Research Square

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Phosphatidylserine liposomes attenuate Crohn's disease through an immunosuppressive mechanism targeting monocytes

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Abstract

Background

Crohn's disease (CD) is a chronic, lifelong inflammatory disorder characterized by recurrent episodes of acute flares and clinical remission. Despite advances in current therapies, many patients continue to experience recurrent inflammation, treatment resistance, and severe complications, highlighting the urgent need to better understand the underlying immunopathological mechanisms and to develop more potent therapeutic interventions.

Methods

Phosphatidylserine (PS), a key phospholipid essential for intestinal homeostasis, serves as a non-conventional upstream immune checkpoint that drives global immunosuppression. In this study, we investigated the relationship between PS levels and disease activity in CD patients. Using phosphatidylserine liposomes (PSL), spherical microscopic vesicles composed of PS phospholipids and designed as advanced biocompatible drug delivery systems, we examined whether PSL attenuates acute and chronic CD through immune regulation targeting monocytes.

Results

Circulating PS levels were significantly decreased in CD patients and showed a negative correlation with disease activity. Inflamed intestinal tissues surgically resected from CD patients exhibited robust infiltration of $CD68^+$ macrophages, along with diminished PS distribution. In TNBS-induced mouse models of acute or chronic colitis, PSL treatment markedly mitigated weight loss, reduced disease activity index (DAI) scores, attenuated colon shortening, and improved pathological histological outcomes. Mechanistically, PSL intervention decreased the abundance of pro-inflammatory Ly6C ($Ly6C^{high}$) monocytes while increasing the population of anti-inflammatory Ly6C patrolling ($Ly6C^{low}$) monocytes. Additionally, PSL treatment suppressed Th17 cell (IL-17α$^+$ CD4$^+$ T cell) expansion and promoted regulatory T cell (Foxp3$^+$ CD4$^+$ T cell) generation. *In vitro* experiments further verified that PSL-treated bone marrow-derived monocytes (BMDMs) presented a higher proportion of $Ly6C^{high}$ monocytes relative to $Ly6C^{low}$ subsets. T cells co-cultured with LPS-primed and PSL-treated monocytes displayed impaired proliferative ability and upregulated expression of immunosuppressive biomarkers. Moreover, PSL activated the TREM2/DAP12/p-STAT6 signaling axis in BMDMs, which is indispensable for PSL-mediated phenotypic switching of monocytes from the pro-inflammatory Ly6C phenotype to the immunosuppressive Ly6C phenotype.

Conclusion

PSL ameliorates TNBS-induced intestinal colitis by facilitating the phenotypic conversion of pro-inflammatory Ly6C monocytes into immunosuppressive Ly6C patrolling monocytes. These findings provide a novel molecular mechanism underlying the anti-inflammatory efficacy of PSL, offering a promising therapeutic candidate for CD treatment.

Introduction

Crohn's disease (CD) is a chronic, immune-mediated inflammatory bowel disease (IBD) that causes relapsing inflammation throughout the gastrointestinal tract [1]. While its prevalence remains highest in developed regions, particularly North America and Europe, the incidence of CD has been steadily increasing in developing countries worldwide [2]. A global epidemiological study reported approximately 6.8 million prevalent IBD cases in 2017, contributing to 0.83 million years of life lost and 1.02 million years lived with disability [3]. Effective clinical management of IBD, including CD and ulcerative colitis (UC), is essential to avert persistent intestinal inflammation, which otherwise leads to irreversible gastrointestinal tract damage and elevates the risk of colorectal cancer (CRC) [4]. Notably, CRC represents the most prevalent malignant neoplasm in CD patients [5]. CD pathogenesis is driven by aberrant and excessive immune activation, wherein dysregulated T cell responses primarily target the intestinal mucosa [6]. These clinical and pathological observations highlight an urgent demand to further elucidate the underlying immunopathological mechanisms of CD and develop more potent therapeutic interventions.

Monocytes serve as central regulators of inflammatory initiation and resolution. In humans, circulating monocytes are categorized into three functionally distinct subsets: classical $CD14^{++}CD16^{-}$, non-classical $CD14^{+}CD16^{++}$ and intermediate $CD14^{+}CD16^{+}$ monocytes [7]. Equivalent monocyte subpopulations have been well characterized in mice, with murine $CD11b^{+}Ly6C^{high}$, $CD11b^{+}Ly6C^{int}$ and $CD11b^{+}$ $Ly6C^{low}$ monocytes corresponding to human classical, intermediate, and non-classical monocytes, respectively [8; 9; 10; 11]. Functionally, murine $CD11b^{+}Ly6C^{high}$ monocytes are defined as pro-inflammatory monocytes, whereas $CD11b^{+}Ly6C^{low}$ monocytes are characterized as patrolling monocytes with anti-inflammatory and reparative properties. Accumulating evidence indicates that phenotypic conversion of $Ly6C^{high}$ pro-inflammatory monocytes into $Ly6C^{low}$ patrolling monocytes is a promising therapeutic strategy for mitigating chronic intestinal inflammation, including IBD [11].

Phosphatidylserine (PS), an essential membrane phospholipid highly enriched in neuronal cell membranes, has recently been recognized as a critical modulator of intestinal homeostasis, with established roles in preserving gut barrier integrity and regulating gut microbial composition. In peripheral blood, PS is predominantly localized to the inner leaflet of the plasma membrane in erythrocytes and platelets, participating in blood coagulation and mediating the clearance of senescent or damaged circulating cells [12]. Additionally, PS acts as a key signaling molecule that modulates monocyte activation, differentiation, and target cell recognition[13]. Under physiological conditions, PS is sequestered on the cytoplasmic side of the plasma membrane. During cellular apoptosis, PS is

externalized to the cell surface, serving as an “eat-me” signal for monocyte-mediated phagocytosis of apoptotic cells. This process actively induces immune tolerance and anti-inflammatory responses, thereby protecting against autoimmune tissue injury [14]. Phagocytosis of apoptotic cells further drives monocyte phenotypic switching from the pro-inflammatory Ly6C^{high} profile to the anti-inflammatory, reparative Ly6C^{low} phenotype [15].

Phosphatidylserine liposomes (PSL) are microscopic spherical vesicles enclosed by phospholipid bilayers that contain functional PS. PSL mimic apoptotic cell surfaces to modulate immune homeostasis, predominantly by inducing immune tolerance and suppressing inflammatory responses. Moreover, PSL can function as advanced targeted drug delivery systems: the inherent recognition of surface PS by monocytes and macrophages enables the precise delivery of therapeutic cargos to inflamed tissues enriched with these immune cells [16; 17; 18]. In the present study, we first analyzed the correlation of circulating PS levels with disease activity and evaluated CD68 expression in surgically resected inflamed intestinal tissues from CD patients. Using TNBS-induced murine models of acute and chronic colitis, we further investigated the regulatory effects of PSL on monocyte phenotypic polarization. In vitro experiments were also performed to explore the molecular mechanisms underlying PSL-mediated immunosuppression.

