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Original Article

Activating Dectin-1/SOCS1 signaling attenuates *pseudomonas aeruginosa*-induced lung injury

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Abstract: Objective: To investigate the role of Dectin-1 in alleviating Pseudomonas aeruginosa (PA)-induced lung injury and its underlying mechanism. Methods: Wild-type and Dectin-1 knockout (KO) C57BL/6 mice were exposed to PAvia intratracheal instillation. PAO1 strains were cultured, inactivated, and quantified. MHS cells were used in vitro. Curdlan was employed to activate Dectin-1 signaling, and SOCS1 expression was modulated through genetic manipulation. Levels of Dectin-1, Syk, p-Syk, SOCS1, p-p65, and p65 were assessed. Lung injury was evaluated using H&E and TUNEL staining, cell counts and protein content in bronchoalveolar lavage fluid (BALF), the lung tissue wet/dry ratio, and seven-day survival rates. Bacterial burden in the lung was assessed by PA colony formation. Inflammatory responses were measured by TNF-α, IL-6, and IL-1β levels in BALF, as well as the p-p65/p65 ratio in lung or cell lysates. Apoptosis rates in cells were determined by flow cytometry. Results: Dectin-1 expression was downregulated in the lungs and MHS cells following PA infection. Dectin-1 depletion exacerbated PA-induced lung injury. Activation of Dectin-1 by curdlan significantly alleviated PA-induced lung injury. PA infection reduced SOCS1 expression, and SOCS1 interference exacerbated the inflammatory response and apoptosis in MHS cells, nullifying the protective effects of curdlan. Overexpression of SOCS1 significantly reduced inflammation and apoptosis in both MHS cells and Dectin-1 KO mice. Conclusions: Activation of Dectin-1 significantly mitigates PA-induced lung injury, with SOCS1 playing a critical role in this process.

Keywords: Dectin-1, SOCS1, macrophages, *pseudomonas aeruginosa*, lung injury

Introduction

Pseudomonas aeruginosa (PA) is a Gram-negative, aerobic bacterium with opportunistic pathogenicity, capable of causing acute and chronic infections in immunocompromised individuals or those with chronic lung disease [1]. It is one of the most common pathogens responsible for hospital-acquired pneumonia [2]. The prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) PA strains has increased in recent years, with reports indicating that these strains now exceed 10% globally [3]. The age-standardized mortality rate for PA infections is as high as 7.4% per 100,000 population affected by bacterial pathogens [4]. Upon invading the host, PA activates macrophages to release inflammatory mediators, leading to immune cell recruitment. This results in a hyperreactive cytokine storm, and the toxins directly secreted by PA can damage the respiratory system and induce lung epithelial cell death [5]. Danger-associated molecular patterns (DAMPs) released from damaged cells further amplify the inflammatory response, potentially exacerbating lung injury [6]. Given PA's multidrug resistance, alternative therapeutic strategies beyond antibiotics are urgently needed [7].

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Macrophages play a crucial role in immune responses to infection, primarily through pattern recognition receptors (PRRs). PRRs recognize pathogen-associated molecular patterns (PAMPs) on pathogens or DAMPs, initiating downstream immune responses [8]. However, an overactive inflammatory response can damage lung tissue. Suppressing pyroptosis and apoptosis, while promoting efferocytosis in macrophages, has been shown to protect against PA infection [9-11]. The C-type lectin receptors (CLRs) are key PRRs expressed in the myeloid lineage [12]. Dectin-1, also known as the β-glucan receptor, is a CLR expressed on macrophages/monocytes, dendritic cells (DCs), neutrophils, and γδ T cells [13], supporting its diverse biological functions upon activation [14]. Dectin-1 activation has been shown to exert immunoregulatory effects during bacterial infections [15, 16]. Moreover, the Dectin-1 agonist, curdlan, is used as an adjuvant therapy [17]. However, the potential protective effects of Dectin-1 in alleviating PA-induced lung injury have yet to be fully explored. This study aims to investigate the role of Dectin-1 in PA pneumonia and uncover the underlying mechanisms. Our findings will provide novel insights into Dectin-1's function in bacterial infections and lay the theoretical foundation for further research in this area.

Materials and methods

Animals, cell lines and bacteria strains

All animal experiments were performed following the ARRIVE guidelines and approved by Shanghai Jiaotong University (license key: SYXK 2018-0027).

Dectin-1 knockout (KO) male mice and wild type C57BL/6 male mice (6-8 weeks old) were purchased from Shanghai Model Organisms Center. The mice were housed on a 12-hour light/dark cycle and had free access to food and water. The MHS cell lines were purchased from the Shanghai Institute of Biochemistry and Cell Biology and cultured in DMEM supplemented with 10% FBS (Gibco), 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Beyotime, C0222) at 37°C in a 5% CO_2 incubator. The culture medium was further supplemented with 0.05 mM β-mercaptoethanol (Mkbio, 60-24-2).

The wild-type PAO1 strain was stored in our laboratory. PAO1 (ATCC 27853) was grown and maintained on Luria-Bertani (LB) plates (BeyoPureTM LB Broth, Beyotime, ST156), prepared by dissolving in ultrapure water, adjusting the pH to 7.4 with NaOH or HCl, adding 1.5% agar (Beyotime, ST004E), and sterilizing. Monoclonal bacteria were transferred to liquid LB medium and incubated at 37°C with shaking (200 rpm) for 16 hours.

PA lung infection model

To prepare the PA bacteria solution, monoclonal PAO1 colonies were inoculated into LB medium and grown for 16 hours. The bacterial suspension was then washed and adjusted to 3×10^7 CFU/mL (1 OD_{600}=1×10^8 CFU) for use.

