



Macropinocytosis enhances foamy macrophage formation and cholesterol crystallization to activate NLRP3 inflammasome after spinal cord injury

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ABSTRACT

After spinal cord injury (SCI), phagocytes endocytose myelin debris to form foam cells, exacerbating the inflammatory response. It has been previously shown that macrophages become foam cells through the phagocytosis of myelin debris via receptor-dependent mechanisms after SCI. Blocking receptor-mediated endocytosis did not completely prevent foam cell formation, so we investigated receptor-independent endocytosis. Here, we revealed that foam cells formed after myelin debris internalization were predominantly macrophages rather than microglia. Receptor-independent macropinocytosis has an important position in foamy macrophage formation through engagement of myelin debris endocytosis after SCI. Mechanistic studies showed that cholesterol crystallization following macropinocytosis-mediated foamy macrophage formation promoted the reactive oxygen species (ROS) production and the NOD-like receptor protein 3 (NLRP3) inflammasome activation, increasing the secretion of interleukin-1 β (IL-1 β). Inhibition of macropinocytosis might reverse this effect, resulting in enhanced axonal regeneration and reduced neural apoptosis, thereby improving outcomes after SCI. Overall, our study revealed a previously unrecognized role for macropinocytosis in foamy macrophages formation after SCI, and confer a promising therapeutic strategy for SCI through focus on macropinocytosis.

1. Introduction

Spinal cord injury (SCI) is a common disorder with many causes and is linked to high levels of disability due to sensory and motor impairment [1–3]. Following the initial mechanical trauma, a prolonged secondary injury phase ensues, primarily driven by cytokines and chemokines

released from the injury site. SCI not only reduces patients' quality of life and affects their physical and mental well-being but also leads to significant financial burdens on the patient, their family, and society. Currently, there are insufficient effective treatments for secondary damage due to complex pathophysiologic processes following SCI [4,5]. Therefore, the treatment of SCI is of great social importance [6,7].

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Demyelination is a significant pathological event that occurs in both acute and chronic SCI. Extensive myelin debris persisted in the lesion area for an extended period. Cholesterol, a major component of myelin, is synthesized into cholesteryl esters and transferred to lipid droplets for storage within macrophages. Continuous demyelination produces myelin debris, giving rise to foamy macrophages with a pro-inflammatory phenotype [8–11]. Macrophages act as phagocytes, which is important in debris clearance at the injury site [12–14]. In multiple sclerosis (MS) characterized by demyelination, microglia in the central nervous system, also remove myelin debris.

In the last decade, research on macrophage endocytosis of myelin debris post-SCI has been insufficient and has primarily focused on receptor-mediated pathways, particularly macrophage scavenger receptor 1 (MSR1) and CD36. However, studies indicate that MSR1 or CD36 knockout does not completely inhibit the development of foamy macrophages after SCI, implying the presence of other endocytic pathways. Non-receptor-dependent macropinocytosis is a type of endocytosis by the formation of large-diameter macropinosomes through the curling of the plasma membrane [15–17]. Recent studies on atherosclerosis have demonstrated that macrophages can internalize low-density lipoprotein (LDL) through macropinocytosis to form foamy macrophages [18]. Furthermore, macropinocytosis inhibitors have better efficacy in preventing the formation of foamy macrophages, which has not been previously reported in SCI models.

Here, we investigated the involvement of macrophage macropinocytosis in foam cell formation following SCI. The spatio-temporal distributions of myelin debris and foam cells were evaluated, indicating that foam cells were primarily derived from macrophages. Our findings also indicated that macropinocytosis was important in developing foamy macrophages, regardless of whether MSR1/CD36 was knocked out. We uncovered that blocking macropinocytosis reduced macrophage NLRP3 inflammasome activation and ROS generation, attenuating the inflammatory response after SCI. Thus, focusing on macropinocytosis may represent a novel strategy for SCI.

2. Materials and methods

2.1. Reagents

The antibodies utilized for western blotting were anti- β -actin (AB0011, Abways, Shanghai, China), anti-Pro-Caspase-1 (sc-398715, Santa Cruz, Dallas, TX, USA), anti-Caspase-1 (3866, Cell Signaling Technology, Beverly, MA, USA), anti-IL-1 β (12703, Cell Signaling Technology), anti-Pro-IL-1 β (18689, Cell Signaling Technology), anti-NLRP3 (68102-1-Ig, Protein Tech, Chicago, IL, USA). The HRP-conjugated secondary antibodies (both anti-rabbit and anti-mouse) were from Amersham (Buckinghamshire, UK). Antibodies against F4/80 (ab6640, Abcam, Cambridge, UK), CD68 (MCA1957, Bio-Rad, Hercules, CA, USA), dMBP (ab5864, Sigma-Aldrich, St. Louis, MO, USA), iba-1 (ab5076, Abcam), Hexb (ab133376, Absin, Shanghai, China), NF200 (ab207176, Abcam), Tau (4019, Cell Signaling Technology), MAP2 (GB11128-2, Servicebio, Wuhan, China), NeuN (ab104224, Abcam), and GFAP (GB11096-100, Servicebio) were utilized for immunofluorescence (IF). DAPI fluoromount-G was from SouthernBiotech (0100-20, Birmingham, LA, USA). Secondary antibodies for IF were donkey anti-mouse Alexa Fluor 488 (ab150105, Abcam), donkey anti-rabbit Alexa Fluor 488 (ab150073, Abcam), donkey anti-mouse Alexa Fluor 555 (ab150106, Abcam), goat anti-rabbit Alexa Fluor 594 (ab150088, Abcam), goat anti-rat Alexa Fluor 594 (ab150160, Abcam), donkey anti-goat Alexa Fluor 594 (ab150132, Abcam), and donkey anti-rat Alexa Fluor 647 (ab150155, Abcam). The antibodies utilized for flow cytometry were anti-CD45-APC-Cy7 (557659, BD Biosciences, San Diego, CA, USA), CD11b-PE-Cyanine7 (25-0112-82, Invitrogen, Waltham, MA, USA), F4/80-PE (565410, BD Biosciences) and iNOS-FITC (610330, BD Biosciences). Dextran to trace macropinocytosis was purchased from Sigma (46945) and Thermo Fisher (D1841). The macropinocytosis

inhibitor in our study was EIPA (HY-101840, MedChem Express, Monmouth Junction, NJ, USA).

2.2. Mice

MSR1^{-/-} and CD36^{-/-} mice were generated as described, together with wild-type (WT) animals of the same background as control mice [19–21]. All animals were housed in a controlled, pathogen-free, environment with a 12-h light/dark cycle and unrestricted food and water. All animal experiments followed the guidelines of the Animal Care and Use Committee of the First Affiliated Hospital of Nanjing Medical University.

2.3. Mouse SCI models

Eight week-old mice (male WT, MSR1^{-/-}, and CD36^{-/-}) were used for construction of the SCI model. All animals were anesthetized with ketamine and xylazine (70 and 7 mg/kg, respectively) and administered intraperitoneally. The spinal cord at T9-T10 was exposed by laminectomy without causing damage to the dura. A rod (5 g) was dropped from 6.5 cm height onto the spinal cord using an impactor (68097, RWD, CA, USA). Control (sham) mice underwent laminectomy without SCI. The animals remained in a temperature-controlled environment during recovery until thermoregulation was restored.

2.4. Cell culture

Bone marrow-derived macrophages (BMDMs) were isolated from femur and tibial bone marrow of four-week-old animals and grown for seven days in RPMI 1640 medium (Gibco, Waltham, MA, USA) with 10 % fetal bovine serum (FBS), 1 % penicillin/streptomycin (P/S), and 25-ng/ml macrophage colony-stimulating factor (M-CSF, 416-ML, R&D Systems).

Primary microglia were obtained by digesting brain tissue from neonatal mice (within 3 days of birth) using 0.25 % trypsin/EDTA. The harvested cells were resuspended in DMEM/F-12 with 1 % P/S and 10 % FBS and cultured in polylysine (PLL)-coated T75 flasks with cell adherent reagent and replacement of the medium every three days. After 10–14 days, mature microglia were collected by shaking flasks gently. Then microglia were cultured in PLL-coated plates for further experiments.

Primary neurons were harvested from the brain tissues of 16-day-old fetal mice using a Primary Neuron Isolation Kit (88280, Thermo Fisher, Waltham, MA, USA).

2.5. Open field test

Mice underwent specialized training in advance with individual placement in an open field for 1 h prior to testing. Data were recorded by two independent researchers who were unaware of the study design. Motor function was assessed by observing ankle movements, trunk instability, stepping, plantar placement, paw position and tail position of mice utilizing the Basso Mouse Scale (BMS), with a total score of 0–9 [22].

2.6. Swimming test

The animals were trained to swim across a transparent rectangular tank. Parameters including dependency on forelimbs, hindlimb movements and alternation, and body angle and instability during swimming were scored according to the Louisville Swim Scale, with each mouse undergoing two trials and the mean scores were calculated.

2.7. Ladder rung walking test

A specially designed horizontal corridor was used to reflect

sensorimotor impairment due to SCI, featuring 1-m-long transparent acrylic panels on both sides and a bottom surface with unevenly spaced metal bars [23]. The apparatus was positioned 15 cm above from the ground. Mice were pre-trained to cross the corridor and were assessed at different time points both prior to and following surgery. Researchers recorded the number of hind limb incorrect placements and calculated their proportion relative of the total steps.

2.8. Electrophysiology

Motor-evoked potentials (MEPs) were measured using electromyography to assess the recovery of motor function on day 28 post-SCI. After anesthesia, the mice were stimulated with a current of 0.5 mA, 0.5 ms, at a frequency of 1 Hz.

2.9. Myelin debris isolation and labeling

Myelin debris was harvested from murine brains by sucrose density gradient centrifugation. The extracted brain tissue was homogenized in 0.32 M sucrose solution followed by the layering of 0.83 M sucrose solution on the top and then centrifugation at 4 °C (100,000×g, 45 min). The myelin debris could be observed at the interface between two sucrose solutions. Subsequently, the myelin debris and carboxyfluorescein succinimidyl ester (CFSE, HY-D0938, MedChemExpress, NJ, USA) were co-incubated for 30 min at 24 °C for fluorescent labeling.

