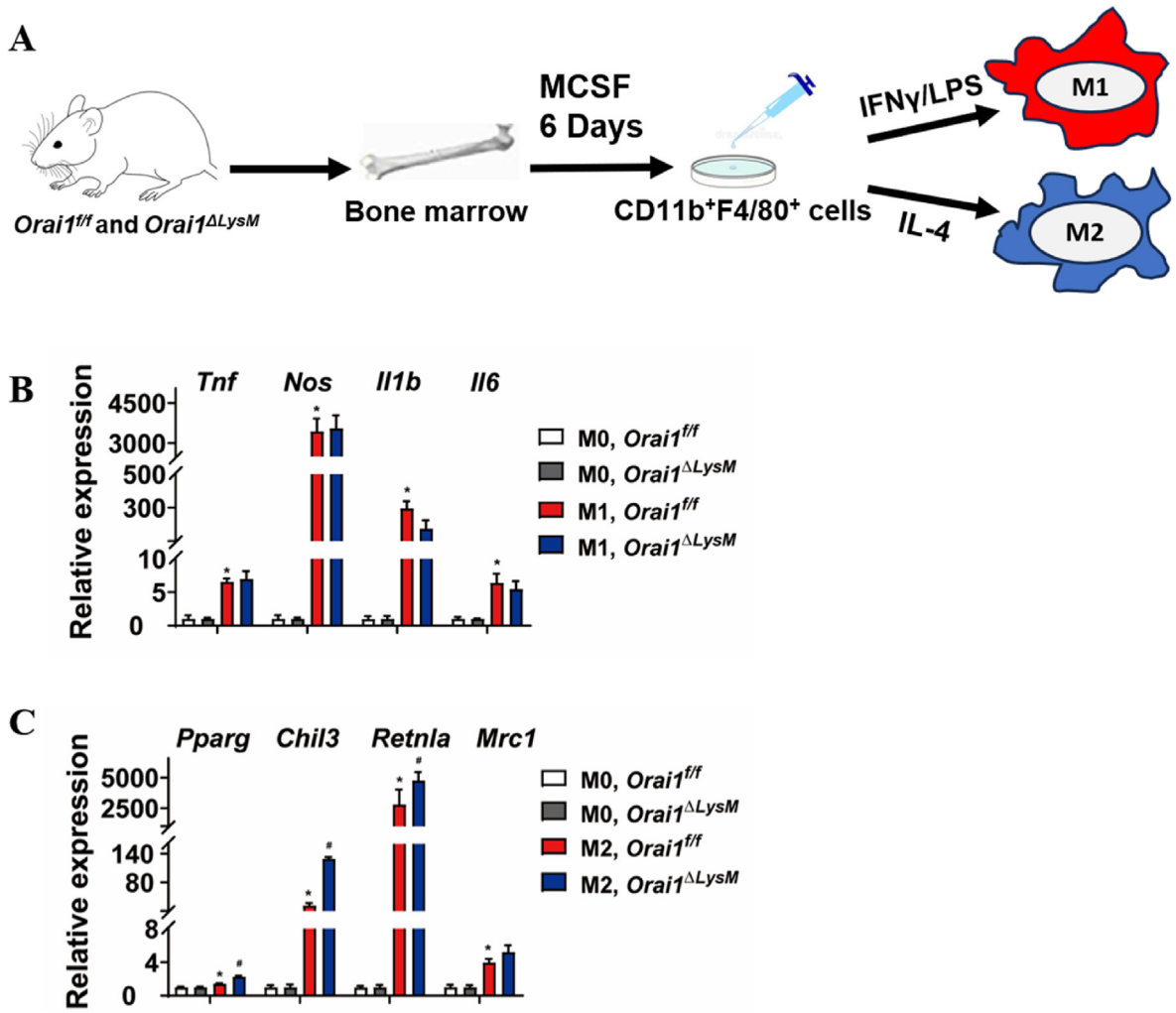

Fig. 2. Effects of myeloid-specific *Orai1* deletion on macrophage polarization *ex vivo*. (A) Schematic diagram for BMDMs isolation and induced polarization from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice. (B) Genes related to M1 polarization and (C) M2 polarization validated by qRT-PCR from the BMDMs of *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice. Data were presented as mean ± SEM, n = 3–5 per condition, *p < 0.05, compared to M0 *Orai1*$^{f/f}$, $^{\#}$P < 0.05, compared to the M2 *Orai1*$^{f/f}$.

line with *Orai1*$^{f/f}$ mice [16] and RT-qPCR result confirmed that the relative expression of *Orai1* were significant down-regulated in BMDMs of *Orai1*$^{\Delta LysM}$ mice (Fig. 1A). Next, we assessed ORAI1-mediated SOCE in macrophages. BMDMs were isolated and cultured for 7 days to maintain as M0 macrophages. Thapsigargin was used in zero external Ca^{2+} to empty Ca^{2+} stores, and allow SOCE by re-introducing external Ca^{2+}. Consistent with current known report [17], we observed that M0 macrophages from myeloid-specific deficient *Orai1* or *Orai1*$^{f/f}$ mice exhibited similar degree of SOCE (Fig. 1B), suggesting that ORAI1 in macrophages exhibited minimal effect on SOCE.