The PA infection animal model was established as previously described [18]. Briefly, mice were anesthetized with pentobarbital sodium (50 mg/kg i.p.), and 50 μL (3×10^7 CFU) of PAO1 was instilled intratracheally while maintaining spontaneous respiration. Mice in the sham group were intratracheally instilled 50 uL sterile PBS. After resuscitation, animals were housed under normal conditions for further experiments. Survival was monitored for 7 days after infection.

3×10^7 Survival was monitored for 7 days post-infection.

Inactivated PA stimulation in cells

Activated PA solution, obtained from overnight growth in liquid LB medium, was collected and diluted to 0.6 OD600. After washing with sterile PBS, the bacteria were irradiated with ultraviolet C (UV, 8 mJ/cm^2) for 15 minutes. To validate inactivation, the irradiated PA solution was plated onto LB plates and incubated. The absence of bacterial colonies confirmed complete inactivation. Cells (approximately 2×10^7 in a 6 cm dish) were treated with 100 μL of inactivated PA solution at 0.6 OD600 and cultured for an additional 24 hours.

Primary antibodies and reagents

Primary antibodies used in this study were as follows: Dectin-1 (Invitrogen, AB_2551734, 1:1000 for WB); Syk (Invitrogen, MA1-19332, 1:1000 for WB); p-Syk (Invitrogen, 44-234G, 1:1000 for WB); p65 (Invitrogen, 51-0500, 1:

1000 for WB); p-p65 (Invitrogen, PA5-121262, 1:2000 for WB); SOCS1 (CST, 55313, 1:1000 for WB); GAPDH (Proteintech, 60004-1-Ig, 1:5000 for WB).

In vitro, Curdlan (Sigma, C7821) was added to the culture medium at 20 μg/mL for 2 hours. In vivo, Curdlan was administered intraperitoneally to mice 1 day before PA infection at a dose of 3 mg per mouse.

Construction and transfection of plasmid, siRNA and Adeno-associated virus (AAV)

Plasmid, siRNA, and AAV of SOCS1 were constructed by Heyuan (Shanghai) Company. The full-length cDNA of SOCS1 (Gene ID: 12703) was subcloned into the pcDNA3.1(+) plasmid with a FLAG tag. The siRNA target sequence for SOCS1 (si-SOCS1) was GCGCGACAGTCGCCAACGGAA. The production and purification of AAV were conducted as previously described [19]. The titer of AAV was quantified via qPCR using the TB Green® PrimeScript™ PLUS RT-PCR Kit (Takara, RR096). Transfection was performed using Lipofectamine™ 2000 (Thermo, 1166-8030) according to the manufacturer's instructions. After 24 hours, the efficiency of knockdown and overexpression was assessed via western blot. AAV-SOCS1 was intravenously injected into mice at a titer of 2×10^{11} vg per mouse in a volume of 100 μL. Mice were subjected to further experiments 7 days after AAV injection, and SOCS1 overexpression in vivo was verified by western blot.

Bronchoalveolar lavage fluid (BALF) extraction

At 24 hours post-PA infection, mice were anesthetized, and BALF was collected via tracheal intubation. Pre-warmed PBS was instilled three times into the lungs and retrieved. The collected BALF was centrifuged at 2500 g for 10 minutes to separate the supernatant and cell pellet. The cells in the BALF were counted using an Advia automated cell counter (Siemens, Berlin, Germany) to assess inflammatory infiltration. Protein content in the BALF supernatant was measured using a bicinchoninic acid (BCA) assay (Beyotime, P0010) to evaluate lung tissue permeability.

Enzyme-linked immunosorbent assay (ELISA)

Cytokine concentrations, including IL-1β, IL-6, and TNF-α, in the BALF were measured using corresponding ELISA kits (eBioscience, E-UNEL-M0064, E-EL-M0044, E-UNEL-M0103) according to the manufacturer's instructions.

Wet-dry weight ratios

The lung wet/dry weight ratio was used to assess the severity of lung edema. Whole lungs from different groups were rapidly harvested and weighed to obtain the wet weight. The tissues were then dried in an oven at 60°C for 48 hours and re-weighed to obtain the dry weight. Lung edema was assessed using the following formula: Ratios = [(wet weight - dry weight)/wet weight] × 100%.

Detection of bacteria burden in lung tissue

Lung tissue was weighed and homogenized using a tissue grinder (Beyotime, E1643). The homogenates were serially diluted and plated onto LB plates. After incubation for 24 hours at 37°C, bacterial colonies on the LB plates were counted.

HE staining

Lung tissue was fixed in 10% phosphate-buffered formalin, embedded in paraffin, and sectioned at 4 μm thickness. The sections were deparaffinized, gradually hydrated, and stained with HE. Images were visualized under a microscope (Olympus, Japan).

TUNEL staining

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining was performed according to the manufacturer's instructions (Beyotime, C1091) to quantify cell apoptosis in lung tissue. Images were acquired using a microscope (Olympus), and TUNEL-positive cells were counted from five random fields.

Flow cytometry

Cell apoptosis was detected by Annexin V/PI staining (BD, 556547) according to the manufacturer's protocol. Fluorescent antibodies against F4/80, T1a, and Dectin-1 were incubated with a single-cell suspension for 30 minutes at room temperature (RT) in the absence of light. After washing with staining buffer (Invitrogen, 00-4222-57), cells were analyzed on a BD FACSCalibur flow cytometer (BD FACSCalibur TM Flow Cytometer, USA). Data were processed using FlowJo software.

Western blot

Lung tissue and cells were lysed using RIPA lysis buffer (Beyotime, P0013B) supplemented with protease and phosphatase inhibitor cocktails (Beyotime, P1045). Protein lysates were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% defatted milk for 2 hours at RT, then incubated overnight at 4°C with primary antibodies. After washing with TBST, membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at RT. Bands were visualized using the ChemiDoc XRS+ imaging system (Bio-Rad).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). Data were presented as means ± SEM from at least three independent experiments. Comparisons between groups were performed using a two-tailed Student's t-test or one-way ANOVA with Tukey's multiple comparison test. Survival rates were analyzed using the Log-rank (Mantel-Cox) test. Statistical significance was considered when $P < 0.05$.