2.10. Transmission electron microscope (TEM) observation

Fresh mouse spinal cord tissues were sequentially fixed with 2 % glutaraldehyde and 1 % osmium tetroxide. Macrophages isolated from spinal cord tissues using fluorescence-activated cell sorting (FACS) were fixed as mentioned above. The fixed tissues were resin-embedded, sliced into ultrathin sections, mounted on copper grids, stained with uranyl acetate and lead citrate, and finally imaged under transmission electron microscope (JEOL-1010, Tecnai G2 Spirit Bio TWIN, FEI, USA).

2.11. Immunofluorescence analysis

Murine spinal cord tissues were prepared into 10 µm thick serial sections after 4 % paraformaldehyde fixation and dehydration in gradient sucrose solution. Sections were blocked for 1 h with 5 % BSA and treated overnight with primary antibodies at 4 °C. Following three washes with PBS, tissues were treated with secondary antibodies (1 h, room temperature), followed by DAPI counterstaining. The samples were evaluated and imaged under microscopy (Zeiss) with quantification by Image J software. The primary antibodies utilized were anti-CD68 (1:200), anti-Hexb (1:200), anti-dMBP (1:2000), anti-NF200 (1:500), anti-F4/80 (1:200), anti-iba-1 (1:200), anti-Tau (1:200), anti-MAP2 (1:200), anti-NeuN (1:200), and anti-GFAP (1:200). Fluorescence-conjugated secondary antibodies: Alexa Fluor 488 conjugated donkey anti-rabbit (1:1000), Alexa Fluor 488 donkey anti-mouse (1:1000), Alexa Fluor 555 donkey anti-mouse (1:1000), Alexa Fluor 594 goat anti-rabbit (1:1000), Alexa Fluor 594 goat anti-rat (1:1000), Alexa Fluor 594 donkey anti-goat (1:1000) and Alexa Fluor 647 donkey anti-rat (1:1000).

To perform Oil Red O (ORO) staining, 0.5 % ORO (O0625, Sigma, USA) solution was preheated to 60 °C. Samples were stained with ORO (20 min) with differentiation of the slides using an 85 % propylene glycol solution and distilled water to remove excess stain. Finally, slides were mounted with a DAPI-containing mounting medium.

2.12. Filipin III staining

To prepare a stock solution, Filipin III (abs42018484, Absin) was dissolved in DMSO to 10 mg/mL. After 4 % paraformaldehyde fixation, samples were stained with 50 µg/ml Filipin III for 1 h at room

temperature before subsequent experiments.

2.13. Dihydroethidium staining

Fresh spinal cord slices were exposed to 2 µmol/L Dihydroethidium (DHE, S0063, Beyotime) staining solution under room temperature for 30 min. Images were acquired using a Zeiss fluorescence microscope.

2.14. Nissl staining

The sections were stained with Nissl staining solution (G1036, Servicebio, Wuhan, China) and then washed to remove excess dye. Glacial acetic acid (0.1 %) was then used for differentiation until the Nissl bodies appeared dark blue. After drying, the sections were evaluated by microscope.

2.15. RNA extraction and RT-qPCR analysis

Total RNA was obtained from cells utilizing RNAiso Plus (9109, TaKaRa, Kusatsu, Shiga, Japan) and subsequently reverse-transcribed through reagent kits (R122-01, Vazyme Biotech, Nanjing, China). RT-qPCR was carried out using the ABI QuantStudio 6 system (Applied Biosystems). Expression was calculated relative to GAPDH by the $2^{-\Delta\Delta Ct}$ method. All primer sequences used are listed in Table 1.

2.16. Western blotting

Cells were lysed in RIPA buffer with complete protease inhibitor tablets. Protein concentrations were measured by the BCA assay kit and were separated on SDS-PAGE and transferred to PVDF membranes. The blots were blocked with 5 % nonfat dried milk for 1.5 h and then treated overnight with primary antibodies at 4 °C: anti-IL-1β (1:1000), anti-Pro-IL-1β (1:1000), anti-Caspase-1 (1:1000), anti-Pro-Caspase-1 (1:1000), anti-NLRP3 (1:1000), anti-IL-1β (1:1000). After washing, the blots were incubated with the corresponding secondary antibodies (1:3000) for 1.5 h at room temperature. The blots were quantified by Image J.

2.17. RNA-seq analysis

BMDMs treated with MD or MD + EIPA were exposed to RNA-seq analysis. Samples were prepared according to the standard sequencing procedures. RNA-seq was performed by an Illumina Nova seq 6000 (Xuran biological, Shanghai, China) following the standard protocols. Gene expression analysis was conducted by HTSeq v0.6.1 and differentially expressed genes (DEGs) were identified with the DESeq R package (1.10.1) with $P < 0.05$. Finally, KOBAS was conducted in Gene Ontology (GO) and KEGG enrichment analysis.

2.18. Flow cytometry analysis

Murine spinal cord tissues were digested for 45 min at 37 °C before filtering using a 200 µm sieve and then incubated with the following antibodies for 30 min at room temperature in the dark: viability dye-ApCy7, anti-CD45-APC-Cy7, CD11b-PE-Cyanine7, F4/80-PE, and iNOS-FITC. We used the Annexin-V-FITC/PI Apoptosis Detection Kit (556547, BD Biosciences, USA) to detect the apoptosis of neurons. Cells were evaluated using flow cytometry and FlowJo v.10 (Treestar, Ashland, OR, USA).

2.19. ELISA

Supernatants of macrophages and spinal cord tissues were drawn and the IL-1β and IL-6 contents were measured using ELISA kits, following the provided directions. Absorbances at 450 nm were read in a microplate reader.

Table 1
The primer sequences used for real time RT-PCR.

Gene	Forward primer	Reverse primer
IL-6	CCACTTCACAAGTCGGAGGCTTA	GCAAGTGCATCATCGTTGTTTCATAC
IL-1 β	TCCAGGATGAGGACATGAGCAC	GAACGTACACACCAGCAGGTTA
GAPDH	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA

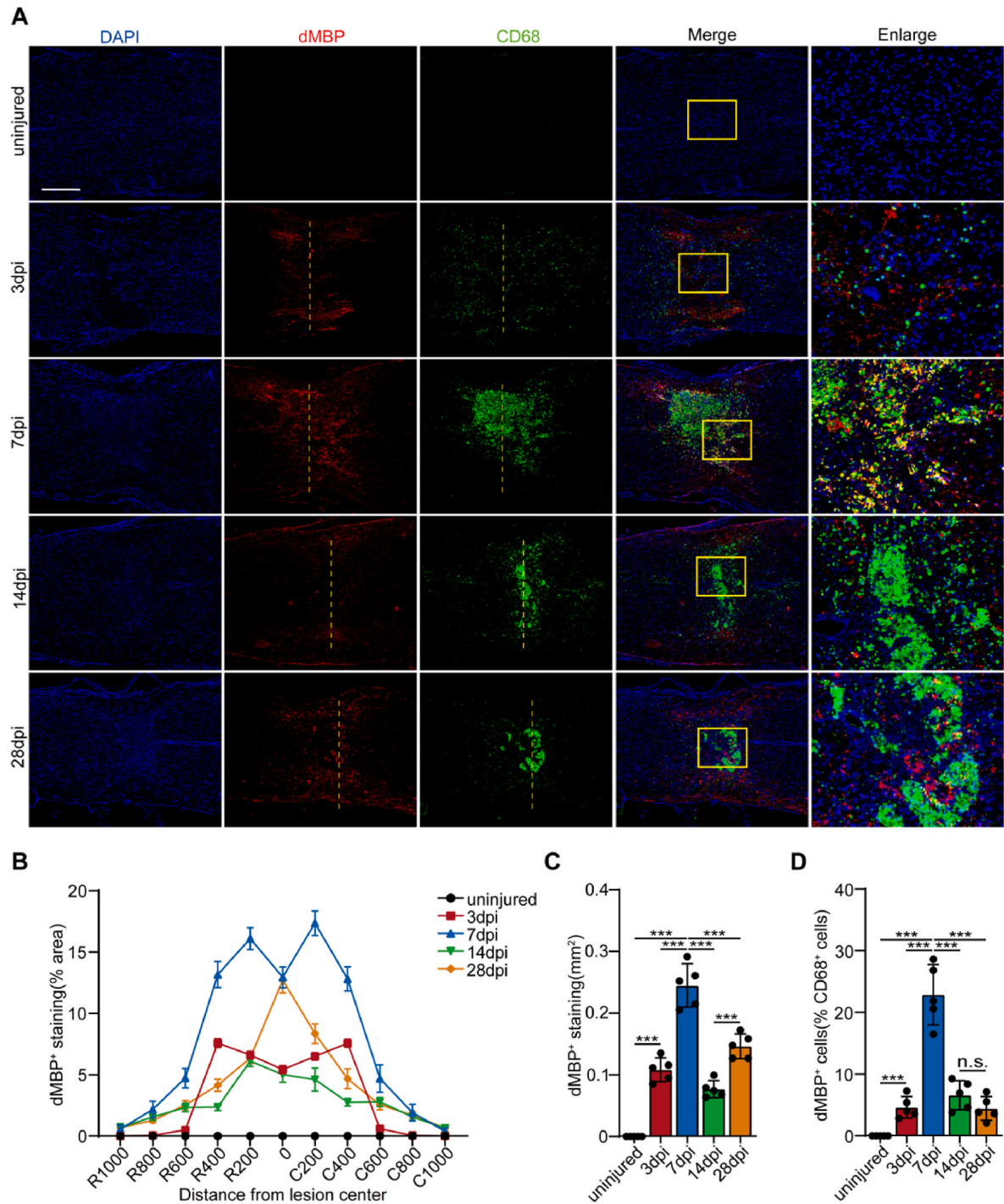


Fig. 1. Phagocytes participate in the clearance of myelin debris after SCI. (A) Phagocytes labeled with CD68 (green), myelin debris labeled with dMBP (red) and nuclei are stained with DAPI (blue). Scale Bar = 500 μ m. Dashed lines indicate the lesion center and the boxed areas are shown magnified. (B) Quantification of dMBP⁺ area of tissue in spinal cord sections taken rostral (R) and caudal (C) at different distances from the lesion center. (C) Quantification of total dMBP⁺ area. (D) Quantification of phagocytes laden with myelin debris in the injury site, n = 5. All data are mean $\pm$ SEM, n.s. indicates no significance, ****P* < 0.001 by one-way ANOVA test with Tukey's test for multiple comparisons (C and D).