3.2. Myeloid cell-specific Orai1 deletion mediates macrophage polarization ex vivo

To evaluate the effects of ORAI1 on macrophage polarization, BMDMs were isolated from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice, respectively. IFN-γ and LPS were used to induce M1 macrophage polarization and IL-4 was used to induce M2 macrophage polarization (Fig. 2A). After stimulation with LPS and IFN-γ, we found that BMDMs derived from *Orai1*$^{f/f}$ mice had an increase in *Tnf*, *Nos*, *Il1b*, and *Il6* expression; while BMDMs from *Orai1*$^{\Delta LysM}$ mice exhibited similarly elevation in those M1-specific cytokines (Fig. 2B). After stimulation with IL-4, we observed that BMDMs derived from *Orai1*$^{f/f}$ mice similarly had an increase in *Pparg*, *Chil3*, *Retnla* and *Mrc1* expression; BMDMs from *Orai1*$^{\Delta LysM}$ mice exhibited further increase in those M2-specific genes, indicating that deletion of *Orai1* in macrophages favors to promote M2 macrophage polarization (Fig. 2C). Taken together, these data suggest that ORAI1 in macrophages exhibited minimal effects on M1 macrophage polarization, but promoted M2 macrophage polarization *ex vivo*.

3.3. Myeloid cell-specific Orai1 deletion does not prevent pancreatic tissue damage in caerulein hyperstimulation pancreatitis

We next assessed the effects of macrophage-specific deletion of *Orai1* on pancreatic tissue damage, using caerulein hyperstimulation pancreatitis model. Caerulein hyperstimulation pancreatitis model is the most widely used and representative experimental model of acute pancreatitis [18]. Compared to *Orai1*$^{f/f}$ mice, overall histological severity score of pancreatic section in *Orai1*$^{\Delta LysM}$ mice was identical (Fig. 3A and B). Similarly, sub-score of edema and inflammatory infiltrate from *Orai1*$^{f/f}$ mice and *Orai1*$^{\Delta LysM}$ mice remained no differences; while compared to *Orai1*$^{f/f}$ mice, there was an increase in sub-score of necrosis in *Orai1*$^{\Delta LysM}$ mice (Fig. 3C). As macrophages and neutrophils are two crucial innate immune