Results

Dectin-1 plays a key role in improving bacterial clearance and attenuating lung injury in PA pneumonia

Dectin-1 is widely distributed across various myeloid cells, indicating its potential for diverse responses and complex signaling pathways. Numerous studies have highlighted the immunoregulatory effects of Dectin-1 during bacterial infections. To investigate the role of Dectin-1 in PA pneumonia, Dectin-1 KO mice with a C57BL/6 background and wild-type (WT) mice were subjected to PA lung infection. Dectin-1 expression was significantly downregulated in WT mice following PA infection (**Figure 1A**, **1B**). Notably, the number of cells in the bronchoalveolar lavage fluid (BALF) from Dectin-1 KO mice was significantly higher compared with WT mice after PA stimulation (**Figure 1C**), as was the protein content (**Figure 1D**) and levels of inflammatory factors (**Figure 1E-G**). The lung tissue wet-to-dry ratios in Dectin-1 KO mice were markedly elevated under PA infection (**Figure 1H**). Consistently, Dectin-1 KO resulted in an increased bacterial burden in lung tissue following PA infection (**Figure 1I**). Histopathological examination of lung tissue sections showed a detrimental impact of Dectin-1 KO, including inflammatory cell infiltration, alveolar septal thickening, blood vessel congestion, and interstitial edema (**Figure 1J**). The seven-day survival rates of Dectin-1 KO mice subjected to PA infection were significantly lower compared to WT mice (**Figure 1K**). These results underscore the importance of Dectin-1 in alleviating PA-induced lung injury.

Dectin-1 agonist improves lung injury and macrophages apoptosis in PA pneumonia

Curdlan, a microbial extracellular polysaccharide, is a specific agonist of Dectin-1, stimulating immune cells for immunomodulation [20]. After intraperitoneal injection of curdlan, WT mice were subjected to PA lung infection to explore the effect of curdlan-induced Dectin-1 activation on PA-induced lung injury. As shown by H&E staining and inflammatory factor analysis in BALF, curdlan significantly alleviated tissue injury and inflammation compared to the PA infection group (**Figure 2A-D**). The apoptosis rate in the lung, reflected by the ratio of TUNEL-positive cells, was decreased following curdlan administration (**Figure 2E**, **2F**). Dectin-1 activation by curdlan was confirmed, as the ratio of Dectin-1/GAPDH and p-Syk/Syk was significantly increased under curdlan stimulation, with or without PA infection (**Figure 2G-I**). We then assessed the prototypical proinflammatory NF-κB signaling pathway. PA infection significantly increased the ratio of p-p65/p65 (Rel A), indicating an increase in NF-κB transcriptional activity, while curdlan administration markedly decreased this ratio (**Figure 2G**, **2J**). The use of curdlan showed significant anti-inflammatory effects and partially mitigated PA-induced lung injury in mice.

Dectin-1 activation attenuates PA induced lung injury by Upregulating SOCS1

Suppressor of SOCS1 plays a critical role in modulating and resolving inflammatory cascades. Mutations in the nuclear localization signal (NLS) of SOCS1 impair its ability to suppress inflammation induced by NF-κB [21]. To investigate the role of SOCS1 in the immunoregulatory effects of Dectin-1 in PA pneumonia,