Materials and Methods

Human clinical sample collection

Adult patients diagnosed with CD were enrolled prospectively between November 2018 and September 2019. CD phenotypes were classified based on the Montreal Classification system [19]. Clinical remission was defined as a Crohn’s Disease Activity Index (CDAI) score of less than 150 [20]. Paraffin-embedded intestinal surgical specimens were obtained from nine patients with CD. Detailed inclusion and exclusion criteria, patient demographic characteristics, disease phenotypes, and sample collection procedures have been described in our previous study [21]. This study was approved by the Ethics Committee of the Sixth Affiliated Hospital of Sun Yat-sen University (SYSU, approval number: 2023ZSLYEC-211). Written informed consent was obtained from all participants prior to enrollment.

Animal Housing and Ethical Approval

BALB/c mice were housed under specific pathogen-free (SPF) conditions at the animal facility of the Sixth Affiliated Hospital of SYSU. All mice were provided with unrestricted access to standard laboratory chow and sterile tap water. Animals were maintained in a temperature-controlled (22 ± 1°C) and humidity-controlled (65–70%) environment under a 12-hour light/dark cycle. Male mice aged 2–3 months with a body weight of 20–25 g were used for all experiments. All animal procedures complied with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Research Ethical Committee of the Sixth Affiliated Hospital of SYSU [22].

Establishment of TNBS-Induced Acute and Chronic Colitis Models

All animal procedures were approved by the Research Ethical Committee of the Sixth Affiliated Hospital of SYSU and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. For TNBS enema administration and tissue harvest, mice were anesthetized with 3%–4% isoflurane for induction and maintained on 1.5% isoflurane with oxygen flow. At the experimental endpoint, mice were euthanized by continuous inhalation of 5% isoflurane for 10 min, and cervical dislocation was performed to verify complete death before colon and spleen tissue collection.

TNBS-induced murine model of acute colitis

To induce acute colitis, BALB/c mice were sensitized with 1% 2,4,6-trinitrobenzene sulfonic acid (TNBS) dissolved in ethanol 7 days prior to induction, followed by intrarectal administration of 100 μL of 2.5% TNBS to induce acute colitis, which mimics human CD [23].

TNBS-induced murine model of chronic colitis

For chronic colitis induction, mice received initial sensitization with 1% TNBS. Repeated intrarectal TNBS administration was performed on days 7, 14, and 21 using gradually increased concentrations of 0.75%, 1.5%, and 2.5%, respectively, to establish persistent chronic intestinal inflammation [24].

In Vitro Culture of Murine Bone Marrow-Derived Monocytes

Bone marrow (BM) cells were isolated from C57BL/6 mice and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% penicillin–streptomycin, and 10 ng/mL macrophage colony-stimulating factor (M-CSF). For inflammatory stimulation, cells were treated with 100 ng/mL lipopolysaccharide (LPS) with or without 50 μg/mL PSL. Fresh medium containing LPS and PSL was replenished every 48 hours. After 5 days of continuous culture, cells were harvested for subsequent phenotypic and functional analyses.

Preparation of Phosphatidylserine Liposomes (PSL)

Brain L-α-phosphatidylserine (PS) and bovine liver L-α-phosphatidylcholine (PC) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). In brief, 25 mg PS and 25 mg PC were fully dissolved in 5 mL chloroform–methanol mixture (90:10, v/v). Organic solvents were removed by rotary evaporation for 2 hours to form a uniform thin lipid film. The dried lipid film was rehydrated with phosphate-buffered saline (PBS) and sonicated at 100 W for 20 minutes. The resulting lipid suspension was sequentially extruded through polycarbonate membranes with pore sizes of 400 nm, 200 nm, and 80 nm to obtain

homogeneous PSL with an average particle size of approximately 100 nm. PC-only liposomes without PS were prepared using an identical protocol and served as blank controls [25].

Lipidomic Profiling of Clinical Plasma Samples

Blood samples were collected from overnight-fasted healthy controls (HCs) and CD patients. Plasma was isolated via centrifugation and stored at -80°C until use. Lipidomic profiling was performed using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS). Lipid species were annotated and quantified using LipidSearch software (Thermo Fisher Scientific, Waltham, MA, USA) [26].

PSL treatment and analysis in TNBS-induced murine models of acute and chronic colitis

PSL Intervention in Acute Colitis Model

Following TNBS-induced acute colitis establishment, mice were randomly divided into three groups: blank group (no TNBS or liposome treatment, n = 6), control liposome group (100 μL control liposome enema, n = 8), and PSL treatment group (10 mg/kg PSL enema, n = 8). Liposome administration was performed on days 1, 3, and 5 after TNBS induction. All mice were euthanized on day 13, and colon tissues were collected for subsequent experiments.

PSL Intervention in Chronic Colitis Model

After TNBS-mediated chronic colitis induction, mice were assigned to three groups: blank group (n = 6), control liposome group (100 μL control liposome enema, n = 10), and PSL treatment group (10 mg/kg PSL enema, n = 10). Interventions were administered on days 8, 10, 12, 15, 17, 19, and 22. All mice were sacrificed on day 24 for tissue collection and downstream analysis.

Body weight, stool consistency, and occult bleeding were monitored daily throughout the experimental period. The disease activity index (DAI) was calculated according to previously established criteria [26]. Body weight changes were calculated relative to the baseline weight measured on day 0.

Histological Analysis

Colon tissues were harvested immediately after euthanasia. The entire colon was rinsed with ice-cold PBS, spread flat, and measured for total length. Colon specimens were fixed in 4% paraformaldehyde and embedded in paraffin. Serial 4-μm tissue sections were prepared and stained with hematoxylin and eosin (H&E). Histological injury scores were evaluated based on the degree of inflammatory cell infiltration, crypt structural damage, epithelial erosion, and tissue edema, following previously reported scoring standards [27].

Quantitative Real-Time PCR

Total RNA was extracted from cultured monocytes, and reverse transcription was conducted using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative real-time PCR was subsequently performed to detect the mRNA expression levels of TNF-α, TGF-β, IL-1β, and iNOS in accordance with the manufacturer's protocols.

Western Blotting Analysis

Cell samples were lysed in 1× SDS sample buffer. All lysates were sonicated for 15 seconds and heated at 100°C for 5 minutes. After centrifugation at 12,000 rpm for 10 minutes at 4°C, protein concentrations were quantified using the DC Protein Assay (Bio-Rad Laboratories). Equal amounts of protein were separated via gradient SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were blocked with 5% skim milk in PBS and incubated overnight at 4°C with primary antibodies against TREM2, DAP12, p-STAT6, and GAPDH (Cell Signaling Technology, Danvers, MA, USA). After washing, membranes were incubated with HRP-conjugated secondary antibodies. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate (Thermo Fisher Scientific, Waltham, MA, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

The concentrations of TNF-α, MCP-1, and MIP-1α in cell culture supernatants were measured using commercial ELISA kits (R&D Systems, Minneapolis, MN, USA), strictly following the manufacturer's instructions.