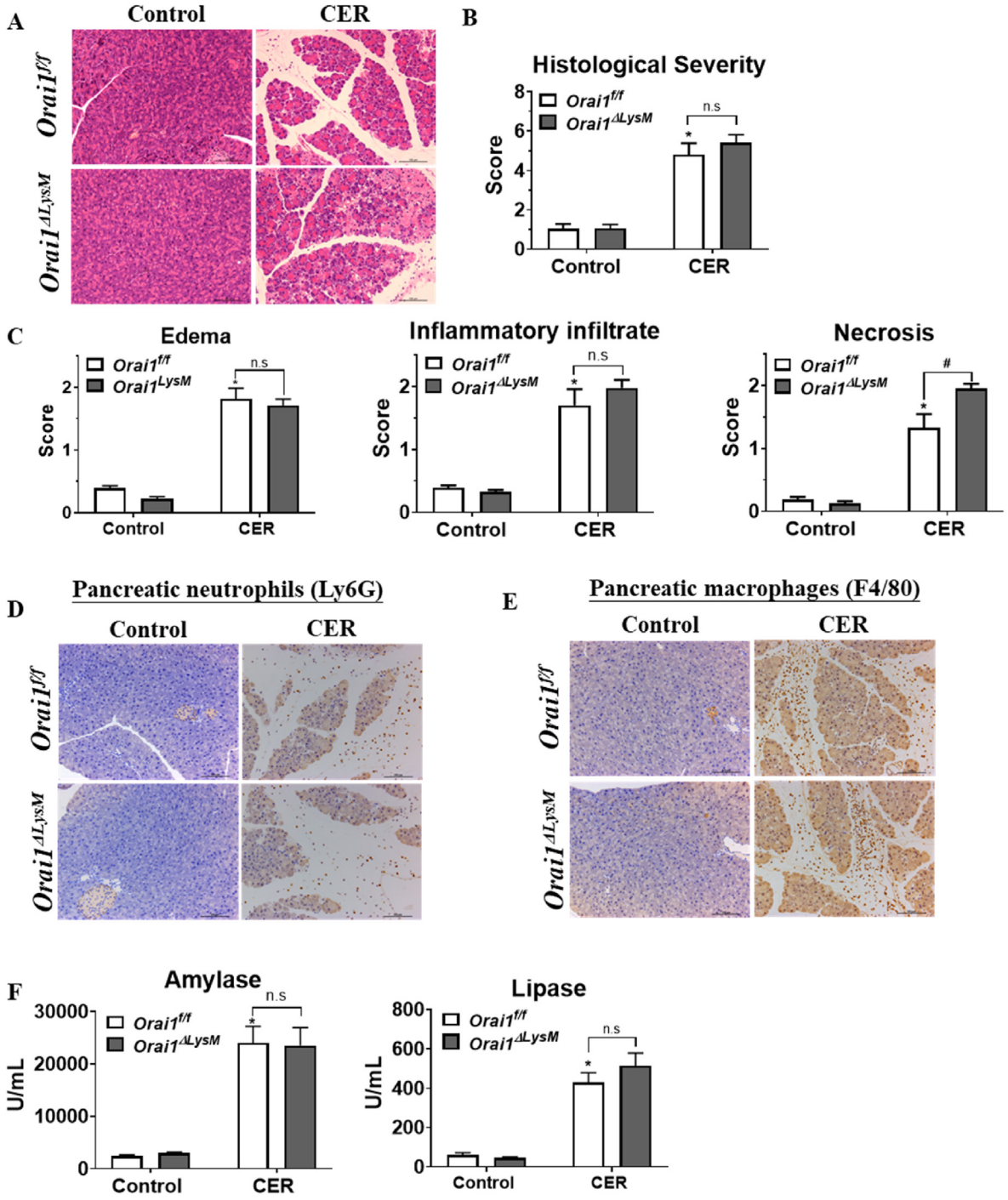

Fig. 3. Effect of myeloid-specific *Orai1* deletion on pancreatic tissue damage in caerulein hyperstimulation pancreatitis. (A) Representative H&E images of the pancreas (original magnification, × 200) from the control and pancreatitis induced by caerulein (CER) from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice. (B) Score of overall histological severity and (C) sub-scores for edema, inflammatory infiltrate, and necrosis. Representative images of immunohistochemical staining of (D) Ly6G and (E) F4/80 for pancreatic tissue from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice. (F) Serum amylase and lipase. The specimens were taken 24 h after the induction of acute pancreatitis. Data were presented as mean ± SEM, n = 6 animals per group, *p < 0.05, compared to *Orai1*$^{f/f}$, $^{\#}$P < 0.05, compared to the *Orai1*$^{f/f}$ CER group, n.s, not significant.

cells that infiltrate into the pancreas in the early stage of AP and further contribute to amplify pancreatic tissue damage [19,20]. We performed immunohistochemistry (IHC) staining of the pancreas, with Ly6G for neutrophil and F4/80 for macrophages. Similarly, we observed that there were no significant differences in pancreatic neutrophil and macrophage infiltration between *Orai1*$^{f/f}$ mice and *Orai1*$^{\Delta LysM}$ mice (Fig. 3D). Serum amylase and lipase levels similarly remained no differences between *Orai1*$^{f/f}$ mice and *Orai1*$^{\Delta LysM}$ mice (Fig. 3E). Collectively, these data suggest that myeloid cell-specific ORAI1 deletion does not prevent pancreatic tissue damage during caerulein hyperstimulation pancreatitis.