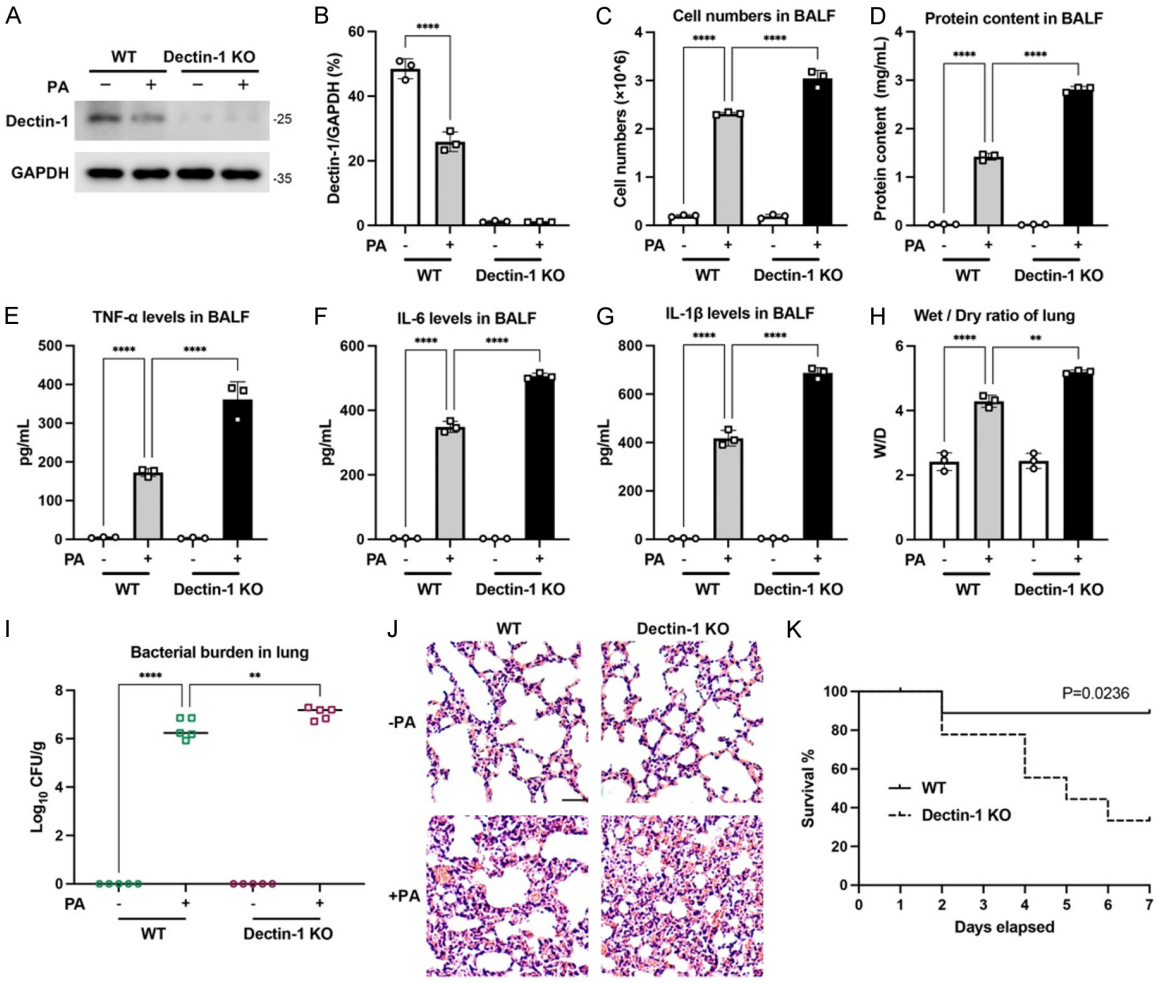

Figure 1. Deletion of Dectin-1 exacerbates *Pseudomonas aeruginosa* (PA) induced lung injury in mice. (A) Dectin-1 expression levels were measured in tissue homogenate using western blot with GAPDH as loading control. C57BL/6 wild type (WT) and Dectin-1 KO mice were subjected to *Pseudomonas aeruginosa* (PA, <3×10^7 CFU, 50 μL per mouse>, +PA) infection or saline (-PA) through intratracheal instillation, bronchoalveolar lavage fluid (BALF) and lung tissue were collected 24 h after infection. N=3 per group. (B) Ratios of Dectin-1/GAPDH were calculated and compared with every other group. (C, D) Cell numbers (C), protein content (D) in BALF were calculated using hemocytometer and BCA assay respectively. (E-G) Inflammatory factors including TNF-α (E), IL-6 (F) and IL-1β (G) in BALF were detected by ELISA assay. (H) Tissue wet/dry ratios were calculated through weighting the whole lung. N=3 per group. (I) Lung tissue homogenate were doubling diluted and painted onto the agar medium and incubated for 18 h, bacteria colony were then counted with n=5 per group. (J) Representative lung histopathology images (H&E staining). Scale bar: 50 μm. (K) Seven days survival rates of mice subjected to PA lung infection in WT and Dectin-1 KO mice with n=9 per group. Data are presented as means ± SEM from n =3 independent experiments. $^{**}P < 0.01$, $^{****}P < 0.0001$, respectively.

we exposed WT and Dectin-1 KO mice to PA infection through the airway (**Figure 3A**, **3B**). Under PA attack, SOCS1 expression in lung tissue significantly decreased, and this depletion was aggravated in the absence of Dectin-1 (**Figure 3A**, **3C**). In MHS cells, stimulation with inactivated PA (iPA) reduced Dectin-1 expression and the p-Syk/Syk ratio (**Figure 3D-F**), along with downregulation of SOCS1 (**Figure 3D**, **3G**), mimicking the in vivo response. SOCS1 knockdown in MHS cells using si-SOCS1 did not affect Dectin-1 expression or the p-Syk/Syk ratio but increased NF-κB activation, as reflected by the elevated p-p65/p65 ratio under iPA stimulation (**Figure 3D-H**). Moreover, apoptosis rates were significantly higher in SOCS1 knockdown MHS cells compared to mock-transfected cells upon iPA stimulation (**Figure 3I**).