2.20. Cholesterol assay

The total and free cholesterol contents in sorted macrophages were examined using the Amplex Red Cholesterol Assay Kit (S0211S, Beyotime, Shanghai, China).

2.21. ROS analysis

BMDM ROS levels were examined using DCFH-DA (S0033S, Beyotime) and analyzed by flow cytometry. Mitochondrial ROS in macrophages isolated from spinal cord tissues were measured using MitoSOX Red (M36008, Thermo Fisher). All procedures were conducted under the instructions of manufacturer.

2.22. OCR analysis

The Seahorse XF96 extracellular flux analyzer was utilized to assess the BMDM oxygen consumption rate (OCR). The culture media were replaced with the media containing 25 mM glucose and 2 mM pyruvate. Subsequent procedures were performed as directed and the data were quantified using Wave software.

2.23. Statistical analysis

Data were analyzed using GraphPad Prism 8 and the results are expressed as mean $\pm$ SEM. T-tests were used for two-group comparisons of normally distributed data. Comparisons among multiple groups used one/two-way ANOVA with the Tukey post hoc test. $P < 0.05$ was considered as significant. Specific information is included in the figure legends.

3. Results

3.1. Phagocytes internalize myelin debris after SCI

Mechanical compression and subsequent demyelination pathology after SCI produce myelin debris in the lesion area. We first used degenerated myelin basic protein (dMBP) immunoreactivity to clarify the spatiotemporal distribution of myelin debris in the spinal cords of the uninjured groups and on days 3, 7, 14, and 28 after SCI (Fig. 1A). Spinal cord tissues from the uninjured groups contained no detectable myelin debris. The amount of myelin debris significantly increased during SCI, with a peak appearing on day 7. Moreover, the quantity of myelin debris in the injury center was markedly greater than that in the perilesional area at all post-injury time points (Fig. 1A–C). Based on previous research indicating the critical role of macrophages and activated microglia in engulfing myelin debris in both SCI and MS models, we analyzed the immunofluorescence co-localization of CD68 and dMBP to investigate myelin debris engulfment by these two phagocytes at various time points following SCI. Our data demonstrated that CD68⁺ phagocytes contained myelin debris at the lesion site that peaked at 7 days post-injury (dpi). However, the internalization of myelin debris by CD68⁺ phagocytes was weakened until 28 dpi (Fig. 1A and D). Macrophage and microglia internalization of myelin debris was further evaluated *in vitro*. We isolated BMDMs and primary microglia and labeled myelin debris with CFSE. Figs. S1A–D demonstrated that after 1, 3, and 6 h of exposure to CFSE labeled myelin debris, *iba-1*⁺ microglia had less CFSE-positive area than F4/80 labeled macrophages, suggesting a lower capacity for internalizing myelin debris in microglia than in BMDMs. The results demonstrate that myelin debris is mainly distributed in the center of the lesion, peaking at 7 dpi, and CD68⁺ phagocytes internalize myelin debris after SCI.

3.2. Internalization of myelin debris leads to foamy macrophages after SCI

Intracellular myelin debris transforms into lipid droplets (LDs) for storage, forming foam-like cells. To explore the spatio-temporal distribution of foam cells after SCI, we used ORO to stain lipid droplet-laden cells. ORO⁺ cells were predominantly located in center of the lesion core, with numbers peaking at 7 dpi (Fig. 2A and C). Furthermore, macrophages (identified as CD68⁺Hexb⁺), in contrast to microglia (Hexb⁺), primarily accumulated in the lesion core after SCI (Fig. 2A and B). Fluorescence co-localization analysis revealed that many macrophages transformed into foam cells. In contrast, microglia exhibited minimal foam cell formation (Fig. 2A and D). The proportion of ORO⁺ foam macrophages peaked at 7 dpi and subsequently reduced at 28 dpi (Fig. 2D). We further confirmed that macrophages internalized myelin debris to form foamy macrophages *in vitro*, using BODIPY 493/503 fluorescent dye. LDs were upregulated in macrophages after myelin debris stimulation, peaking at 48 h post-stimulation and remaining until 72 h (Figs. S1E and F). Our results indicated that macrophages are crucial for foam cell formation after SCI.

3.3. Macrophage macropinocytosis involves in myelin debris internalization after SCI

Previous studies have indicated that macrophages internalize Low Density Lipoprotein (LDL), the primary carrier of cholesterol, through macropinocytosis in atherosclerosis [18]. Following spinal cord injury, macrophages similarly endocytose cholesterol-rich myelin debris. To examine the involvement of macropinocytosis in this process, we administered 70 kDa FITC-Dextran (a macropinosome marker) intrathecally to the mice 2 h before execution (Fig. 3A). Flow cytometry (FC) analysis demonstrated that macropinocytosis in the spinal cord tissues peaked at 7 dpi (Fig. S2A). We further analyzed the FC data and found that macrophages internalized FITC-Dextran. The maximum concentration of intracellular dextran was observed at 7 dpi, indicating the occurrence and highest activity of macropinocytosis (Fig. 3B and C). Furthermore, we performed IF staining of spinal cord tissues seven days post-injury, revealing the presence of dextran in macrophages (Fig. 3D). However, we found astrocytes, neurons, microglia, and oligodendrocytes had no co-localization with FITC-Dextran (Fig. S2B). The flow-sorted macrophages were used for IF staining to confirm the presence of dextran in the macrophages (Fig. 3E). In addition, TEM revealed that macrophages internalize myelin debris via macropinocytosis at 7 dpi (Fig. 3F). Macrophages sorted at 7 dpi were also observed to engulf myelin debris through macropinocytosis (Fig. 3G). In the *in vitro* experiments, we incubated CFSE labeled myelin debris and 70 kDa Rhod-Dextran with BMDMs both in the presence and absence of the macropinocytosis inhibitor 5-[N-ethyl-N-isopropyl] amiloride (EIPA). As illustrated in Fig. 3H and I, FC showed that the macropinocytosis inhibitor markedly reduced the fluorescent levels of myelin debris and dextran within BMDMs, indicating that the suppression of macropinocytosis diminishes the internalization of myelin debris by BMDMs. In conclusion, the results demonstrate the involvement of macropinocytosis in macrophage internalization of myelin debris after SCI.

3.4. Inhibiting macropinocytosis decreases foamy macrophage formation

Kong et al. have indicated that blocking the receptor-mediated endocytosis pathway only partially suppresses foam cell development after SCI [24]. Macropinocytosis, a non-receptor-mediated endocytosis, has recently been identified as an important contributor to foam cell formation in atherosclerosis, although its effect after SCI remains unknown [18]. To further clarify the relative contribution of macropinocytosis and receptor-mediated endocytosis in foamy macrophage formation after SCI, WT, MSR1^{-/-}, and CD36^{-/-} mice were exposed to

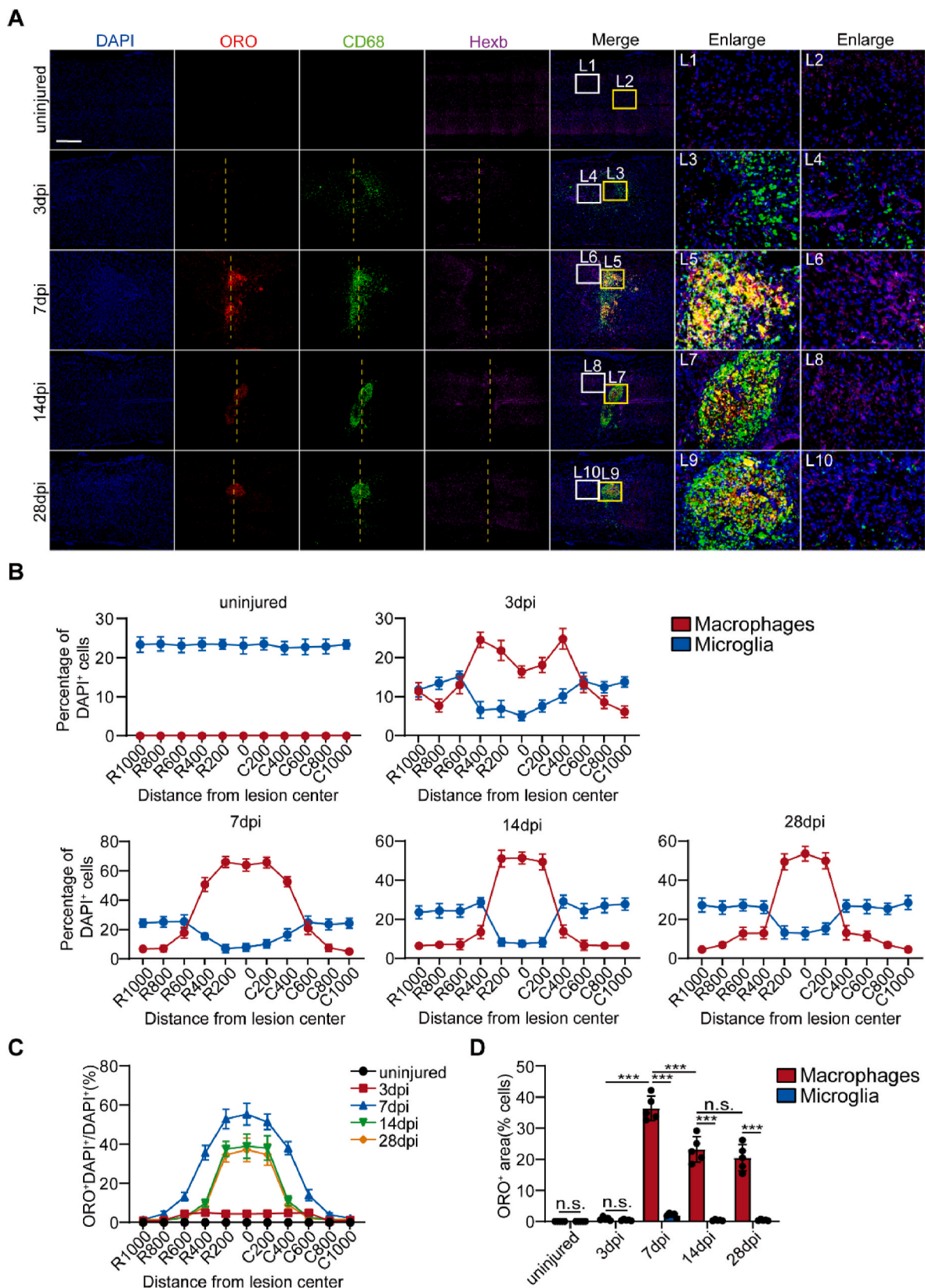


Fig. 2. Spatio-temporal distribution of foam cells after SCI.