Flow Cytometry Analysis

For immune phenotyping, single-cell suspensions were stained with fluorophore-conjugated antibodies targeting Ly6C, Ly6G, and CD11b for myeloid subset analysis, and CD3, CD4, CD8, PD-1, and Foxp3 for T-cell profiling. Cultured monocytes were stained with antibodies against Ly6C, Ly6G, CD11b, F4/80, and iNOS. All flow cytometry antibodies were purchased from BioLegend (San Diego, CA, USA). Stained samples were acquired using a FACSCanto II flow cytometer (BD Biosciences), and data analysis was performed using FlowJo software (Ashland, OR, USA).

T-cell Proliferation Assay

Mouse primary T cells were isolated from splenocytes using the EasySep™ Mouse T Cell Isolation Kit (STEMCELL Technologies). Purified T cells were labeled with carboxyfluorescein succinimidyl ester (CFSE, Invitrogen, Carlsbad, CA, USA) and stimulated with plate-bound anti-mouse CD3 antibody (5 μg/mL). CFSE-labeled T cells were co-cultured with pre-treated monocytes at a 1:1 ratio for 72 hours. The proliferation capacity and expression levels of Foxp3 and PD-1 in CD4 T cells were analyzed via flow cytometry.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, Boston, MA, USA). Two-group comparisons were analyzed by unpaired Student's t-test, while multiple-group comparisons

were evaluated by one-way analysis of variance (ANOVA). All data are presented as mean ± standard error (SE). A p value < 0.05 was considered statistically significant.

Results

Circulating PS Levels Are Negatively Correlated with Disease Activity in CD Patients

Patients with active CD exhibited significantly reduced circulating PS levels compared with healthy individuals, and PS levels were markedly restored after remission-inducing therapy (Fig. 1A). Lipidomic analysis further confirmed a significant negative correlation between plasma PS abundance and CD disease activity, as quantified by the CDAI (Fig. 1B).

Given the well-established anti-inflammatory properties of PS [28], and the central role of unrestrained inflammation in CD pathogenesis, we further analyzed the association of PS levels with clinical inflammatory markers. Circulating PS concentrations were negatively correlated with erythrocyte sedimentation rate (ESR) (Fig. 1C). Among all examined immune cell subsets, PS levels showed the strongest negative correlation with monocyte abundance (Fig. 1D). To further verify local PS distribution in intestinal lesions, immunofluorescence co-staining for PS and the monocyte/macrophage marker CD68 was performed on paired inflamed and non-inflamed intestinal tissues from nine CD patients. Inflamed intestinal regions displayed robust CD68 immune cell infiltration accompanied by diminished PS distribution, while non-inflamed regions presented minimal inflammatory infiltration and abundant PS expression (Fig. 1E).

PSL Exerts Therapeutic Effects in TNBS-Induced Acute and Chronic Colitis Models

Since TNBS-induced acute colitis recapitulates the pathological and clinical features of human acute CD [23], we established a murine acute colitis model to evaluate the therapeutic efficacy of PSL (Fig. 2A). Compared with the control PC liposome treatment group, PSL intervention significantly alleviated colitis-associated body weight loss (Fig. 2B), reduced DAI scores (Fig. 2C), and attenuated colon shortening (Fig. 2D). Moreover, histological examination demonstrated that PSL-treated mice had markedly lower intestinal tissue injury scores than control mice (Fig. 2E).

Chronic CD is a persistent, lifelong inflammatory condition that dominates clinical disease progression. As TNBS-induced chronic colitis closely mimics the pathological characteristics of human chronic CD [23; 24], we further constructed a murine chronic colitis model to explore the therapeutic potential of PSL (Fig. 3A). Consistent with the acute colitis results, PSL-treated mice exhibited improved body weight recovery and decreased DAI scores compared with control mice (Fig. 3B–C). Although no significant difference in colon length was observed between the two groups (Fig. 3D), PSL treatment effectively reduced intestinal histological damage (Fig. 3E). Collectively, these results demonstrate that PSL

treatment ameliorates both acute and chronic TNBS-induced colitis, supporting its therapeutic potential for CD intervention.

PSL Modulates Innate and Adaptive Immune Responses in Acute and Chronic Colitis

PSL is known to induce immune tolerance and mitigate inflammatory responses by suppressing dendritic cell maturation, reducing pro-inflammatory cytokine secretion, and upregulating anti-inflammatory mediators[29; 30]. To elucidate the immunomodulatory mechanisms underlying PSL-mediated colitis protection, we analyzed peripheral immune cell profiles in TNBS-induced colitis models. In acute colitis mice, PSL treatment significantly decreased the peripheral proportions of neutrophils and monocytes (Fig. 4A–C). Specifically, PSL inhibited the expansion of pro-inflammatory Ly6C monocytes ($Ly6C^{high}$) and promoted the accumulation of anti-inflammatory Ly6C monocytes ($Ly6C^{low}$) (Fig. 4D–G). In terms of adaptive immunity, PSL treatment increased the frequency of immunosuppressive $Foxp3^+$ CD4 regulatory T cells (Tregs) and reduced the proportion of pro-inflammatory IL-17α$^+$ CD4 Th17 cells (Fig. 4H–K).

To investigate the protective role of PSL in TNBS-induced acute and chronic colitis, we examined peripheral blood immune cells. The results showed that in the TNBS-induced murine model of acute colitis, PSL significantly reduced the proportions of peripheral blood neutrophils and monocytes (Fig. 4A-C), including reduced inflammatory $Ly6C^{high}$ monocytes and increased $Ly6C^{low}$ monocytes (Fig. 4D-G). Further studies showed increased proportions of regulatory T cells (Foxp3 + CD4+ T cells) and a reduced proportion of Th17 cells (IL-17α + CD4 + T cells) in PSL-treated mice compared with control mice (Fig. 4H-K).

In the chronic colitis model, neutrophil proportions were significantly elevated in the control group relative to healthy blank mice, yet PSL treatment did not notably alter neutrophil abundance (Fig. 5A–B). Nevertheless, PSL retained its regulatory effect on monocyte polarization, effectively reducing Ly6C pro-inflammatory monocytes and increasing Ly6C patrolling monocytes (Fig. 5C–F). Consistent with the acute colitis findings, PSL treatment upregulated Foxp3 Tregs and suppressed Th17 cell expansion in chronic colitis (Fig. 5G–I). Additionally, PSL intervention increased the proportion of PD-1-expressing T cells (Fig. 5J). These findings indicate that PSL alleviates intestinal inflammation by modulating monocyte phenotypic switching and balancing Treg/Th17 immune homeostasis in both acute and chronic colitis.

PSL Promotes Anti-Inflammatory Monocyte Polarization and Immunoregulatory T Cell Responses In Vitro

To validate the direct regulatory effect of PSL on monocytes, we performed in vitro experiments using murine bone marrow (BM)-derived monocytes. LPS stimulation alone induced a pro-inflammatory

phenotype, characterized by an increased proportion of Ly6C^{high} monocytes and a decreased proportion of Ly6C^{low} monocytes. In contrast, combined LPS and PSL treatment reversed this phenotypic shift, reducing Ly6C^{high} monocyte abundance and enriching Ly6C^{low} monocyte**s (**Fig. 6A–D**)**.