3.4. Myeloid cell-specific Orai1 deletion does not prevent pancreatic tissue damage in biliary acute pancreatitis

Next, we further evaluate the effect of myeloid cell-specific

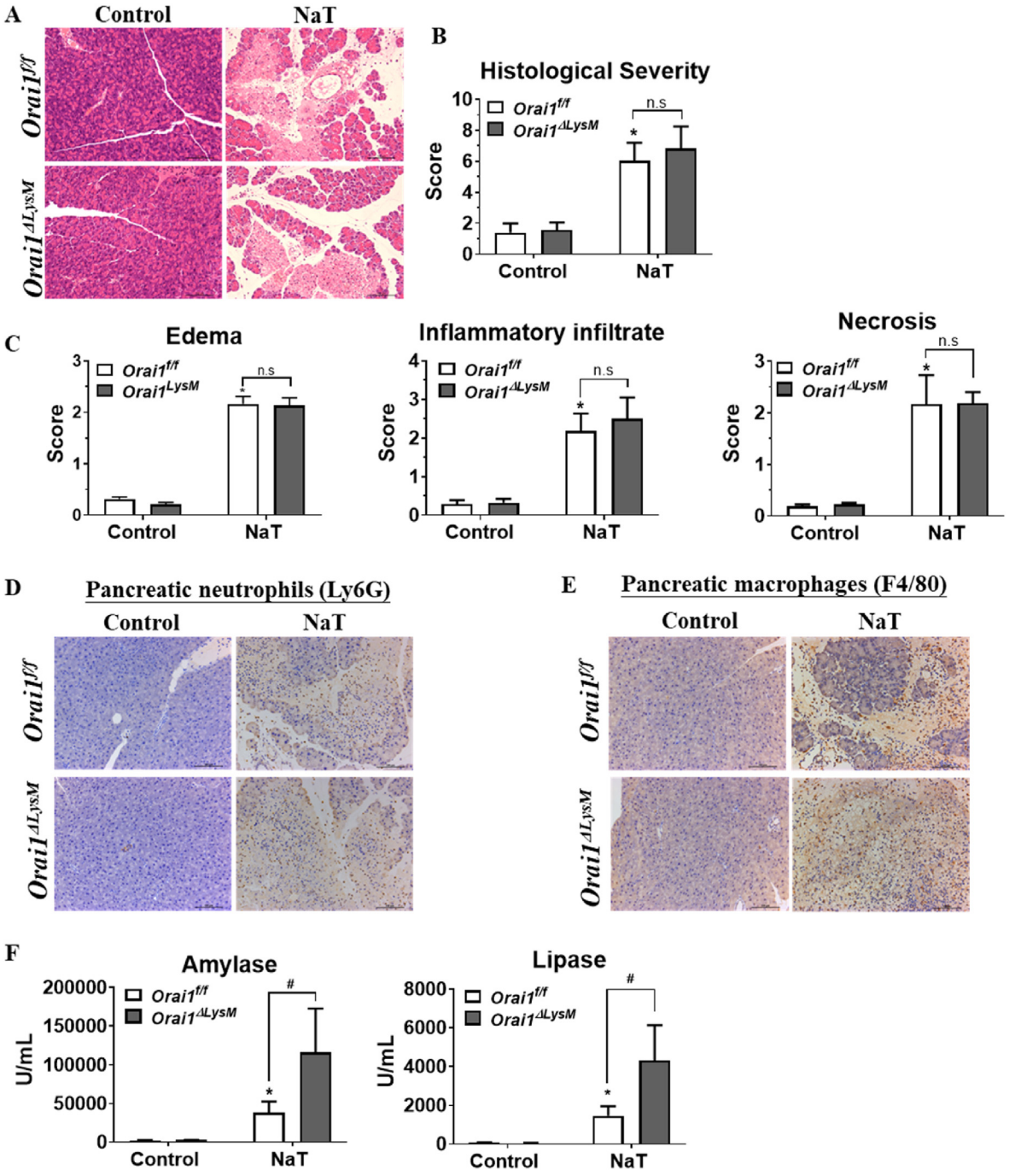

Fig. 4. Effect of myeloid-specific *Orai1* deletion on pancreatic tissue damages in biliary AP induced by sodium taurocholate (NaT). (A) Representative H&E images of the pancreas (original magnification, × 200) from the control and pancreatitis induced by retrograde biliopancreatic infusion of NaT from $Orai1^{f/f}$ and $Orai1^{\Delta LysM}$ mice. (B) Score of overall histological severity and (C) sub-scores for edema, inflammatory infiltrate, and necrosis. Representative images of immunohistochemical staining of (D) Ly6G and (E) F4/80 for pancreatic tissue from $Orai1^{f/f}$ and $Orai1^{\Delta LysM}$ mice. (F) Serum amylase and lipase. The specimens were taken 24 h after the induction of acute pancreatitis. Data were presented as mean ± SEM, n = 6 animals per group, *p < 0.05, compared to $Orai1^{f/f}$, #P < 0.05, compared to the $Orai1^{f/f}$ NaT group, n.s, not significant.

Orai1 deletion on a more severe and clinically representative model of acute pancreatitis, namely biliary acute pancreatitis. Consistent with what we observed in caerulein hyperstimulation pancreatitis, overall histological severity score of the pancreas, including all the sub-score of edema, inflammatory infiltrate, and necrosis were not significantly different between $Orai1^{f/f}$ mice and $Orai1^{\Delta LysM}$ mice (Fig. 4A–C). Similarly, immunostaining of pancreatic infiltration of neutrophils by Ly6G and macrophages by F4/80 did not exhibit significant differences between $Orai1^{f/f}$ mice and $Orai1^{\Delta LysM}$ groups (Fig. 4D and E). However, compared to $Orai1^{f/f}$ mice, serum amylase and lipase levels were significantly higher in $Orai1^{\Delta LysM}$ mice (Fig. 4F). Collectively, these data further suggest that myeloid cell-specific *Orai1* deletion does not prevent pancreatic tissue damage in a clinically representative model of acute pancreatitis.