(A) Macrophages identified as $CD68^+$ (green) and $Hexb^-$ (purple), microglia identified as $Hexb^+$, lipid drops labeled with Oil Red O (red) and nuclei are stained with DAPI (blue). Scale Bar = 500 μm . Dashed lines indicate the lesion center. Magnified images of boxed areas are shown on the right. (B) Quantification of the distribution of macrophages and microglia measured by the percentage of $DAPI^+$ cells at different distances from the lesion center. (C) Quantification of the distribution of ORO^+ cells in indicated groups. (D) Quantification of macrophages and microglia laden with lipid drops of uninjured murine spinal cord and spinal cord tissues at 3, 7, 14, and 28 dpi, $n = 5$. All data are mean $\pm$ SEM, n.s. indicates no significance, $***P < 0.001$ by two-way ANOVA with Tukey's test for multiple comparisons (D).

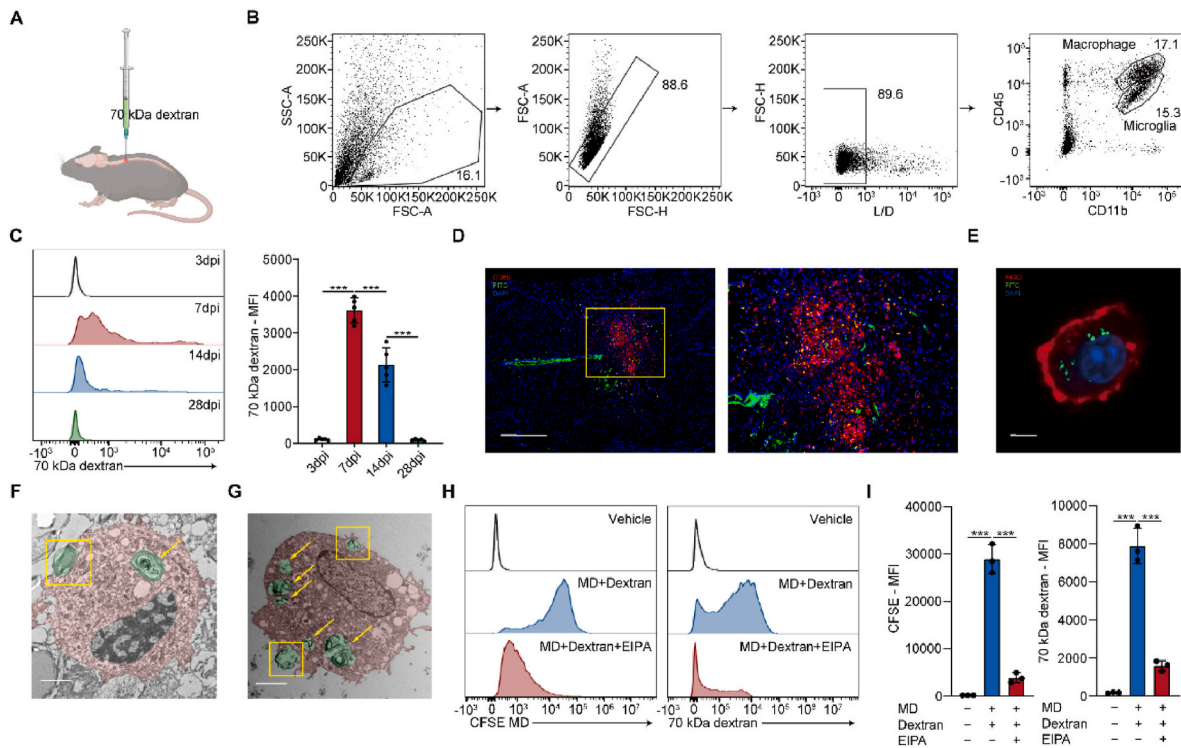


Fig. 3. Macrophages internalize myelin debris through macropinocytosis after SCI.

(A–E) WT mice were treated with intrathecal 70 kDa FITC-dextran (1 mg/ml) 2 h before execution. (H–I) BMDMs were pre-treated with or without EIPA for 20 min, followed by exposure to CFSE-MD and Rhod-dextran for 3 h. (A) Schematic representation of intrathecal injection of fluorescent dextran in mice. (B and C) Flow cytometric analysis of macrophages ($CD45^{hi} CD11b^{+}$) isolated from spinal cord in the injury site. Mean fluorescent intensity of FITC-dextran in macrophages collected at 3, 7, 14, and 28 dpi, $n = 5$. (D) Representative images of macrophages laden with FITC-dextran in the injury site at 7 dpi. Scale Bar = 500 μm . Magnified image of the boxed area is shown below. (E) Representative images of FITC-dextran (Green)-containing macrophages isolated from spinal cord labeled with F4/80 (red), nuclei are stained with DAPI (blue). Scale Bar = 5 μm . (F) TEM images of macrophages in the lesion site of mice at 7 dpi. Yellow boxes indicate macrophages internalized myelin debris through macropinocytosis, while yellow arrows indicate internalized myelin debris. Scale Bar = 2 μm . (G) TEM images of collected macrophages sorted from spinal cord at 7 dpi. Yellow boxes indicate macrophages internalized myelin debris through macropinocytosis, while yellow arrows indicate internalized myelin debris. Scale Bar = 2 μm . (H and I) Quantification of CFSE-MD and Rhod-dextran in BMDMs by FC analysis, $n = 3$. All data are mean $\pm$ SEM, *** $P < 0.001$ by one-way ANOVA with Tukey's test for multiple comparisons (C and I). MD, myelin debris.

intrathecal injection of macropinocytosis inhibitor EIPA. IF results demonstrated that the percentage of ORO^{+} macrophages was reduced in $MSR1^{-/-}$ and $CD36^{-/-}$ mice compared to that in WT controls (Figs. S3A and B). Notably, the percentage of ORO^{+} macrophages decreased significantly in EIPA-treated WT, $MSR1^{-/-}$, and $CD36^{-/-}$ mice compared to that in Saline-treated controls (Fig. 4A and B). We isolated macrophages from spinal cord tissues by FC and prepared cell slides to validate these results. The LDs of the macrophages were detected using BODIPY 493/503. Compared to WT controls, the number of LDs decreased in $MSR1^{-/-}$ and $CD36^{-/-}$ macrophages (Figs. S3C and D). Similarly, the levels of LDs decreased significantly in EIPA-treated WT, $MSR1^{-/-}$, and $CD36^{-/-}$ macrophages compared to Saline-treated controls (Fig. 4C and D). We next confirmed that the percentage of $dMBP^{+}CD68^{+}$ phagocytes decreased in the $MSR1^{-/-}$ or $CD36^{-/-}$ groups compared to WT controls (Figs. S4A and B). Furthermore, EIPA inhibited the endocytosis of myelin debris by $CD68^{+}$ phagocytes *in vivo* and BMDMs *in vitro*, which may be responsible for the reduction in foam cells (Figs. S4C–F). In conclusion, macropinocytosis plays a vital role in foamy macrophage formation after SCI.

3.5. Inhibition of macrophage macropinocytosis alleviates NLRP3 inflammasome activation and IL-1 β production after SCI

To further investigate the effect of macropinocytosis in SCI, we performed the RNA-sequencing (RNA-seq) of the control, myelin debris (MD)-treated, and MD combined with EIPA treated BMDMs to analyze the differentially expressed genes (DEGs). All expressed genes with fold

changes >1.5 and $P < 0.05$ between groups were categorized as DEGs. Fig. 5A revealed that 2833 DEGs were both upregulated in MD-treated BMDMs compared to controls and decreased in MD + EIPA groups compared to MD-treated ones. A total of 1847 DEGs were down-regulated in MD-treated BMDMs but upregulated in MD + EIPA groups. Notably, the expression of typical inflammation-related factor genes, including IL-1 β and IL-6, was markedly upregulated in MD-treated groups compared to controls, and decreased in MD + EIPA groups compared to those MD treated ones (Fig. 5B). GO biological process analysis revealed that the DEGs were primarily associated with IL-1 production, acute inflammatory response, NLRP3 inflammasome, cholesterol metabolism and ROS generation (Fig. 5C). KEGG pathway analysis revealed that inflammatory pathways, including NF- κ B and TLR signaling, were the most dominant pathways regulated by EIPA (Fig. 5D). Furthermore, both *in vitro* and *in vivo* studies showed that upregulation of IL-1 β could be suppressed by EIPA treatment, whereas IL-6 showed a minor decrease in the EIPA groups. Moreover, EIPA treatment had no effect on IL-1 β secretion of macrophages without MD stimulation (Fig. 5E–G). These studies indicate a potential regulatory role for macropinocytosis in the NLRP3 inflammasome and IL-1 β pathway. Therefore, we investigated whether macropinocytosis triggered Caspase-1 proteolysis and IL-1 β secretion. Western blotting (WB) results showed that MD treatment significantly increased NLRP3 levels in BMDM lysates, and the level of NLRP3 was attenuated by EIPA treatment. Furthermore, EIPA treatment prevented activation of Caspase-1 and the secretion of IL-1 β (Figs. S5A and B). We further investigated the effect of macropinocytosis on macrophage polarization during