At the molecular level, LPS stimulation significantly upregulated the expression of pro-inflammatory markers (TNF-α, IL-1β, iNOS) and chemokines (MCP1, MIP1α) in BM-derived monocytes **(**Fig. 6E–I**)**. Combined LPS and PSL treatment markedly suppressed the expression and secretion of these pro-inflammatory factors, while increasing the expression of the anti-inflammatory cytokine TGF-β **(**Fig. 6J**).**

We further established a monocyte–T cell co-culture system to investigate whether PSL-educated monocytes regulate T cell function. T cells co-cultured with LPS-primed and PSL-treated monocytes exhibited impaired proliferative capacity and elevated expression of immunosuppressive markers (Foxp3 and PD-1) compared with T cells co-cultured with LPS-only treated monocytes **(**Fig. 6K, M–N**).**

Mechanistically, western blot analysis confirmed that combined LPS and PSL treatment activated the TREM2/DAP12/p-STAT6 signaling axis in BM-derived monocytes, whereas LPS stimulation alone failed to trigger this pathway **(**Fig. 6O**)**. These results identify the TREM2/DAP12/p-STAT6 pathway as a key mechanism mediating PSL-induced monocyte immunosuppressive polarization [31].

Discussion

Previous studies showed that, in patients with IBD, PS is characteristically externalized on the surface of colonic capillaries and elevated in microparticles. Annexin A5 (ANXA5), which has a high affinity for PS, exhibits strong colon-targeting ability and effectively alleviates experimental colitis by reducing inflammatory cell infiltration through inhibition of endothelial cell activation in a PS-binding-dependent manner [32]. In line with these previous findings, our clinical data revealed that circulating PS levels were negatively correlated with disease activity and intestinal CD68 myeloid infiltration in CD patients. Functionally, our *in vivo* colitis models demonstrated that PSL, which mimic apoptotic cell surfaces, effectively alleviate intestinal inflammation by driving the phenotypic conversion of pro-inflammatory Ly6C monocytes into immunosuppressive Ly6C patrolling monocytes. Collectively, these results uncover a novel immunomodulatory mechanism of PSL and provide a potential therapeutic target for CD treatment.

Murine monocytes can be classified into two functionally distinct subsets, Ly6C^{high} and Ly6C^{low}, which have opposing roles. Ly6C^{high} monocytes serve as early pro-inflammatory responders, rapidly infiltrating damaged tissues and amplifying local inflammation, whereas Ly6C^{low} monocytes primarily mediate immune surveillance, tissue repair, and inflammatory resolution [33; 34]. In the context of IBD, Ly6C monocytes migrate from the peripheral blood to inflamed colonic tissues in a CCR2-dependent manner, acting as pivotal pro-inflammatory effectors that exacerbate intestinal injury and disease progression[35]. Consistent with these reports, the present study verified that reprogramming monocyte

polarization from the Ly6C^{high} pro-inflammatory phenotype to the Ly6C^{low}, anti-inflammatory phenotype is sufficient to suppress intestinal inflammatory responses in CD-associated colitis.

Beyond modulating monocyte phenotypic switching, PSL exerts broad anti-inflammatory effects by reshaping the inflammatory cytokine profile. Prior studies have shown that PSL inhibits the secretion of pro-inflammatory mediators (TNF-α, IL-1β) while elevating the expression of anti-inflammatory cytokines such as TGF-β and IL-10 [36; 37]. Our *in vitro* experiments further validated these regulatory effects. In LPS-stimulated bone marrow-derived monocytes, combined PSL treatment significantly reduced the expression of pro-inflammatory markers (TNF-α, IL-1β, iNOS) and decreased the production of pro-inflammatory chemokines (MCP-1, MIP-1α), while enhancing TGF-β expression. These findings confirm that PSL effectively suppresses monocyte-driven inflammatory cascades, which is central to its protective effects against intestinal inflammation.

Ly6C^{high} inflammatory monocytes are critical for adaptive immune activation, as they promote T cell proliferation and effector differentiation via antigen cross-presentation and pro-inflammatory cytokine secretion in injured tissues[38]. In contrast, Ly6C^{low} patrolling monocytes exert immune-regulatory functions by inhibiting excessive T cell activation and facilitating inflammatory resolution through IL-10 secretion[34; 39]. In our co-culture system, T cells incubated with PSL-educated monocytes displayed impaired proliferative capacity and upregulated expression of immunosuppressive markers (Foxp3 and PD-1). These results indicate that PSL-mediated monocyte reprogramming inhibits Ly6C^{hi} monocyte-dependent adaptive immune hyperactivation, thereby restoring intestinal immune homeostasis.

Mechanistically, PSL mimics apoptotic cell surfaces and engages the TREM2/DAP12 signaling axis in myeloid cells. TREM2 is a key surface receptor that recognizes externalized PS on apoptotic cells and cellular debris. Upon PS-induced TREM2 activation, the intracellular adaptor protein DAP12 is phosphorylated, triggering downstream activation of SYK and PI3K signaling cascades. These signaling events ultimately induce STAT6 phosphorylation and activation[40; 41; 42]. The activated TREM2/DAP12/p-STAT6 pathway is well documented to drive macrophage and monocyte reprogramming toward a reparative phenotype, characterized by enhanced phagocytic activity, reduced pro-inflammatory cytokine release (TNF-α, IL-6), and increased secretion of anti-inflammatory mediators (IL-10, TGF-β)[42; 43]. In this study, we confirmed that PSL treatment specifically activates the TREM2/DAP12/p-STAT6 signaling axis in bone marrow-derived monocytes. This pathway is therefore indispensable for PSL-induced monocyte phenotypic switching from a pro-inflammatory Ly6C^{high} state to an immunosuppressive Ly6C^{low} state.

In conclusion, our study demonstrates that PSL ameliorates TNBS-induced colitis by triggering TREM2/DAP12/p-STAT6-dependent monocyte reprogramming, which shifts the immune landscape from pro-inflammatory Ly6C ^{high} monocyte dominance to immunosuppressive Ly6C ^{low} monocyte enrichment. This novel immunomodulatory mechanism highlights the therapeutic potential of PSL for the clinical treatment of CD.

Declarations

Data availability

The corresponding author will provide all study data and materials upon reasonable request.

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Conflict of interest:

The authors declare no conflicts of interest with the contents of this article.