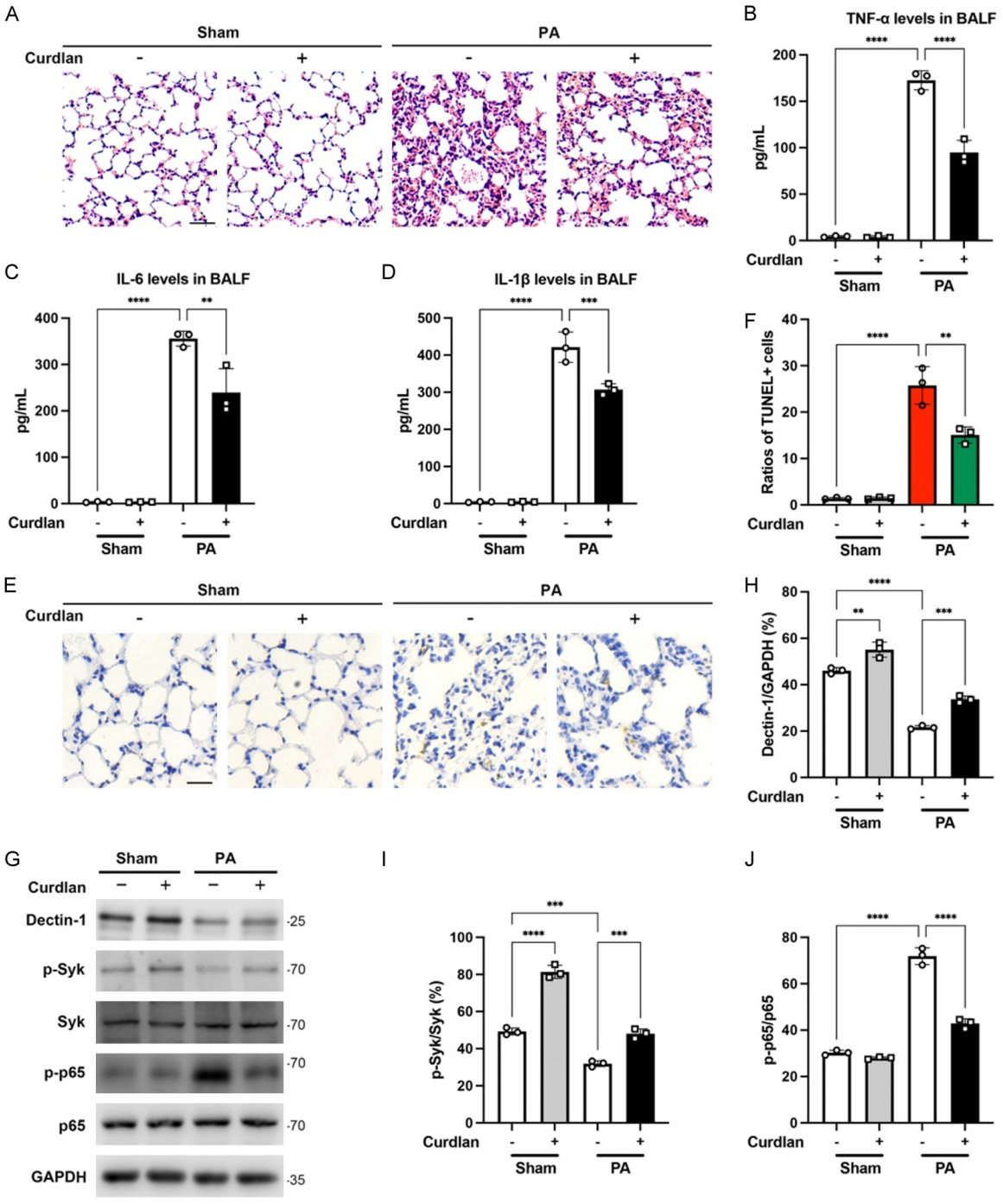

Figure 2. Activating Dectin-1 signaling attenuates lung injury induced by PA in mice. (A) Representative lung histopathology images (H&E staining). Scale bar: 50 μm. C57BL/6 WT mice were intraperitoneally injected with curdlan (+Curdlan) at 3 mg per mouse 1 day before PA infection, or with saline (-Curdlan), then they were subjected to 50 μL PA solution (PA) or equal volume saline (Sham) intratracheal instillation. After 24 h, mice were sacrificed for sampling. (B-D) Inflammatory factors including TNF-α (B), IL-6 (C) and IL-1β (D) in BALF were detected by ELISA assay. (E) Representative TUNEL staining images. Scale bar: 50 μm. (F) Percentage of TUNEL$^+$ cells in lung tissue section were quantified from five random fields. (G) Dectin-1, p-Syk, Syk, p-p65, p65 expression in Lung tissue homogenate were detected by western blot with GAPDH as loading control. (H-J) Dectin-1/GAPDH (H), p-Syk/Syk (I), p-p65/p65 (J) ratios were calculated and compared with every other group. Data are presented as means ± SEM from n =3 independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, respectively.