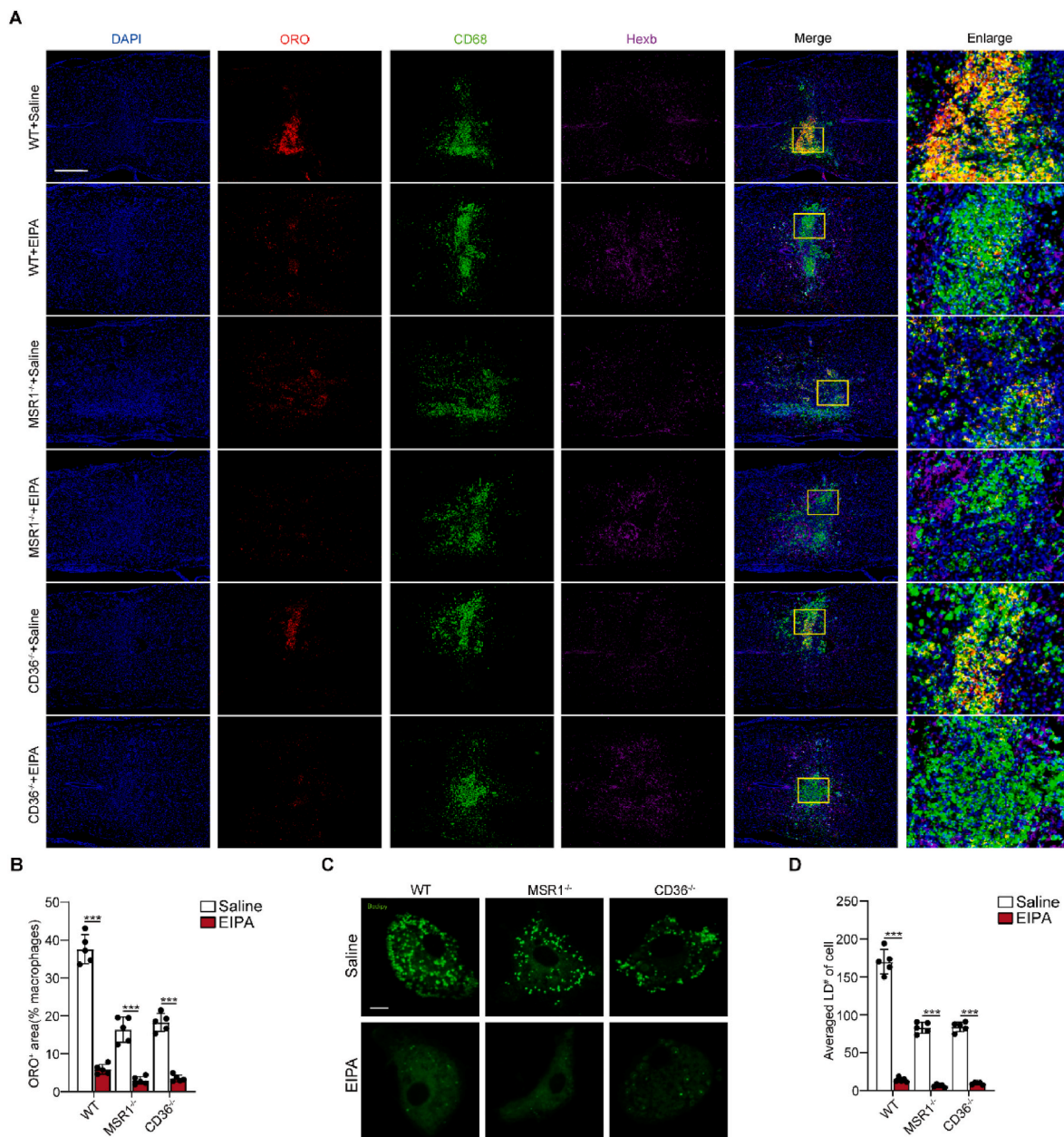


Fig. 4. Macropinocytosis promotes foamy macrophage formation.

(A) Representative images of spinal cord tissues of WT, MSR1^{-/-} and CD36^{-/-} mice at 7 dpi. Macrophages identified as CD68⁺ (green) and Hexb⁻ (purple), microglia identified as Hexb⁺, lipid droplets labeled with Oil Red O (red) and nuclei are stained with DAPI (blue). Scale Bar = 500 μ m. Magnified images of boxed areas are shown on the right. (B) Quantification of macrophages laden with lipid droplets in the injury site, n = 5. (C and D) Representative images of macrophages isolated from spinal cord in indicated groups at 7 dpi. Lipid droplets stained with Bodipy (green). Scale Bar = 10 μ m. Quantifications show numbers of intracellular lipid droplets, n = 5. All data are mean $\pm$ SEM, ****P* < 0.001 by two-way ANOVA with Tukey's test for multiple comparisons (B and D).

SCI. The number of M1-like macrophages at the injury site was markedly decreased in the EIPA-treated groups at 7 dpi (Fig. 5H and I). In summary, inhibition of macrophage macropinocytosis alleviates the pro-inflammatory response by inhibiting the NLRP3 inflammasome and IL-1 β .

3.6. Macropinocytosis inhibitor alleviates NLRP3 inflammasome activation and IL-1 β production by reducing cholesterol accumulation

Previous studies have elucidated the regulatory mechanisms underlying cholesterol accumulation, NLRP3 inflammasome activation, and IL-1 β production in foamy macrophages, particularly in demyelinating conditions such as multiple sclerosis (MS). As myelin is formed largely

by cholesterol, excess myelin debris in macrophages results in foam cell formation and triggers cholesterol overload, leading to the formation of cholesterol crystals and NLRP3 inflammasome activation [25]. To further investigate the link between cholesterol overload, cholesterol crystal formation and macrophage macropinocytosis, the mice were exposed to EIPA or the cholesterol efflux agonist GW3965 treatment after SCI surgery. Subsequently, macrophages were isolated from the spinal cord tissues using FACS at 7 dpi. We found that both treatment groups showed a decrease in total and free cholesterol levels in macrophages, with the EIPA treatment group exhibiting a more significant decrease (Fig. 6A and B). Additionally, we analyzed the levels of free cholesterol in sorted macrophages using Filipin III staining. The FC and IF analysis were consistent with previous results (Fig. 6C–F).

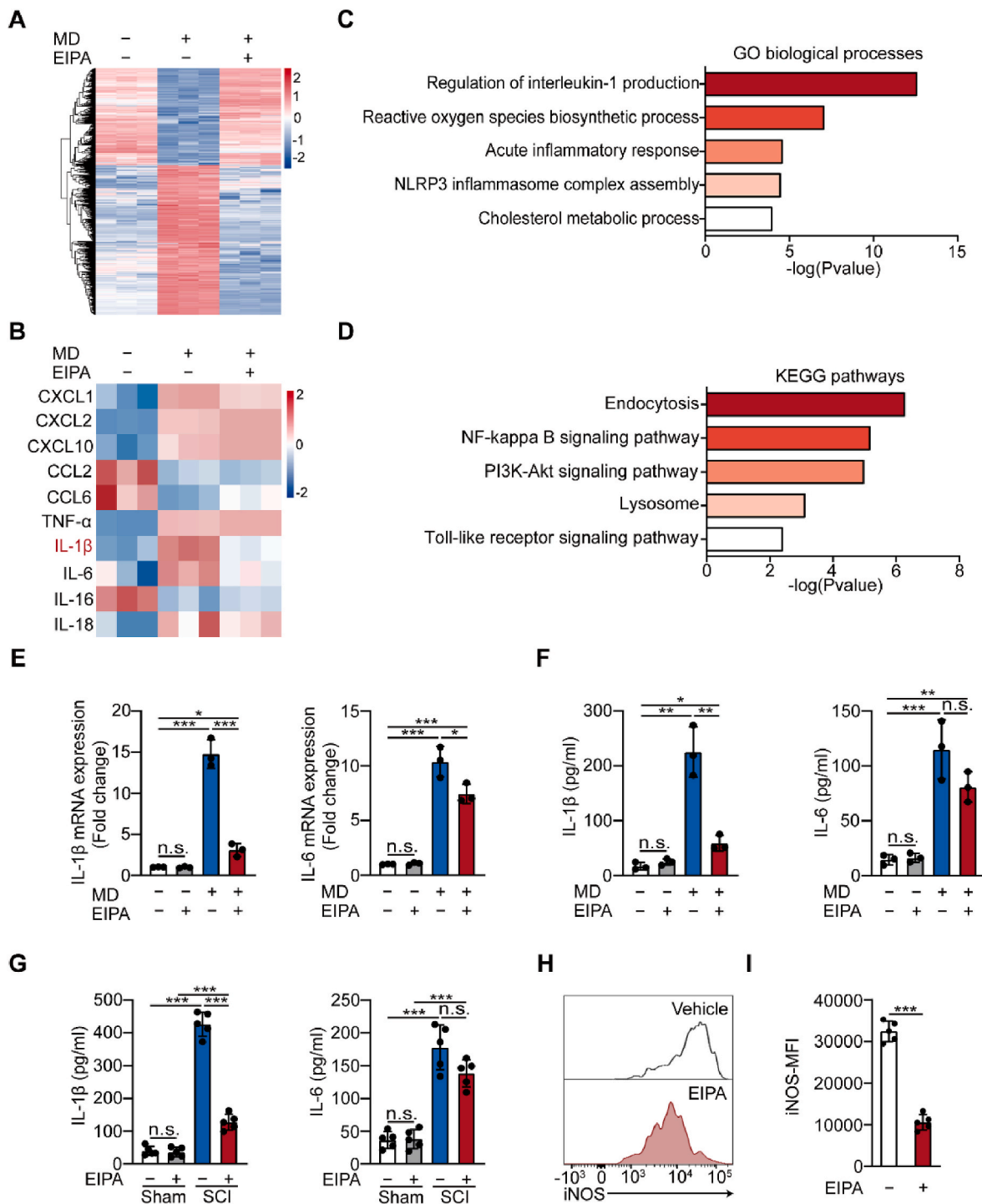
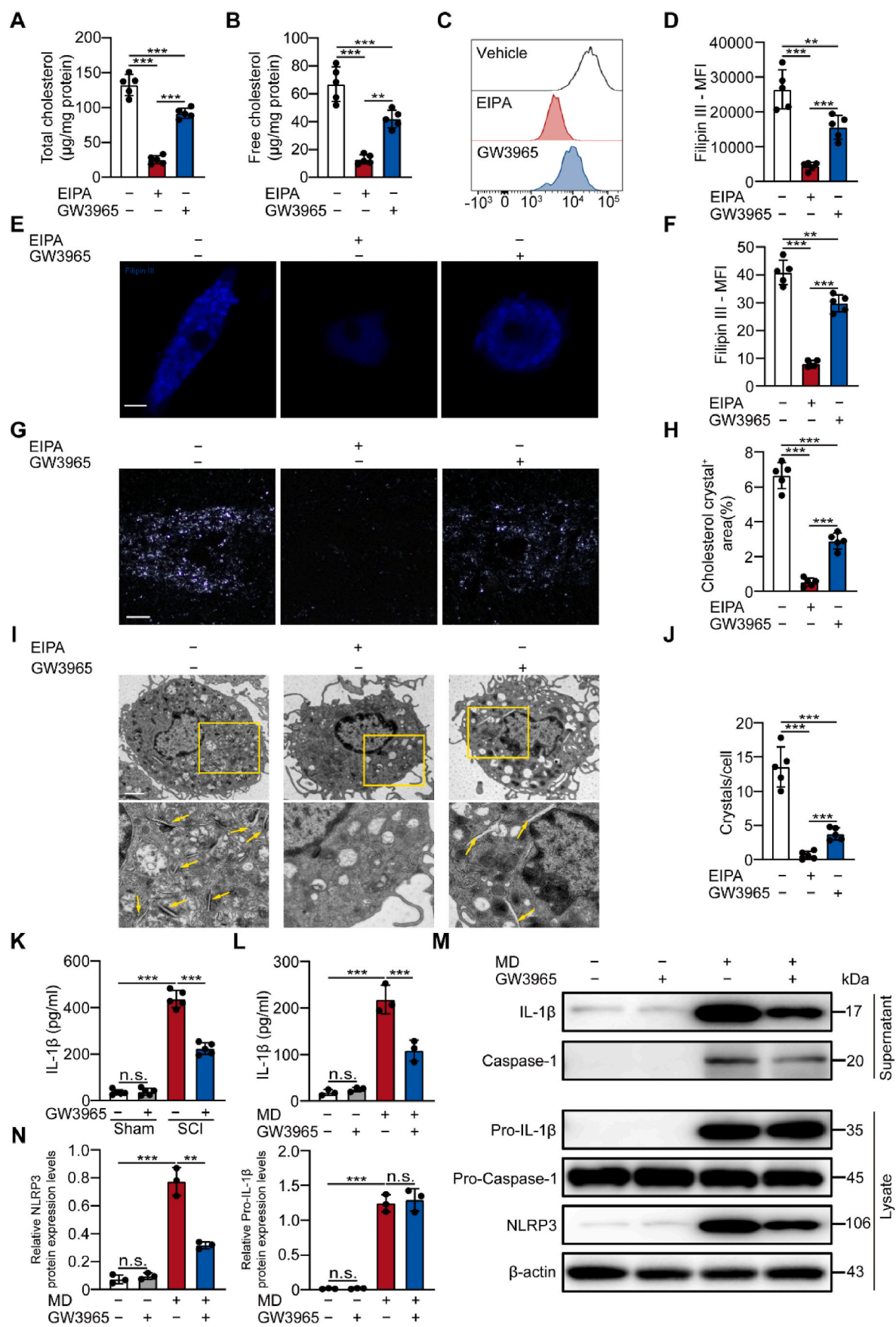