References

1. Baumgart DC, Sandborn WJ. Crohn's disease. Lancet. 2012;380:1590–605.
2. Li N, Shi RH. Updated review on immune factors in pathogenesis of Crohn's disease. World J Gastroenterol. 2018;24:15–22.
3. G.B.D.I.B.D., Collaborators. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020;5:17–30.
4. Zhang Y, Chu X, Wang L, Yang H. Global patterns in the epidemiology, cancer risk, and surgical implications of inflammatory bowel disease. Gastroenterol Rep (Oxf). 2024;12:goae053.
5. Uchino M, Ikeuchi H, Hata K, Minagawa T, Horio Y, Kuwahara R, Nakamura S, Watanabe K, Saruta M, Fujii T, Kobayashi T, Sugimoto K, Hirai F, Esaki M, Hiraoka S, Matsuoka K, Shinzaki S, Matsuura M, Inoue N, Nakase H, Watanabe M. Intestinal cancer in patients with Crohn's disease: A systematic review and meta-analysis. J Gastroenterol Hepatol. 2021;36:329–36.
6. Marks DJ, Rahman FZ, Sewell GW, Segal AW. Crohn's disease: an immune deficiency state. Clin Rev Allergy Immunol. 2010;38:20–31.
7. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB. Nomenclature of monocytes and dendritic cells in blood. Blood. 2010;116:e74–80.
8. Yuan R, Geng S, Chen K, Diao N, Chu HW, Li L. Low-grade inflammatory polarization of monocytes impairs wound healing. J Pathol. 2016;238:571–83.

9. Geng S, Chen K, Yuan R, Peng L, Maitra U, Diao N, Chen C, Zhang Y, Hu Y, Qi CF, Pierce S, Ling W, Xiong H, Li L. The persistence of low-grade inflammatory monocytes contributes to aggravated atherosclerosis. Nat Commun. 2016;7:13436.
10. Narasimhan PB, Marcovecchio P, Hamers AAJ, Hedrick CC. Nonclassical Monocytes in Health and Disease. Annu Rev Immunol. 2019;37:439–56.
11. Lessard AJ, LeBel M, Egarnes B, Prefontaine P, Theriault P, Droit A, Brunet A, Rivest S, Gosselin J. Triggering of NOD2 Receptor Converts Inflammatory Ly6C(high) into Ly6C(low) Monocytes with Patrolling Properties. Cell Rep. 2017;20:1830–43.
12. Wesseling MC, Wagner-Britz L, Huppert H, Hanf B, Hertz L, Nguyen DB, Bernhardt I. Phosphatidylserine Exposure in Human Red Blood Cells Depending on Cell Age. Cell Physiol Biochem. 2016;38:1376–90.
13. Callahan MK, Halleck MS, Krahling S, Henderson AJ, Williamson P, Schlegel RA. Phosphatidylserine expression and phagocytosis of apoptotic thymocytes during differentiation of monocytic cells. J Leukoc Biol. 2003;74:846–56.
14. Furuta Y, Zhou Z. How do necrotic cells expose phosphatidylserine to attract their predators-What's unique and what's in common with apoptotic cells. Front Cell Dev Biol. 2023;11:1170551.
15. Kitamura T, Doughty-Shenton D, Cassetta L, Fragkogianni S, Brownlie D, Kato Y, Carragher N, Pollard JW. Monocytes Differentiate to Immune Suppressive Precursors of Metastasis-Associated Macrophages in Mouse Models of Metastatic Breast Cancer. Front Immunol 8 (2017) 2004.
16. Wu Z, Ma HM, Kukita T, Nakanishi Y, Nakanishi H. Phosphatidylserine-containing liposomes inhibit the differentiation of osteoclasts and trabecular bone loss. J Immunol. 2010;184:3191–201.
17. Zhang L, Habib AA, Zhao D. Phosphatidylserine-targeted liposome for enhanced glioma-selective imaging. Oncotarget. 2016;7:38693–706.
18. Garcia-Loza I, Perna-Barrull D, Aguilera E, Almenara-Fuentes L, Gomez-Munoz L, Greco D, Vila M, Salvado M, Mancera-Arteu M, Olszowy MW, Petriz J, Dalmases M, Rodriguez-Vidal S, Barneda-Zahonero B. Vives-Pi, Targeting macrophages with phosphatidylserine-rich liposomes as a potential antigen-specific immunotherapy for type 1 diabetes. J Autoimmun. 2024;145:103196.
19. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV Jr., Pena AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol 19 Suppl A (2005) A5–36.
20. Best WR, Becktel JM, Singleton JW, Kern F Jr.. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology. 1976;70:439–44.
21. Yang Q, Diao N, Ma F, Huang Z, Lin M, Liu X, Guo Q, Li P, Tang J, Gao X, Chao K. PI(4,5)P2 alleviates colitis by inhibiting intestinal epithelial cell pyroptosis through NNMT-mediated RBP4 m6A modification. Cell Death Dis. 2024;15:923.

22. Li P, Zhou D, Xie Y, Yuan Z, Huang M, Xu G, Huang J, Zhuang Z, Luo Y, Yu H, Wang X. Targeting G-quadruplex by TMPyP4 for inhibition of colorectal cancer through cell cycle arrest and boosting anti-tumor immunity. Cell Death Dis. 2024;15:816.
23. Antoniou E, Margonis GA, Angelou A, Pikouli A, Argiri P, Karavokyros I, Papalois A, Pikoulis E. The TNBS-induced colitis animal model: An overview. Ann Med Surg (Lond). 2016;11:9–15.
24. Silva I, Pinto R, Mateus V. Preclinical Study in Vivo for New Pharmacological Approaches in Inflammatory Bowel Disease: A Systematic Review of Chronic Model of TNBS-Induced Colitis. J Clin Med 8 (2019).
25. Huynh ML, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. J Clin Invest. 2002;109:41–50.
26. Yang Q, Huang Z, Diao N, Tang J, Zhu X, Guo Q, Chao K, Gao X. Lipidomics reveals significant alterations associated with exclusive enteral nutrition treatment in adult patients with active Crohn's disease. Ann Transl Med. 2022;10:1062.
27. Gong Z, Zhao S, Zhou J, Yan J, Wang L, Du X, Li H, Chen Y, Cai W, Wu J. Curcumin alleviates DSS-induced colitis via inhibiting NLRP3 inflammsome activation and IL-1beta production. Mol Immunol. 2018;104:11–9.
28. Yeom M, Hahm DH, Sur BJ, Han JJ, Lee HJ, Yang HI, Kim KS. Phosphatidylserine inhibits inflammatory responses in interleukin-1beta-stimulated fibroblast-like synoviocytes and alleviates carrageenan-induced arthritis in rat. Nutr Res. 2013;33:242–50.
29. Gaitonde P, Peng A, Straubinger RM, Bankert RB, Balu-Iyer SV. Phosphatidylserine reduces immune response against human recombinant Factor VIII in Hemophilia A mice by regulation of dendritic cell function. Clin Immunol. 2011;138:135–45.
30. Ramani K, Miclea RD, Purohit VS, Mager DE, Straubinger RM, Balu-Iyer SV. Phosphatidylserine containing liposomes reduce immunogenicity of recombinant human factor VIII (rFVIII) in a murine model of hemophilia A. J Pharm Sci. 2008;97:1386–98.
31. Che Y, Yu Z, Ji S, Yang D. Decoding TREM2 Signaling Pathways: Linking Macrophage Glycolysis to Inflammatory Diseases in the CNS. Neurol Neuroimmunol Neuroinflamm. 2026;13:e200527.
32. Zhang X, Song L, Li L, Zhu B, Huo L, Hu Z, Wang X, Wang J, Gao M, Zhang J, Hua Z. Phosphatidylserine externalized on the colonic capillaries as a novel pharmacological target for IBD therapy. Signal Transduct Target Ther. 2021;6:235.
33. Alkhani A, Levy CS, Tsui M, Rosenberg KA, Polovina K, Mattis AN, Mack M, Van Dyken S, Wang BM, Maher JJ, Nijagal A. Ly6c(Lo) non-classical monocytes promote resolution of rhesus rotavirus-mediated perinatal hepatic inflammation. Sci Rep. 2020;10:7165.
34. Yang P, Liu L, Sun L, Fang P, Snyder N, Saredy J, Ji Y, Shen W, Qin X, Wu Q, Yang X, Wang H. Immunological Feature and Transcriptional Signaling of Ly6C Monocyte Subsets From Transcriptome Analysis in Control and Hyperhomocysteinemic Mice. Front Immunol. 2021;12:632333.