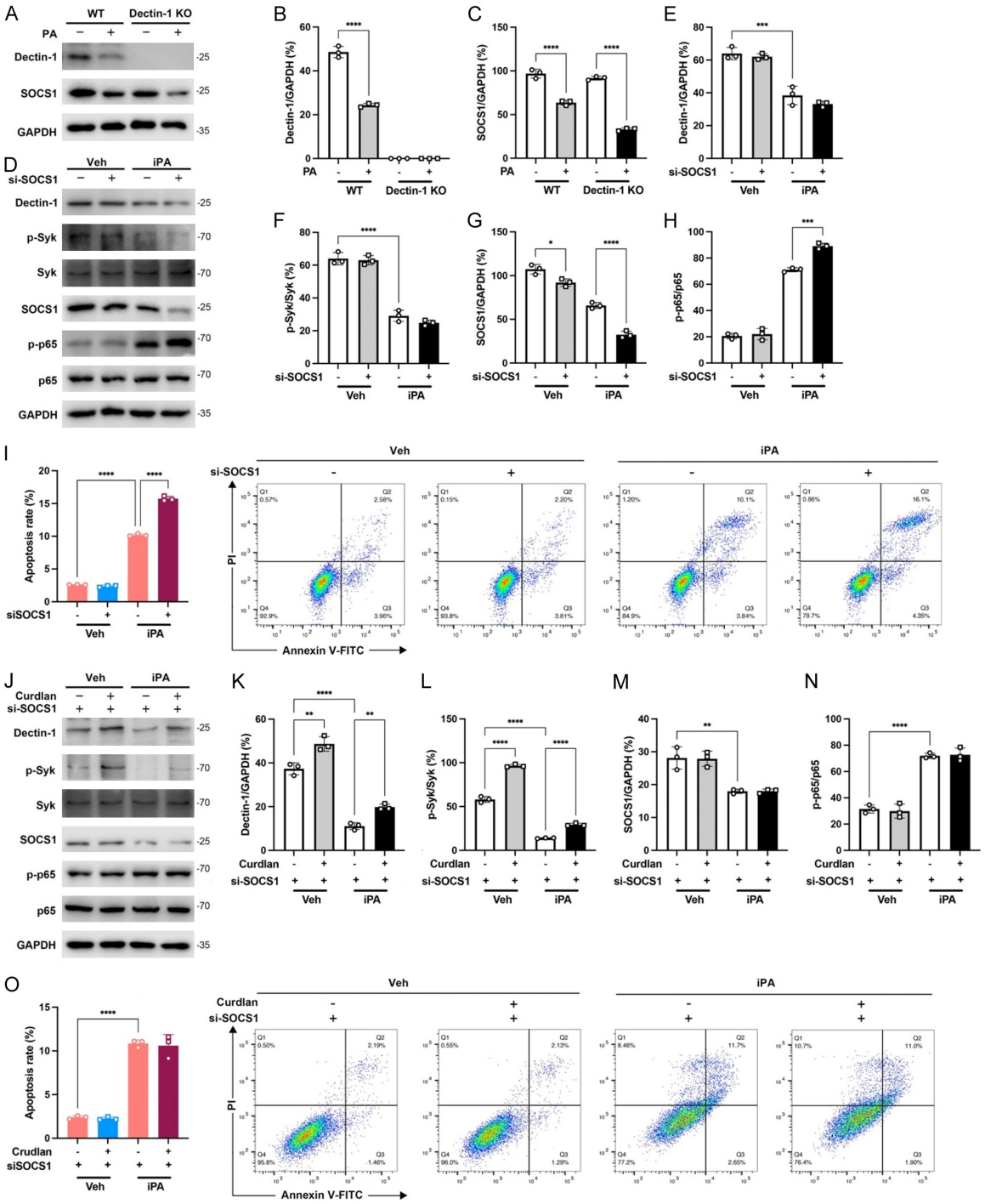

Figure 3. SOCS1 serves as a main mediator in Dectin-1 signaling to attenuates lung injury induced by PA. (A) Dectin-1, SOCS1 expression in lung tissue were detected by western blot with GAPDH as loading control. C57BL/6 wild type (WT) and Dectin-1 KO mice were subjected to PA (+PA) or saline (-PA) intratracheal instillation, after 24 h, lung tissues were retrieved and homogenized for further detection. (B, C) Dectin-1/GAPDH (B), SOCS1/GAPDH (C) ratios were calculated and compared with every other group. (D) The expression of Dectin-1, p-Syk, Syk, SOCS1, p-p65, p65 were assessed in MHS cell lysate via western blot with GAPDH as loading control. Small interfere RNA (siRNA) of SOCS1 (+si-SOCS1) and si-scramble (-si-SOCS1) were transfected into MHS cells for 24 h, then cells were stimulated with iPA (<0.6 OD diluted in 100 μL sterile PBS>, iPA) or with vehicle control (Veh) for another 24 h. (E-H) Dectin-1/GAPDH (E), p-Syk/Syk (F), SOCS1/GAPDH (G), p-p65/p65 (H) ratios were calculated and compared with every other group. (I) Apoptosis of MHS cells in each group were assessed by Annexin V and PI staining via flow cytometry. Annexin V and PI double positive cells were recognized as apoptotic cells and their ratios were quanti-

fied and compared. (J) Dectin-1, p-Syk, Syk, SOCS1, p-p65, p65 expression were detected in MHS cell lysate by western blot with GAPDH as loading control. MHS cells were transfected with si-SOCS1 (+si-SOCS1) for 24 h, then cells were stimulated with curdlan (<20 μg/mL>, +Curdlan) or vehicle control (-Curdlan), after 2 h incubation, cells were subjected to iPA (<iPA, 0.6 OD diluted in 100 μL sterile PBS>, iPA) or vehicle control (Veh) and co-incubated with curdlan for another 24 h. (K-N) Dectin-1/GAPDH (K), p-Syk/Syk (L), SOCS1/GAPDH (M), p-p65/p65 (N) ratios were calculated and compared with every other group. (O) Apoptosis of MHS cells in each group were assessed by Annexin V and PI staining via flow cytometry. Apoptosis rates (ratios of Annexin V and PI double positive cells) were quantified and compared. Data are presented as means ± SEM from n =3 independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, respectively.

To further validate the role of SOCS1, we administered curdlan to SOCS1 knockdown MHS cells to investigate the Dectin-1/SOCS1 signaling pathway. SOCS1 interference did not affect Dectin-1 expression or the p-Syk/Syk ratio, even though iPA downregulated both (**Figure 3J-M**). However, SOCS1 knockdown counteracted the beneficial effects of curdlan, as indicated by unchanged p-p65/p65 ratios in the combination of curdlan and si-SOCS1 treatment (**Figure 3J**, **3N**). Flow cytometry confirmed increased apoptosis (**Figure 3O**). These findings underscore the pivotal role of SOCS1 as a downstream mediator in the Dectin-1/Syk signaling axis.

SOCS1 compensation alleviates PA induced lung injury independent of Dectin-1 expression

To further confirm the role of SOCS1, we overexpressed it in MHS cells and in Dectin-1 KO mice using SOCS1-FLAG plasmids and adeno-associated virus (AAV-SOCS1), respectively. In vitro, SOCS1 overexpression protected MHS cells from iPA-induced damage, as evidenced by reduced p-p65/p65 ratios and apoptosis rates (**Figure 4A**, **4C-E**). Notably, SOCS1 overexpression did not affect Dectin-1 expression levels (**Figure 4A**, **4B**). In vivo, SOCS1 overexpression (confirmed by western blot in **Figure 4F**, **4G**) significantly attenuated the inflammatory response in PA-infected Dectin-1 KO mice. The p-p65/p65 ratios (**Figure 4F**, **4H**), as well as the levels of inflammatory factors such as TNF-α (**Figure 4I**), IL-6 (**Figure 4J**), and IL-1β (**Figure 4K**) in BALF, were all significantly reduced. H&E staining revealed alleviated tissue injury (**Figure 4L**), and the number of TUNEL+ cells in lung tissue sections was also reduced (**Figure 4M**, **4N**). These results demonstrate the protective role of SOCS1 in PA-induced lung infection and injury, providing a strong theoretical basis for the identification of new therapeutic targets for PA-induced lung injury.