Fig. 5. Macropinocytosis inhibitor suppresses NLRP3 inflammasome activation and IL-1 β secretion of foamy macrophages

(A-F) BMDMs were pre-treated with or without EIPA for 20 min, followed by exposure to MD for 48 h. (A) RNA-seq analysis identified the 4680 DEGs between control, MD and MD + EIPA groups. (B) Heatmap of classical genes of cytokines and chemokines between control, MD and MD + EIPA groups. (C and D) The GO and KEGG analysis for these 4680 DEGs in biological processes and signaling pathway. (E) Relative mRNA levels of IL-1 β and IL-6 in BMDMs, $n = 3$. (F) The amount of secreted IL-1 β and IL-6 in the supernatant of BMDMs were detected by ELISA, $n = 3$. (G-I) WT mice were treated with 3 days of EIPA (1 mg/ml) or Saline at 4 dpi and executed at 7 dpi. (G) Quantitative ELISA of IL-1 β and IL-6 of spinal cord tissues in the injury site, $n = 5$. (H and I) Quantification of iNOS $^{+}$ macrophages in the lesion site by FC analysis, $n = 5$. All data are mean $\pm$ SEM, n.s. indicates no significance, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by one-way ANOVA with Tukey's test for multiple comparisons (E to G) and two-tailed Student's t -test (I). DEG, differentially expressed gene.

Furthermore, polarized light microscopy revealed that EIPA was more effective in inhibiting cholesterol crystallization compared to GW3965 (Fig. 6G and H). TEM results further confirmed these findings, as the number of cholesterol crystals was markedly lower in the EIPA-treated group than in the GW3965 group (Fig. 6I and J). The above results

suggested that blocking macropinocytosis inhibits cholesterol crystallization after foamy macrophage formation. Our previous studies demonstrated that decreasing myelin debris internalization by blocking macropinocytosis suppressed macrophage NLRP3 inflammasome activation and IL-1 β production. We further used the cholesterol efflux



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Fig. 6. Inhibition of cholesterol crystal formation decreased NLRP3 activation and IL-1 β release

(A–K) WT mice were exposed to a 3-day EIPA/GW3965/Saline injection intrathecally at 4 dpi. (A and B) Cholesterol quantitation of macrophages isolated from spinal cord was performed by Amplex Red cholesterol assay kit. (C and D) Free cholesterol of isolated macrophages was detected through Filipin III staining. (E and F) Representative images of isolated macrophages in indicated groups at 7 dpi. Free cholesterol stained with Filipin III (blue). Quantification of mean fluorescence intensity of immunofluorescence images. Scale Bar = 10 μ m. (G and H) Representative image of polarized light microscope of the spinal cord at 7 dpi and quantification of cholesterol crystal-positive area. Scale Bar = 100 μ m. (I and J) Transmission electron microscopy images of macrophages isolated from spinal cord tissues in indicated groups on 7 dpi. Magnified images of the boxed area are shown below. Yellow arrows indicate cholesterol crystals. Scale bars = 2 μ m. Numbers of cholesterol crystals in macrophages at 7 dpi, $n = 5$. (K) IL-1 β levels in spinal cord tissues from sham, sham + GW3965, SCI, and SCI + GW3965 groups, $n = 5$. (L–N) BMDMs were treated with MD for 48 h, or BMDMs treated with GW3965 1 h before adding MD. (L) The amount of secreted IL-1 β in the supernatants of cell culture was detected by ELISA, $n = 3$. (M–N) Western blotting analysis of IL-1 β and Caspase-1 expression in the supernatants of BMDMs treated with MD or MD + GW3965, and quantification of NLRP3 and Pro-IL-1 β expression in the lysate of BMDMs, $n = 3$. All data are mean $\pm$ SEM, n.s. indicates no significance, ** $P < 0.01$, *** $P < 0.001$ by one-way ANOVA with Tukey's test for multiple comparisons (A, B, D, F, H, J, K, L, N).

agonist GW3965 to verify the relationship between cholesterol crystals, NLRP3 inflammasome activation, and IL-1 β release. GW3965 treatment significantly reduced the IL-1 β level in injured spinal cord tissue. Furthermore, GW3965 treatment did not affect IL-1 β level in Sham groups (Fig. 6K). *In vitro* results were in line with those *in vivo* (Fig. 6L). WB analysis revealed that the level of NLRP3 was markedly decreased in the GW3965 + MD treatment group compared to that in the MD treatment group (Fig. 6M and N). In conclusion, the inhibition of macrophagocytosis prevents cholesterol crystallization after foamy macrophage formation, which can alleviate inflammatory responses induced by NLRP3 inflammasome activation and IL-1 β production.

3.7. Blocking macrophagocytosis reduces ROS production

Our previous RNA-seq analysis showed significant changes in ROS levels after EIPA treatment, we further studied the effects of EIPA on ROS production. Mice were exposed to EIPA or GW3965 treatment after SCI surgery. DHE staining revealed that both EIPA and GW3965 treatment decreased ROS production compared to vehicle groups, while EIPA treatment group exhibiting a more significant decrease (Fig. 7A and B). Consistently, mean fluorescence intensity of DCFH-DA was also decreased in EIPA + MD and GW3965 + MD groups compared to MD groups (Fig. 7C and D). We further sorted out macrophages from SCI mice by FACS and detected the level of MitoSOX. Immunofluorescence staining analysis were consistent with previous results (Fig. 7E and F). Accordingly, EIPA treatment also increased the level of OCR, an index of mitochondrial oxidative phosphorylation (OXPHOS, Fig. 7G and H).