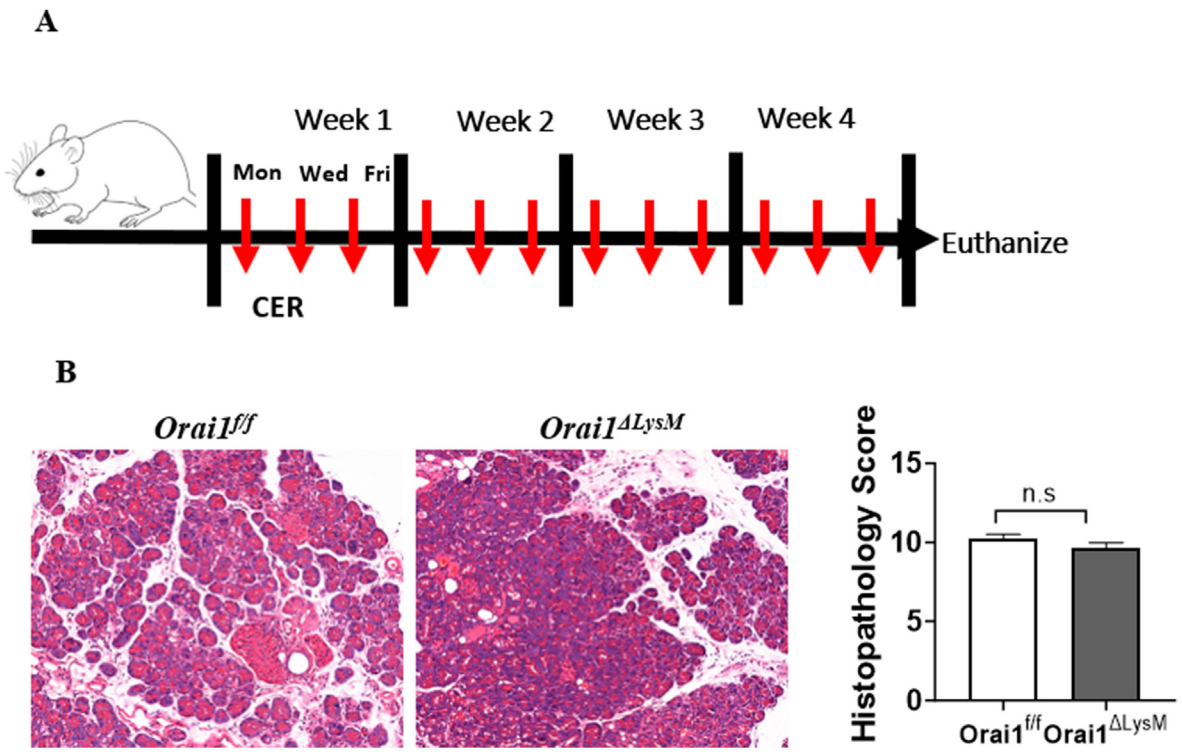

Fig. 5. Effect of myeloid-specific *Orai1* deletion on pancreatic tissue damage in experimental model of chronic pancreatitis induced by repetitive injections of caerulein. (A) Schematic diagram for induction of chronic pancreatitis. **(B)** HE staining and histological score of the pancreas from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice (scale bar: 50 μm). Data were presented as mean ± SEM, n = 6 animals per group, n.s, not significant compared to *Orai1*$^{f/f}$.

3.5. Myeloid cell-specific Orai1 deletion does not prevent pancreatic tissue damage in experimental chronic pancreatitis induced by repetitive injections of caerulein

To further evaluate whether Orai1 mediates pancreatic tissue damage and repair in the setting of long-term repetitive pancreatic inflammation, we used a mouse model of chronic pancreatitis induced by repeated intraperitoneal injection of caerulein for four weeks was studied (Fig. 5A). Compared to *Orai1*$^{f/f}$ mice, overall histological severity score of pancreatic section in *Orai1*$^{\Delta LysM}$ mice was identical (Fig. 5B). These data further suggest that myeloid cell-specific *Orai1* deletion does not alleviate pancreatic tissue damage in the setting of persistent chronic pancreatic inflammation.

3.6. Myeloid cell-specific Orai1 deletion does not reduce sepsis-associated tissue damage, but leads to an increase in serum pro-inflammatory cytokines

Since acute pancreatitis and sepsis are similar in that the extent of tissue damage or organ dysfunction is driven by dysregulated systemic activation of immune responses, which often determines disease outcome [21,22]. Therefore, we also assessed the impact of myeloid-cell specific *Orai1* deletion on sepsis-associated tissue damage. As in acute pancreatitis, we observed that myeloid cell-specific ORAI1 did not affect overall survival rate, nor sepsis-associated lung, kidney, and liver tissue damage (Fig. 6A and B). However, compared to *Orai1*$^{f/f}$ mice, several pro-inflammatory cytokines, including serum IL-6, TNF-α, and IL-1β were consistently higher in *Orai1*$^{\Delta LysM}$ mice (Fig. 6C). Collectively, these data suggest that myeloid-cell specific *Orai1* deletion does not reduce sepsis-associated lung injury, but significantly increased serum levels of pro-inflammatory cytokines, with unknown reasons.

3.7. Early treatment with Orai1 inhibitor CM4620 reduces sepsis-associated systemic inflammatory response

CM4620 is a selective Orai1 inhibitor currently under active clinical development and has been demonstrated clinical safety and potential efficacy in patients with hypoxemia secondary to systemic inflammatory response syndrome in acute pancreatitis settings (ClinicalTrials.gov Identifier: NCT03709342). We further explored whether early treatment with Orai1 inhibition would offer protective benefits against the systemic inflammatory response associated with sepsis. In LPS induced sepsis model, mice were treated with CM4620 (20 mg/kg; 2 IP injections), and then serum and tissue specimens collected at 24 h following the LPS injection (Fig. 7A). Although we did not observe significant impact on overall survival rates with CM4620 treatment (Fig. 7B), a significantly decrease in serum levels of inflammatory markers, such as IL-6, TNF-α, and IL-1β was observed, which indicates a reduction in systemic inflammatory responses (Fig. 7C). These data suggest that early intervention with Orai1 inhibit can to some extent mitigate the systemic inflammatory response in experimental model of sepsis.