Discussion

In this study, we found that the activation of Dectin-1 expressed on macrophages could mitigate lung injury induced by PA infection. This protective effect is likely due to the upregulation of SOCS1 expression.

PA exhibits resistance to a wide range of antibiotics through intrinsic, acquired, and adaptive mechanisms, posing a significant challenge to antibiotic therapy. Furthermore, the growing concerns over the overuse and misuse of antibiotics have intensified the need for alternative therapeutic strategies [22]. Recently, non-antibiotic approaches, including quorum sensing and bacterial lectin inhibition, iron chelation, nanoparticles, and vaccines, have shown promising therapeutic effects, either alone or in combination with other therapies [23]. Other chemical compounds have also proven effective. For example, Wagener et al. [24] reported that α-Tocopherol, a lipid-soluble antioxidant, inhibited RhoA and PAI-1 activation by blocking T3SS exoenzymes injected into alveolar epithelial cells, thereby increasing survival in PA-infected mice. Targeting key molecules such as the cannabinoid-2 receptor [7], triggering receptor expressed on myeloid cells 2 (TREM2) [9], and CLEC5A [25] has also been demonstrated to mitigate lung injury and inflammation induced by PA pneumonia.

Beyond its well-established role as a classical antifungal receptor, Dectin-1 is recognized as a versatile receptor involved in various biological processes, including allergy, cancer, autoimmune diseases, sterile inflammation, and aging [13]. This multifaceted role makes Dectin-1 an attractive target for further investigation and therapeutic intervention. For instance, Elcombe et al. [16] reported that activating Dectin-1 via zymosan upregulated IL-10 production through the MSK1/2 and CREB-dependent pathway. Additionally, Dectin-1/Syk signaling has been