3.8. Inhibition of macrophagocytosis suppresses foam cell formation to improve functional recovery

To investigate the therapeutic effects of macrophagocytosis inhibitors in SCI, the Louisville Swim Scale, Basso Mouse Scale (BMS), and ladder rung walking test were used to estimate recovery of motor function in EIPA-treated mice following SCI. The sham groups showed no significant difference in the BMS and swimming scores. However, the BMS and swimming scores were markedly higher in the EIPA-treated groups than in the untreated groups from 7 to 28 dpi (Fig. 8A and B). In addition, the ladder rung walking test demonstrated fewer hind limb errors in the EIPA-treated group after SCI (Fig. 8C). Electrophysiological analysis at 28 dpi revealed higher MEP amplitudes and lower latencies in the EIPA-treated group, indicating better electrophysiological recovery (Fig. 8D and E). Nissl staining depicted higher neural survival in the EIPA-treated groups (Fig. 8F and G). To further illustrate the impact of conditioned medium from BMDMs on neurons *in vitro*, we conducted flow cytometry assay and observed that neuronal apoptosis was significantly increased in the media from MD-treated BMDMs compared to the control groups. In contrast, the media from MD + EIPA treatment BMDMs significantly decreased the apoptosis rate (Fig. 8H and I). Furthermore, we immunostained the 200 kDa Neurofilament 200 (NF200) to assess neuronal regenerative capacity. IF analysis indicated that the reduction in NF200 was less in the EIPA-treated groups than in the control groups following SCI (Fig. 8J and K). Microtubule-associated protein 2 (MAP2) and Tau

were used *in vitro* to detect axon lengths. MAP2 is a neuron-specific cytoskeletal protein that acts as a marker for neuronal dendrites. Tau is a microtubule-associated protein that is strongly expressed in axons. IF staining revealed that the media from MD-treated BMDMs resulted in shorter axons than the control groups. Conversely, media from MD + EIPA-treated BMDMs increased axon length compared with that in the MD-treated group (Fig. 8L and M). It has been found that EIPA can significantly suppress IL-1 β secretion. To confirm the relationship between IL-1 β and functional recovery after SCI, we blocked this effect using IL-1 β -neutralizing antibodies. Mice received intrathecal injections of IL-1 β -neutralizing antibodies (20 μ g) for three days at 4 dpi. IL-1 β in MD-treated macrophage supernatant was blocked by neutralizing antibodies (10 μ g/ml) *in vitro*. Both *in vitro* and *in vivo* experiments indicated that reduced neuronal apoptosis and axonal regeneration was facilitated after IL-1 β -neutralization, bringing about improved motor function (Figs. S6A–M). Collectively, blocking macrophagocytosis can decrease IL-1 β production by macrophages after SCI, which is beneficial for protecting neurons and recovering neurological functions.

4. Discussion

SCI is a devastating disease that causes severe dysfunction and affects quality of life with no effective interventional options available [1,26,27]. Here, we describe a novel non-receptor-dependent mechanism whereby macrophagocytosis modulates myelin debris clearance following SCI. We demonstrated that macrophagocytosis is important for engulfing myelin debris to form foamy macrophages after SCI, which was not disrupted by receptor-mediated endocytosis. Macrophagocytosis inhibition decreases NLRP3 inflammasome activation and ROS production by suppressing foamy macrophage formation and the subsequent cholesterol overload-induced cholesterol crystallization. In summary, our findings indicate that the inhibition of macrophagocytosis protects SCI models.

Endocytosis, including caveolae, macrophagocytosis, and receptor-mediated endocytosis, plays a vital role in normal cell life and function [28–30]. In recent years, targeting the effect of receptor-mediated processes has been considered a beneficial therapeutic approach for SCI, but these strategies have limited efficacy [31–33]. Here, we provide evidence that macrophagocytosis is involved in the macrophage internalization of myelin debris generated after SCI. Although compelling evidence is provided that EIPA, a commonly used macrophagocytosis inhibitor, was used in this study to explore the function of macrophagocytosis in debris engulfment, the contribution of other types of endocytosis in SCI cannot be excluded [34,35]. Moreover, due to the specific requirements of cells in capturing and processing different types of extracellular materials, each type of endocytosis is specialized for internalizing particles of particular sizes [36]. It remains unknown whether the internalization of variably sized myelin debris through different endocytosis pathways produces distinct effects [12,37].

The functions of foamy macrophages in inflammation are not well understood. In atherosclerosis models, the contribution of foamy macrophages is linked with both pro-inflammatory and anti-inflammatory activities. Previous studies have described that Liver X receptors

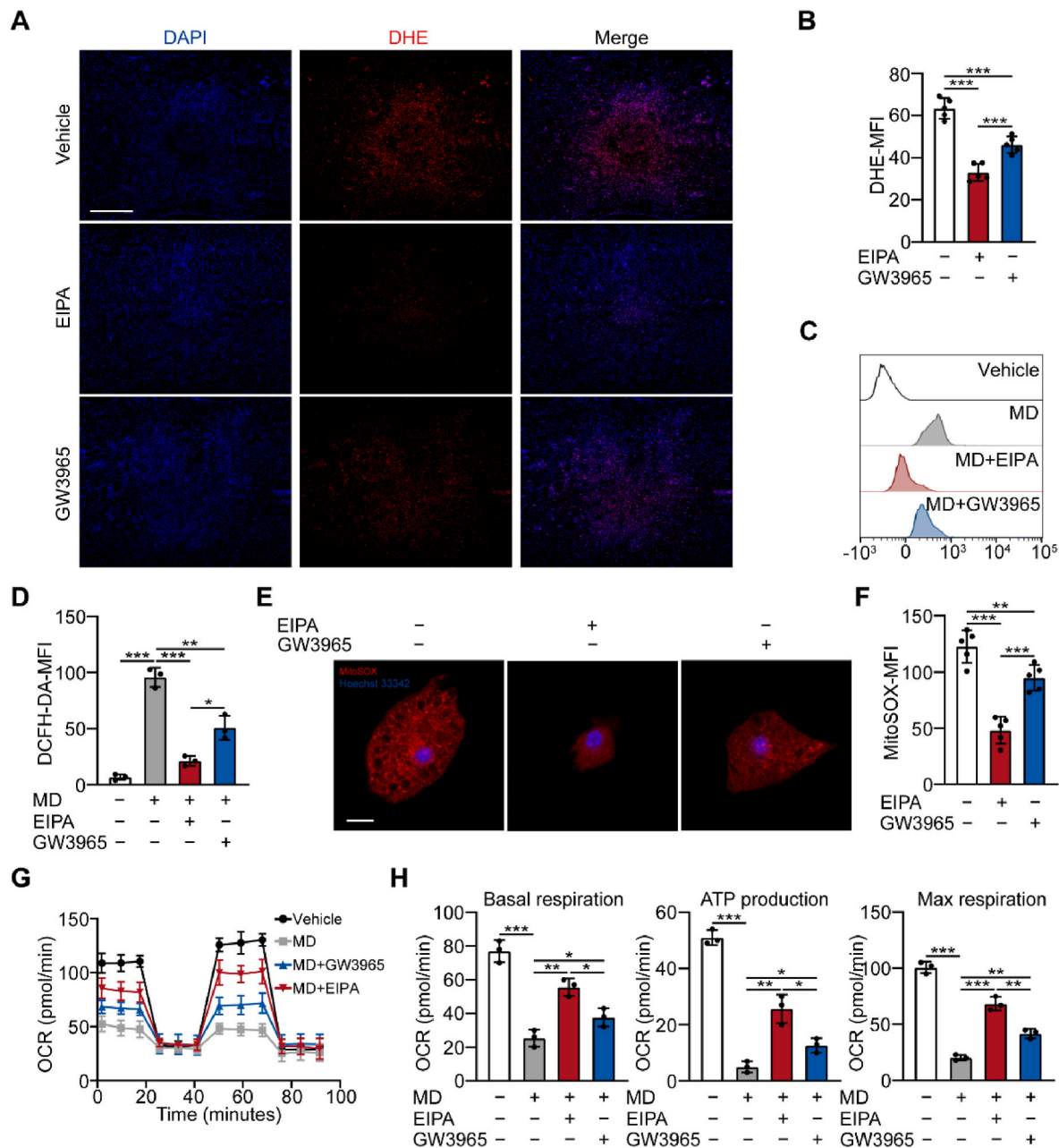


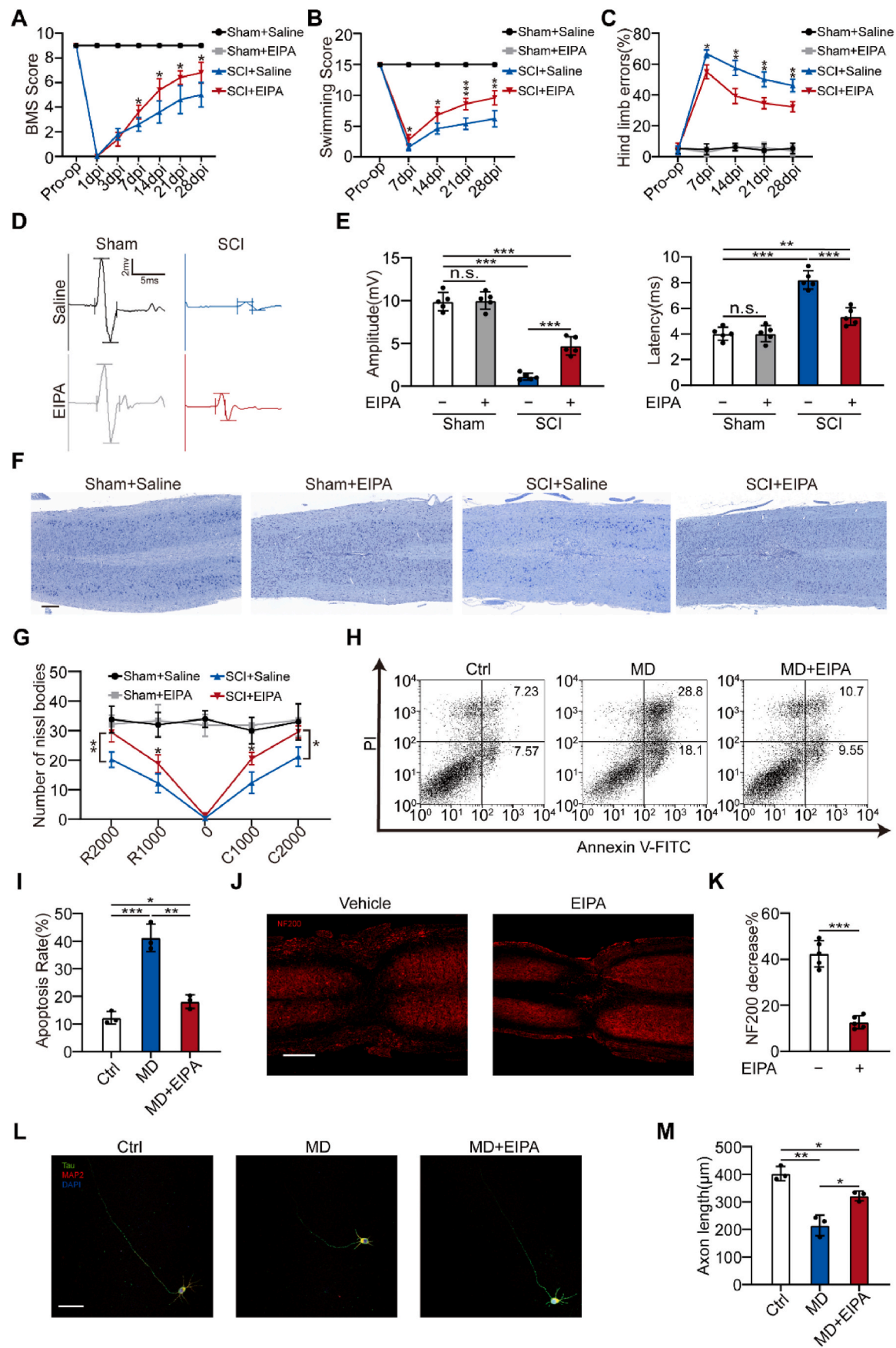
Fig. 7. Blocking macropinocytosis reduced ROS production by decreasing cholesterol accumulation in macrophages after SCI.