4. Discussion

The degree of pancreatic tissue damage is paralleled with pancreatitis severity, which was determined by pancreatic necrosis and persistent organ failure [23,24]. Macrophage is a crucial regulator in specifically amplifying pancreatic tissue damage [25,26]. In this study, we generated myeloid cell-specific *Orai1* deficient mice to examine the effects of ORAI1 in macrophages on macrophage Ca^{2+} signal and its polarization and on pancreatic tissue damage, using two representative mouse models of AP. Firstly, we observed that ORAI1 exhibited minimal role in Ca^{2+} influx in M0

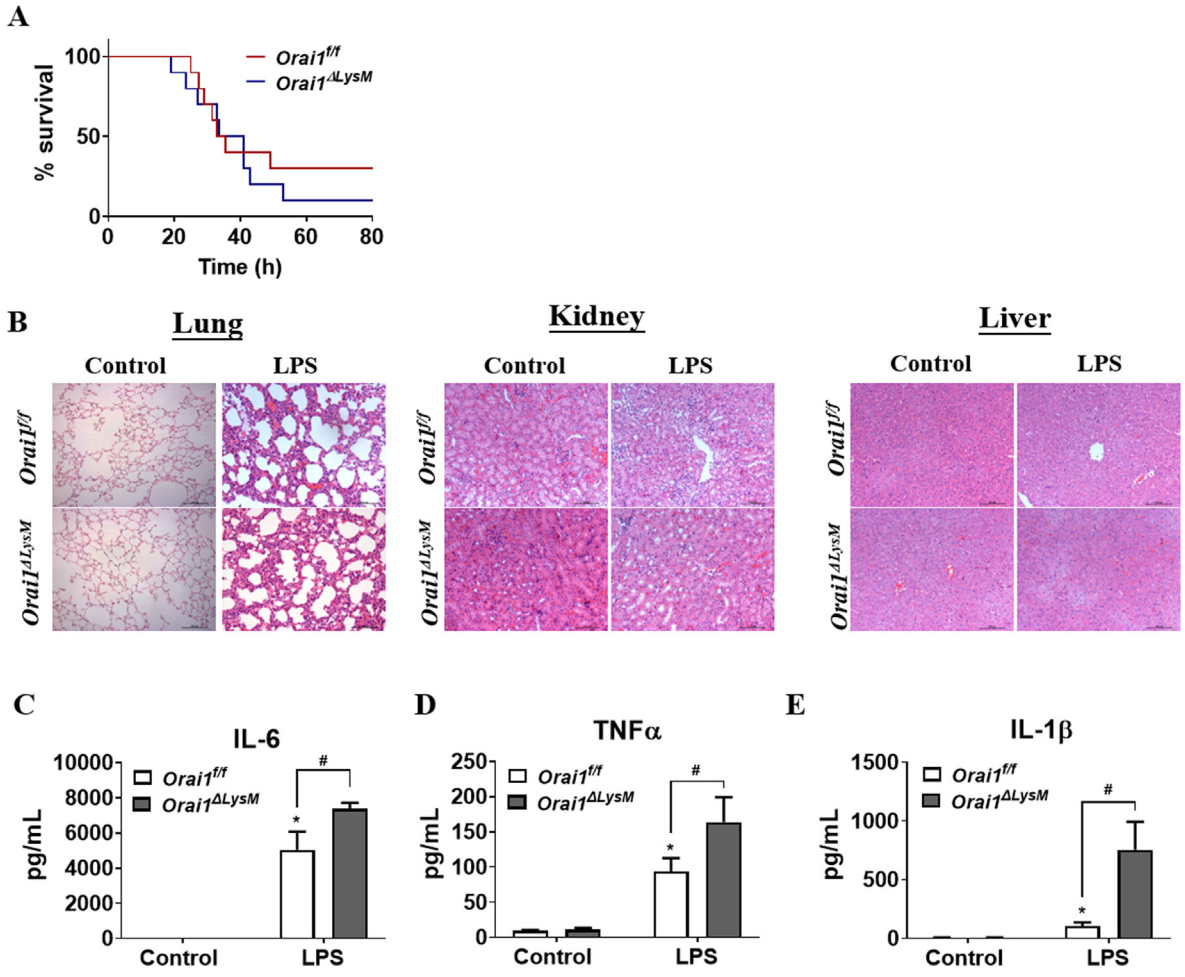

Fig. 6. Effect of myeloid-specific *Orai1* deletion in tissue damage in a model of sepsis induced by lipopolysaccharide (LPS). (A) The survival curves from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice in a model of sepsis induced by LPS. Mice were intraperitoneally injected with 40 mg/kg of LPS and were monitored for survival up to 80 h. (B) Representative images of lung, kidney and liver H&E from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice. (C) Serum IL-6, TNF-α and IL-1β. Specimens were obtained 24 h after the induction of sepsis for the analysis. Data were presented as mean ± SEM, n = 6 animals per group, *p < 0.05, compared to *Orai1*$^{f/f}$; #P < 0.05, compared to the LPS injection group.