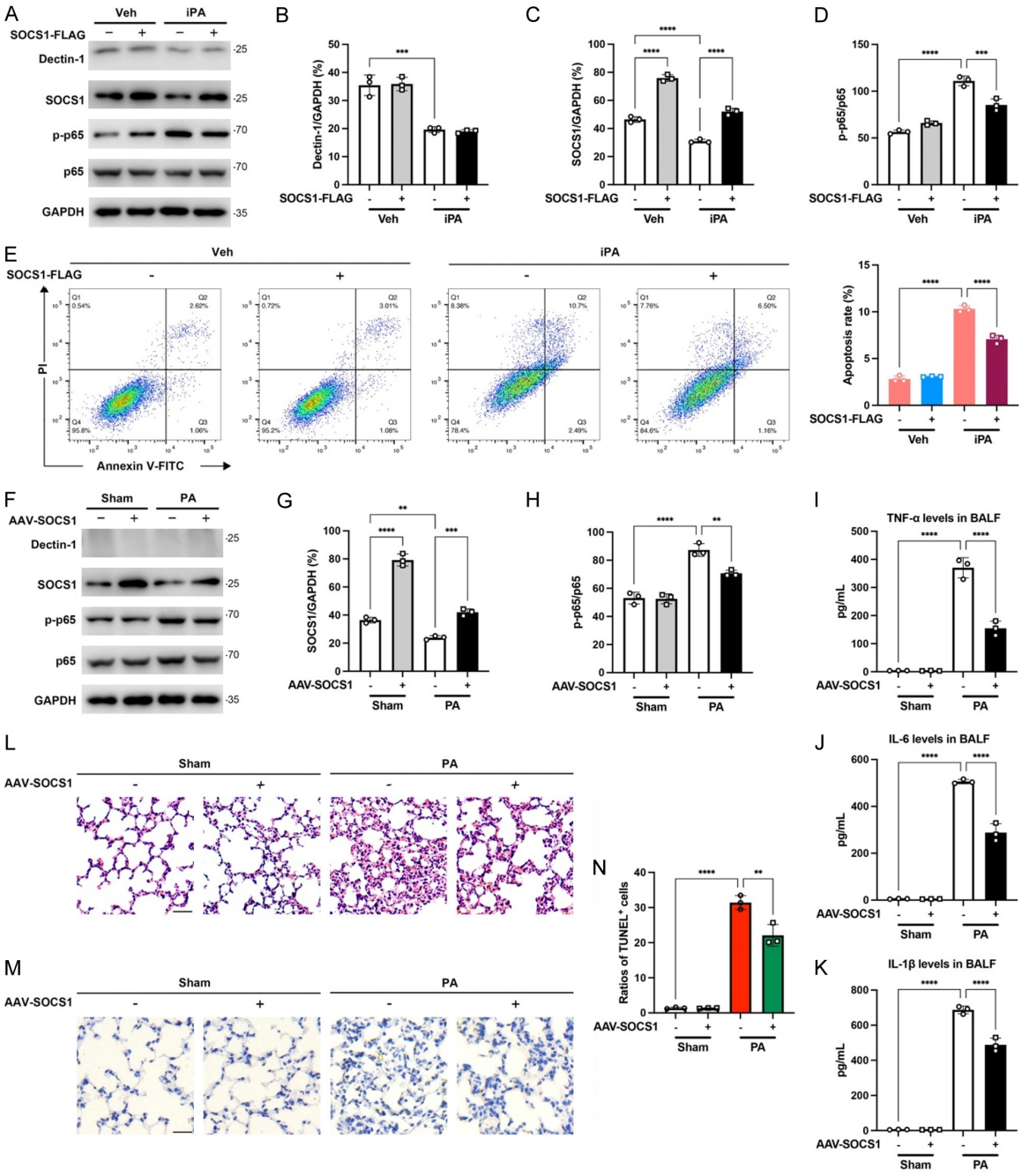

Figure 4. Overexpression of SOCS1 rescues PA induced lung injury in vitro and in vivo. (A) Dectin-1, p-Syk, Syk, SOCS1, p-p65, p65 expression were detected by western blot in MHS cell lysate with GAPDH as loading control. MHS cells were transfected with SOCS1 plasmid (+SOCS1-FLAG) or empty vector (-SOCS1-FLAG) to overexpress SOCS1 for 24 h, then cells were stimulated with iPA (<0.6 OD diluted in 100 μL sterile PBS>, iPA) or with vehicle control (Veh) for another 24 h. (B-D) Dectin-1/GAPDH (B), SOCS1/GAPDH (C), p-p65/p65 (D) ratios were calculated and compared with every other group. (E) Apoptosis of MHS cells in each group were assessed by Annexin V and PI staining via flow cytometry. Apoptosis rates (ratios of Annexin V and PI double positive cells) were quantified and compared with every other group. (F) Dectin-1, SOCS1, p-p65, p65 expression were detected by western blot in lung tissue homogenate with GAPDH as loading control. C57BL/6 Dectin-1 KO mice were intravenously injected with AAV-SOCS1 (<2×10^{11} vg per mouse in 100 μL>, +AAV-SOCS1) or AAV-scramble (-AAV-SOCS1) to overexpress SOCS1 in vivo. After two weeks, mice were intratracheally instilled with PA (<3×10^7 CFU, 50 μL per mouse>, PA) or saline (Sham). After 24 h, BALF and lung tissue were retrieved. (G, H) SOCS1/GAPDH (G), p-p65/p65 (H) ratios were calculated and compared with every other group. (I-K) Inflammatory factors including TNF-α (I), IL-6 (J) and IL-1β (K) in BALF were detected by ELISA assay. (L) Representative lung histopathology images (H&E staining). Scale

bar: 50 μm. (M) Representative TUNEL staining images. Scale bar: 50 μm. (N) Percentage of TUNEL$^+$ cells in lung tissue section were quantified from five random fields. Data are presented as means ± SEM from n =3 independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, respectively.

shown to suppress C5a receptor (C5aR) function by associating with FcγRIIB, which ultimately inhibits C5a-dependent inflammatory responses in vivo [26]. Consistent with these findings, we observed significantly worsened PA-induced lung injury in Dectin-1 KO mice. However, administration of its agonist, curdlan, effectively reversed this lung damage. These results underscore the essential role of Dectin-1 in protecting against PA-induced lung injury.

Dectin-1 exhibits a diverse distribution pattern, supporting its broad range of biological functions [14]. In this study, we assessed its expression on macrophages in mice with PA pneumonia. Our findings revealed that the primary alterations in Dectin-1 and phosphorylated Syk expression occurred on macrophages. As a key component of the innate immune system, macrophages are widely recognized as frontline defenders against invading pathogens due to their ability to recognize, engulf, and eliminate pathogens [27]. Given these characteristics, it is plausible that Dectin-1 elicits its primary response on macrophages. Unfortunately, we did not find related research on this aspect, which remains an important area for future investigation.

The downstream signaling pathways of Dectin-1 are highly diverse and are often categorized based on their reliance on the proximal adaptor molecule Syk [14]. Syk-dependent pathways include PI3K/AKT [28], phospholipase C-γ2 (PLC-γ2) induced Ca^{2+}-dependent activation of nuclear factor of activated T cells (NFAT) and ERK [29], as well as the CARD9/BCL10/MALT1 complex-mediated signaling [30] and CARD9/IRF pathways [31], all of which are responsible for cytokine and interferon production. In addition, Dectin-1 activates Syk-independent pathways, such as the Raf-1-mediated alternative NF-κB activation and PI3K/AKT pathways, which are involved in T-helper cell differentiation and phagocytosis [32, 33]. Several studies have also noted that Dectin-1 induces SOCS1 in modulating TLR crosstalk to reduce NF-κB activation [34], Van et al. reported that intact guar gum activated Dectin-1 and downstream JAK and cSrc pathways, leading to the upregulation of SOCS1 and subsequent anti-inflammatory regulation [35]. As an immunoregulator, SOCS1 was confirmed to play a key role in Dectin-1/Syk-mediated attenuation of PA-induced lung injury in our study.

However, there are some limitations in our research. First, we did not examine the expression of Dectin-1 on other immune cells, such as neutrophils and dendritic cells. This is due to the lack of studies specifically addressing the relationship between Dectin-1 and PA in these immune cell types. Current literature predominantly focuses on macrophages [15, 36]. Second, we did not assess other immune regulatory signaling pathways in this study, nor did we investigate Syk-independent pathways triggered by Dectin-1. Further research is needed to unravel the complex signaling network involved in PA-induced lung injury.

In conclusions, activation of Dectin-1 through curdlan significantly alleviated lung injury induced by PA infection, with SOCS1 playing a critical role in this process. This study highlights the crucial role of Dectin-1 in PA lung infection and provides insights into the underlying mechanisms. Furthermore, it presents a potential therapeutic target for treating PA-induced lung injury.

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Disclosure of conflict of interest

None.

Abbreviations

PA, *Pseudomonas aeruginosa*; MDR, multidrug-resistant; XDR, extensively drug-resistant; DAMPs, Danger-associated molecular patterns; PRRS, pattern recognition receptors; PAMPs,

pathogen-associated model molecules; CLRs, C-type lectin receptors; DCs, dendritic cells; LB, Luria-Bertani; UV, ultraviolet C; AAV, adeno-associated virus; BALF, bronchoalveolar lavage fluid; BCA, bicinchonininc acid; ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin and eosin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; RT, room temperature; PVDF, polyvinylidene fluoride; WT, wild type; iPA, UV-inactivated PA; SOCS1, suppressor of cytokine signaling 1; NLS, nuclear localization signal; TREM2, triggering receptors expressed on myeloid cells 2; C5aR, C5a receptor; Syk, spleen tyrosine kinase; PLC-γ2, Phospholipase C-γ2; NFAT, nuclear factor of activated T cells.

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