35. Zigmond E, Varol C, Farache J, Elmaliah E, Satpathy AT, Friedlander G, Mack M, Shpigel N, Boneca IG, Murphy KM, Shakhar G, Halpern Z, Jung S. Ly6C hi monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. Immunity. 2012;37:1076–90.
36. Ramachandran P, Pellicoro A, Vernon MA, Boulter L, Aucott RL, Ali A, Hartland SN, Snowdon VK, Cappon A, Gordon-Walker TT, Williams MJ, Dunbar DR, Manning JR, van Rooijen N, Fallowfield JA, Forbes SJ, Iredale JP. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A. 2012;109:E3186–95.
37. Olimpieri T, Poerio N, Ponsecchi G, Di Lallo G, D'Andrea MM, Fraziano M. Phosphatidylserine liposomes induce a phagosome acidification-dependent and ROS-mediated intracellular killing of Mycobacterium abscessus in human macrophages. Front Cell Infect Microbiol. 2024;14:1443719.
38. Padgett LE, Araujo DJ, Hedrick CC, Olingy CE. Functional crosstalk between T cells and monocytes in cancer and atherosclerosis. J Leukoc Biol. 2020;108:297–308.
39. Bibi A, Yu Z, Cui L, Yang G. Harnessing monocyte dynamics for treatment of multiple sclerosis; insights from experimental model studies. Immunother Adv. 2025;5:ltaf003.
40. Konishi H, Kiyama H. Microglial TREM2/DAP12 Signaling: A Double-Edged Sword in Neural Diseases. Front Cell Neurosci. 2018;12:206.
41. Luoying W, Xingcheng Y, Donghang C, Mengtuan L, Ping W, Lijuan Z, Yupeng L, Tianyi Z, Hongyu C, Song W, Zijun Z, Hanyu Z, Shengchao C, Cong F, Xiaoyun S. Macrophage activation of the TREM2-DAP12-SYK pathway shapes the adipose tissue microenvironment in obesity and unveils the therapeutic potential of natural compounds egcg and SMRR. Front Immunol. 2025;16:1694985.
42. Kiguchi N, Kobayashi Y, Saika F, Sakaguchi H, Maeda T, Kishioka S. Peripheral interleukin-4 ameliorates inflammatory macrophage-dependent neuropathic pain. Pain. 2015;156:684–93.
43. Seon GM, Kim IG, Um SW, Chung EJ, Yang HC. Mitigation of radiation-induced esophageal fibrosis by macrophage-targeted phosphatidylserine-containing liposomes with partial PEGylation. Bioact Mater. 2025;52:574–87.

Figures

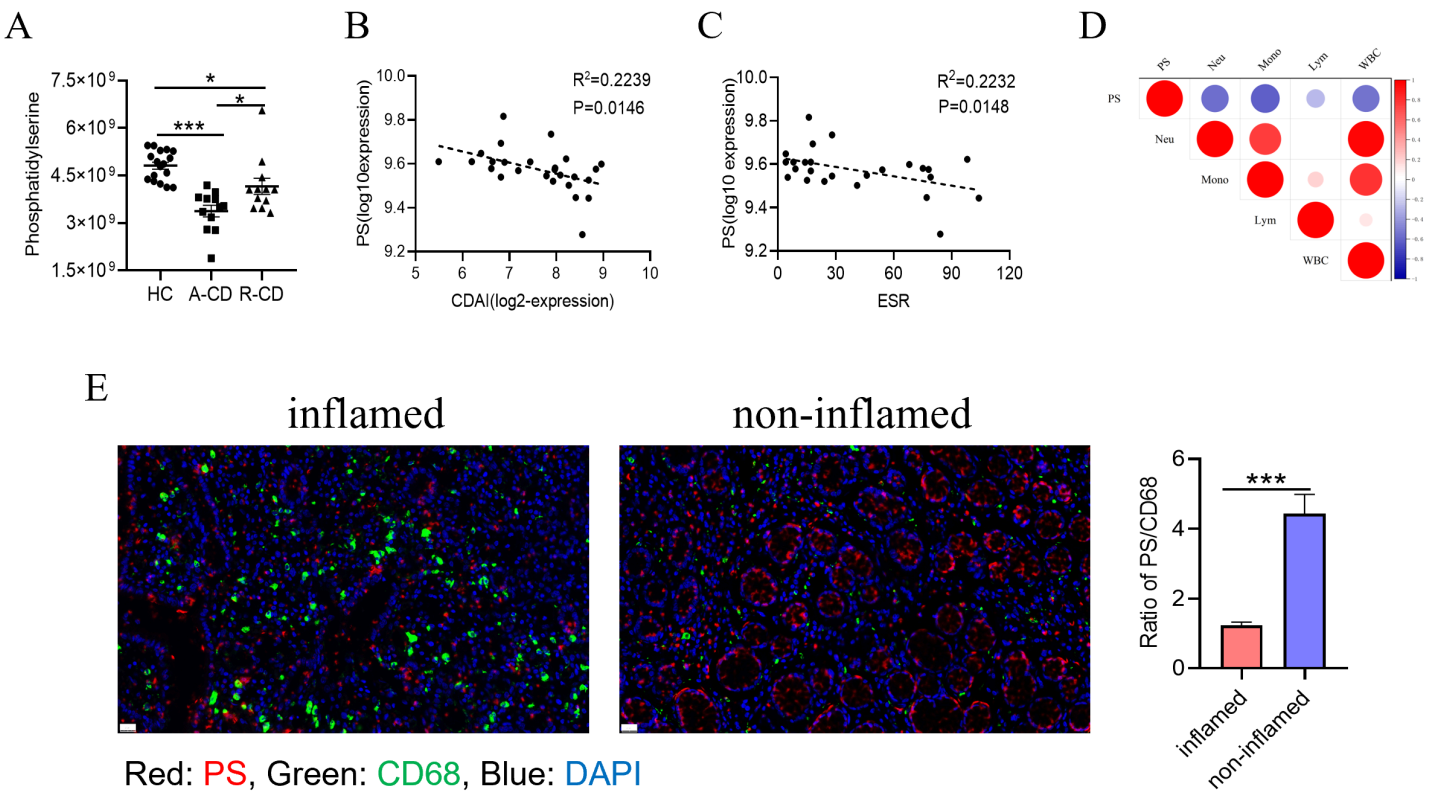


Figure 1

Phosphatidylserine (PS) negatively correlates with disease activity and intestinal inflammation in CD patients

A. Circulating PS levels were significantly decreased in patients with active CD (A-CD) compared with healthy controls (HC), and were markedly restored in patients after remission-inducing therapy (R-CD). Sample sizes: HC = 17, A-CD = 12, R-CD = 12. * $P < 0.05$.