macrophages, along with previous reports [27], suggesting that a redundant role of SOCE in macrophages and other Ca^{2+} influx channels, such as transient receptor potential cation channel subfamily C member 1 (TRPC1), may compensate the deletion of ORAI1 [28–30] or may be cooperate with ORAI1 in mediating SOCE in macrophages [27,31]. Furthermore, using isolated BMDMs isolated from *Orai1*$^{f/f}$ and *Orai1*$^{\Delta LysM}$ mice, we found that myeloid cell-specific *Orai1* deletion (*Orai1*$^{\Delta LysM}$) primarily mediates macrophages towards M2 polarization, which is consistent with the results of most previous studies [32,33]. Furthermore, previous studies have provided evidence that Orai1 and TRPC1 channels play differential roles in mediating polarization. Specifically, Nascimento Da Conceicao et al. showed that ORAI1-mediated Ca^{2+} influx primarily promote IL-4 to induce macrophages towards M2 polarization [12]; while Chauhan et al. found that TRPC1-mediated Ca^{2+} influx is one of the important factors in regulating macrophages towards M1 polarization [27].

Secondly, we found that myeloid cell-specific *Orai1* deletion (*Orai1*$^{\Delta LysM}$) did not affect pancreatic tissue damage in two representative models of experimental AP, namely caerulein hyperstimulation and biliary AP induced by retrograde biliopancreatic infusion of sodium taurocholate. Similarly, tissue damage in a model of sepsis remained unchanged in *Orai1*$^{\Delta LysM}$ mice. As discussed above, the minimal effects of ORAI1 in macrophages on intracellular Ca^{2+} signals and M1 macrophage polarization could partly explain the lack of the effects for myeloid cell-specific *Orai1* deletion on pancreatic tissue damage in AP and sepsis-associated tissue damage. On the other hand, it should be noted that myeloid cell compartment, including not only macrophages, but also neutrophils and monocytes. The net effects of myeloid-specific *Orai1* deletion on tissue damage should include the effects of ORAI1 in neutrophils and monocytes. Consistently, using a neutrophil-specific *Orai1* deficient mice, we recently showed that ORAI1 in neutrophils did not primarily contribute to mediating pancreatic local tissue damage and inflammation, but is crucial for pancreatitis-associated lung injury [11]. Another study by Saul et al. showed that ORAI channels control SOCE and play an important role in reactive oxygen species (ROS) production and bacterial killing in primary human monocytes. Specifically, upon infection, a switch from redox-sensitive ORAI1 to redox-insensitive ORAI3 and these alterations shifted the channel assembly towards a more redox-insensitive configuration and limit monocyte immune response [34], further suggesting that deletion of ORAI1 would only partly contribute to mediating intracellular Ca^{2+} signals and intrinsic functions of myeloid cell compartment.

Interestingly, we found that in a model of sepsis, although myeloid-specific *Orai1* deletion did not affect overall survival and multiple tissue damage, several pro-inflammatory cytokines in