(A and B, E and F) WT mice were exposed to a 3-day EIPA/GW3965/Saline injection intrathecally at 4 dpi. (A) Evaluation of ROS accumulation was detected using DHE staining. Scale Bar = 500 μ m. (B) Quantitative analyses of DHE mean fluorescence intensity, $n = 5$. (C and D, G and H) BMDMs were pre-treated with or without EIPA/GW3965, followed by exposure to MD for 48 h. (C and D) The levels of ROS in BMDMs were determined using DCFH-DA, $n = 5$. (E and F) Representative immunofluorescence images of MitoSOX in isolated macrophages at 7dpi. Scale Bar = 10 μ m. Quantifications show mean fluorescence intensity of MitoSOX, $n = 5$. (G and H) Mitochondrial function of BMDMs was evaluated by OCR, $n = 3$. All data are mean $\pm$ SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by one-way ANOVA with Tukey's test for multiple comparisons (B, D, F, and H).

(LXRs) participate in foam cell formation and suppress inflammatory gene expression [38–40]. However, foamy macrophage formation is a key step in initiating an inflammatory response in atherosclerotic plaques [41–43]. Moreover, recent studies on MS have suggested that foamy macrophages can acquire anti-inflammatory and wound-healing properties and display a unique M2-like phenotype [44]. Conversely, foamy macrophages exhibit an M1-like phenotype in the center of MS lesions, at least for a certain period [11]. Foamy macrophages in SCI have recently been identified as pro-inflammatory, neurotoxic, and defective in apoptotic/necrotic cell clearance, which further leads to delayed wound healing [24]. The present study demonstrates that

excessive intracellular lipids after myelin debris engulfment in SCI form foamy macrophages, which further activate the NLRP3 inflammasome and the production of pro-inflammatory cytokines, thereby contributing to neuronal apoptosis. Our findings are consistent with those of previous studies, indicating that foamy macrophages progressively produce pro-inflammatory cytokines and induce neuronal apoptosis after SCI.

Numerous studies have indicated that excess cholesterol accumulation induces ubiquitous toxicity, which is involved in the progression of various diseases, such as atherosclerosis, non-alcoholic steatohepatitis, and diabetes [45]. Non-metabolized free cholesterol in foamy macrophages crystallizes into cholesterol crystals. These crystals then activate



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Fig. 8. Inhibition of macropinocytosis reduced spinal cord damage and motor function deficits after SCI.

(A–G, J and K) WT mice were exposed to a 3-day EIPA/Saline injection intrathecally at 4 dpi. (A–C) Statistical analysis of the BMS (A), swimming score (B), and hind limb errors (C) in different groups over a 28-day period, $n = 5$. (D and E) Electrophysiological analyses of mice in indicated groups at day 28 post-SCI, $n = 5$. (F and G) Representative images and quantification of Nissl staining in different groups, Scale Bar = 200 μm , $n = 5$. (H and I) Flow cytometry analysis of neuronal apoptosis in primary neurons treated with the conditioned medium from BMDMs that incubated with MD or MD + EIPA through Annexin V-FITC/PI double staining, $n = 3$. (J and K) Representative IF staining and quantification of NF 200 in indicated groups at 28 dpi, Scale Bar = 500 μm , $n = 5$. (L and M) Representative IF images of neurons and quantification of axon length treated with the conditioned medium from different groups of BMDMs, Scale Bar = 50 μm , $n = 3$. All data are mean $\pm$ SEM, n.s. indicates no significance, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by two-way ANOVA with Tukey's test for multiple comparisons (A to C, E and G), one-way ANOVA with Tukey's test for multiple comparisons (I and M) and two-tailed Student's t -test (K).

NLRP3 inflammasome and promote IL-1 β production [46]. The present results showed that cholesterol crystals formed in foamy macrophages, further activating NLRP3 inflammasome and exerting a pro-inflammatory effect. Zheng et al. identified needle-like cholesterol crystals in foamy macrophages of spinal cord lesions using TEM and IF assays, and efficient cholesterol clearance prevented NLRP3 inflammasome activation, which is consistent with our results [46]. Research has demonstrated a strong association between NLRP3 inflammasome and various central nervous system diseases, including cerebrovascular diseases, neurodegenerative diseases, and MS [47]. The NLRP3 inflammasome plays a vital role in the central nervous system and is associated with numerous biological processes, including mitochondrial autophagy, glial activation, oxidative stress, and inflammatory responses [48]. However, the specific regulatory mechanisms that control its activation in the central nervous system remain unclear. Mounting evidence indicates that stimuli such as ATP, ROS, cathepsin B, and mitochondrial Ca^{2+} overload activate the NLRP3 inflammasome and cleave Pro-IL-1 β into mature IL-1 β [49]. Furthermore, cholesterol crystals can activate NLRP3 inflammasome through K^{+} efflux, complement activation, and ROS production [50–52]. Our study identified that excessive internalization of myelin debris led to the foamy macrophage formation, and subsequent crystal formation due to cholesterol accumulation promoted ROS generation and activated the NLRP3 inflammasome, exacerbating SCI. Inhibition of macropinocytosis decreases the number of foamy cells and leads to a reduction in IL-1 β release after SCI. Thus, targeting macropinocytosis inhibitors may represent a novel therapeutic strategy for SCI.

We provide insights into the specific involvement of macropinocytosis in foamy macrophage formation during SCI. However, this study had some limitations. We acknowledge that the effect of EIPA in cells other than macrophages in the spinal cord tissues remains unknown and other types of endocytosis might affect foamy cell formation. We did not perform lineage tracing to investigate macropinocytosis in macrophages in murine SCI models. Whether EIPA has lasting effects on recovery after injury still remains unclear. In addition, we demonstrated that macropinocytosis might act as a new therapeutic target for spinal cord injuries despite the absence of clinical data to support this claim.

5. Conclusions

In brief, our study demonstrated that continual macropinocytosis promotes foamy macrophage formation and activates the NLRP3 inflammasome. Identifying the previously unrecognized role of macropinocytosis in the pathophysiology of SCI has enriched our understanding of the associations between myelin debris and foamy macrophages. Thus, targeting macropinocytosis might be a therapeutic approach in SCI management.

CRediT authorship contribution statement

Chenxi Zhang: Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shujie Zhao:** Writing – review & editing, Project administration, Funding acquisition. **Zhenfei Huang:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Ao Xue:** Methodology, Investigation,

Data curation. **Hao Liu:** Methodology, Investigation, Formal analysis. **Siming Dai:** Software, Methodology. **Ziyang Zheng:** Resources. **Yin Li:** Software, Resources. **Xiaodong Guo:** Validation, Supervision. **Jun Gu:** Data curation. **Feng Zhang:** Formal analysis. **Fubing Wang:** Software. **Yongxiang Wang:** Validation, Supervision. **Xiaohua Zhou:** Supervision. **Shujun Zhang:** Validation. **Hanwen Zhang:** Formal analysis. **Jun Shen:** Visualization, Validation. **Jian Chen:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Data curation, Conceptualization. **Guoyong Yin:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

The study received ethical approval from the Ethics Committee of Nanjing Medical University, and written informed consent was obtained from all participants.

Availability of data and materials

The RNA sequencing data have been submitted to the NCBI Sequence Read Archive (SRA) with Bioproject accession number PRJNA1078745 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1078745>). The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The author declares no conflicts of interest.

12. Acknowledgments

Not applicable.

Abbreviations

BMDM	bone marrow derived macrophage
BMS	Basso Mouse Scale
BSA	bovine serum albumin
CFSE	carboxyfluorescein succinimidyl ester
DEGs	differentially expressed genes
DHE	dihydroethidium
dMBP	degenerated myelin basic protein
EIPA	5-[N-ethyl-N-isopropyl] amiloride
FACS	fluorescence-activated cell sorting
FBS	fetal bovine serum
FC	flow cytometry

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GO	Gene Ontology
IF	immunofluorescence
IL-1 β	interleukin-1 β
KEGG	Kyoto Encyclopedia of Genes and Genomes
LDs	lipid droplets
LDL	low-density lipoprotein
LXRs	Liver X receptors
MAP2	microtubule-associated protein 2
M-CSF	macrophage colony-stimulating factor
MD	myelin debris
MEP	motor-evoked potential
MS	multiple sclerosis
MSR1	macrophage scavenger receptor 1
NF200	Neurofilament 200
NLRP3	NOD-like receptor protein 3
OCR	oxygen consumption rate
ORO	Oil Red O
PLL	polylysine
P/S	penicillin/streptomycin
PVDF	polyvinylidene difluoride
qPCR	Quantitative PCR
RNA-seq	RNA-sequencing
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute
SCI	spinal cord injury
SEM	standard error of mean
TEM	transmission electron microscopy
WB	Western blotting
WT	wild type

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2024.103469>.

Data availability

Data will be made available on request.

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