B. Correlation analysis revealed a significant negative association between plasma PS levels and disease activity (CDAI scores) in CD patients.

C. Plasma PS concentrations were negatively correlated with erythrocyte sedimentation rate (ESR).

D. PS levels were negatively correlated with peripheral monocyte abundance.

E. Immunofluorescence staining showed increased CD68 myeloid infiltration and decreased PS distribution in inflamed intestinal tissues relative to non-inflamed regions. PS (red), CD68 (green). Scale bar = 25μm. Right panel: Quantitative analysis of the PS/CD68 fluorescence ratio. ****P <0.0001; n=3 per group.

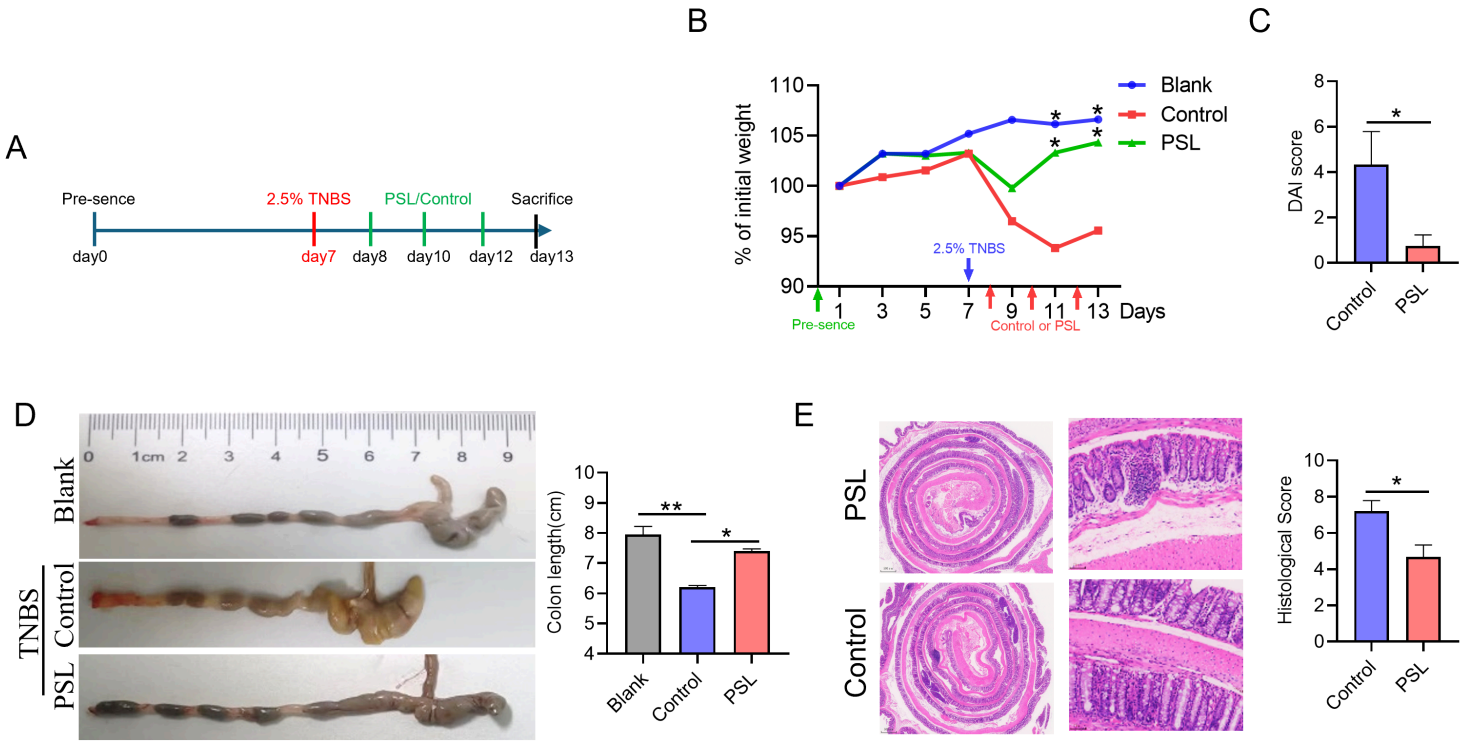


Figure 2

PSL exerts protective therapeutic effects in a TNBS-induced murine model of acute colitis.

A. Schematic illustration of the establishment of the TNBS-induced acute colitis mouse model.

B. PSL treatment significantly attenuated colitis-associated body weight loss compared with the control PC liposome group. n = 6–8 mice per group.

C. PSL treatment markedly reduced disease activity index (DAI) scores. *$P < 0.05$.

D. PSL intervention effectively alleviated colon shortening in colitic mice. Right panel: Quantitative analysis of colon length (cm). *$P < 0.05$, **$P < 0.01$.

E. Mice receiving PSL treatment exhibited significantly decreased histological injury scores. Right panel: Quantitative statistics of histological scores. *$P < 0.05$.