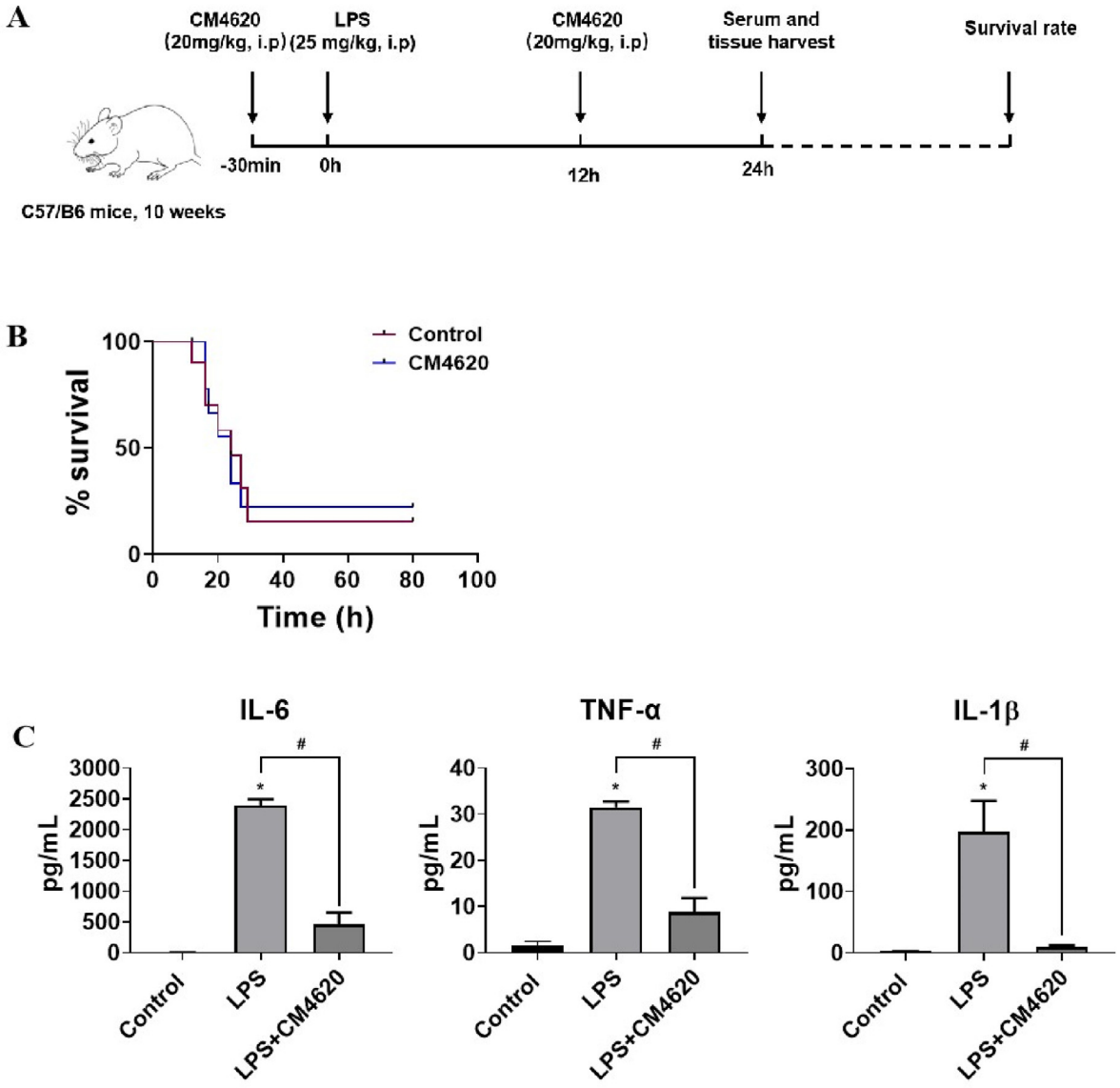

Fig. 7. Effect of Orai1 inhibitor CM4620 in tissue damage in a model of sepsis.
(A) Experimental Design: Mice received an intraperitoneal injection of 25 mg/kg of LPS; CM4620 (20 mg/kg) was given intraperitoneally every 12 h; Serum and tissue samples were collected 24 h following LPS induction, and survival rates were monitored for a duration of up to 80 h. (B) Survival Analysis: The survival rates of subjects in both experimental groups post-LPS induction were plotted in a Kaplan-Meier curve. (C) Serum levels of IL-6, TNF-α, and IL-1β. Data were presented as mean ± SEM, n = 4–8 animals per group, *P < 0.05, compared to the control; #P < 0.05, compared to the group treated with CM4620.

serum, including IL-6, TNF-α, and IL-1β from *Orai1*$^{\Delta LysM}$ mice were significantly elevated, suggesting an increase in systemic inflammatory responses. This phenomenon may be partly explained by myeloid-specific *Orai1* deletion may enhance the ability of macrophages to secrete pro-inflammatory cytokines including IL-6, IL-1β. This process can be an independent of macrophage polarization, adhesion and phagocytosis. However, when the ORAI1 inhibitor CM4620 was used in experimental model of sepsis in wild-type C57B6/J mice, serum levels of pro-inflammatory cytokines were downregulated significantly. This phenomenon indicated that ORAI1 in most of immune cells in mice still plays a crucial role in promoting inflammation in the sepsis model. Although the complexity and cell-specific effects of ORAI1 in the regulation of inflammation, these results further highlight the promise of Orai1 inhibitor to treat pancreatitis and sepsis-associated systemic inflammation.

In conclusion, in this study, we generated myeloid-specific *Orai1* deficient mice and found that ORAI1 is not a predominant SOCE channel in macrophages and play a limited role in mediating pancreatic tissue damage in AP and sepsis-associated organ damage. Future studies are required to identify the crucial SOCE channels in macrophages and their cooperation with ORAI1 during AP.

Author contributions

FC and LW designed and supervised the study and obtained funding, and interpreted the data. XZ, MN, and LL acquired and analyzed the data. WT and MN drafted the manuscript. LW and FC revised the manuscript. XG and GW participated in the intellectual discussions. All the authors approved the final edited version.

Declaration of competing interest

All the authors have no conflict of interest.

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Abbreviations

AP	acute pancreatitis
BMDMs	bone marrow-derived macrophages
CER	caerulein
IFN	interferon
IHC	immunohistochemistry
IL	interleukin
LPS	lipopolysaccharide
NOS	nitric oxide synthetase
ROS	reactive oxygen species
SEM	standard error of the mean
SOCE	store-operated calcium entry
TNF	tumor necrosis factor
TRPC1	transient receptor potential cation channel subfamily C member 1

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