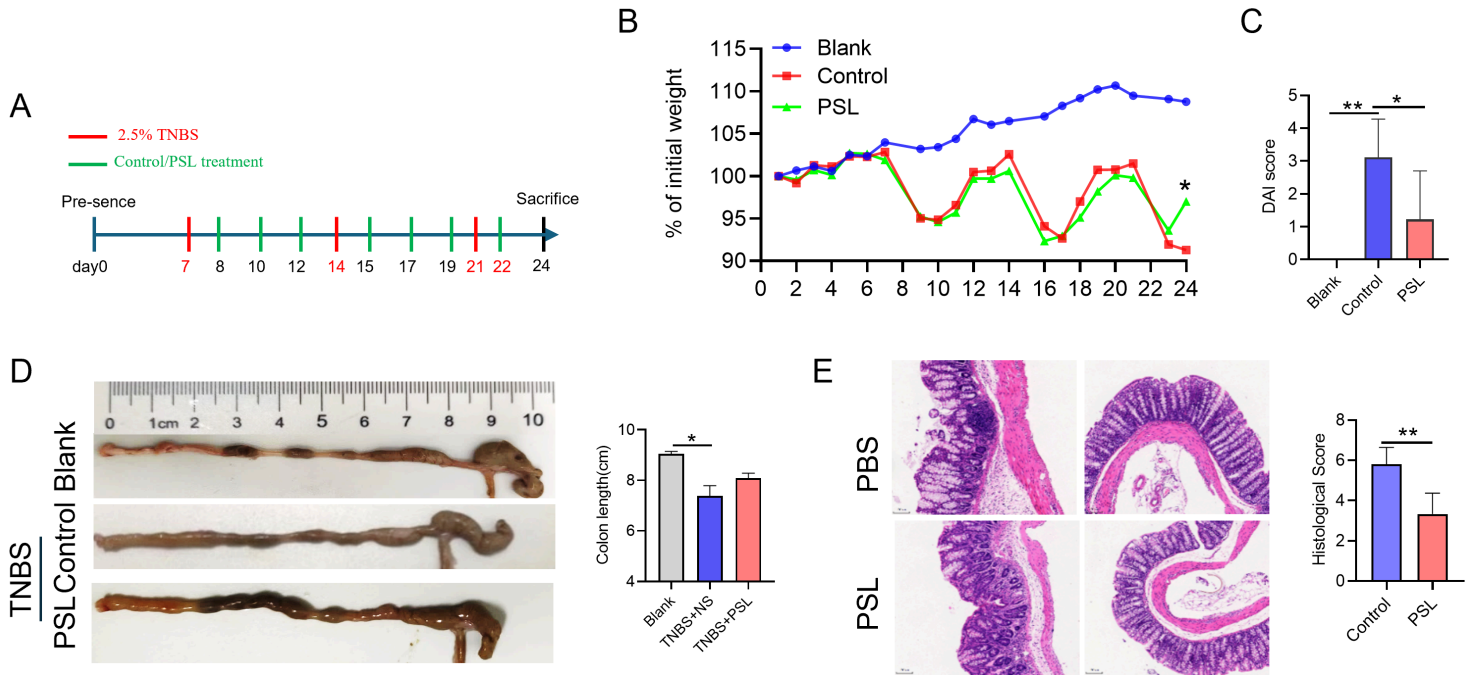


Figure 3

PSL ameliorates disease severity in a TNBS-induced murine model of chronic colitis.

A. Schematic workflow for the establishment of the TNBS-induced chronic colitis mouse model.

B. PSL treatment significantly mitigated body weight loss relative to the PC liposome control group. n = 10 mice per group.

C. PSL intervention markedly decreased disease activity index (DAI) scores. *$P < 0.05$, **$P < 0.01$.

D. PSL treatment alleviated colon shortening in chronic colitic mice. Right panel: Quantitative analysis of colon length (cm). *$P < 0.05$.

E. PSL-treated mice exhibited significantly reduced intestinal histological damage scores. Right panel: Quantitative statistics of histological scores. **$P < 0.01$.

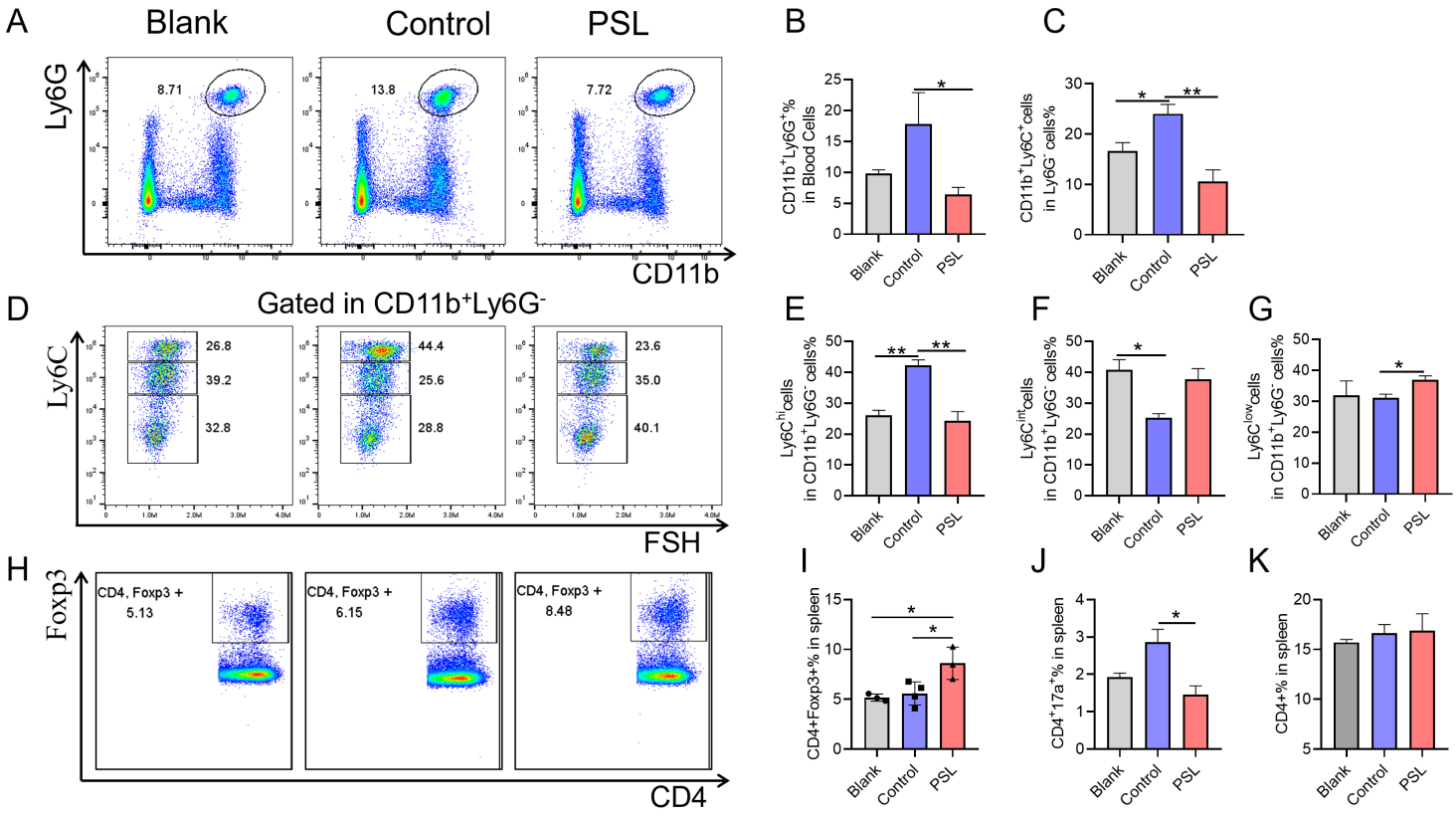


Figure 4

PSL modulates innate and adaptive immune responses in TNBS-induced acute colitis.

A. Representative flow cytometry plots showing the proportions of peripheral blood neutrophils ($CD11b^+Ly6G^+$) in each experimental group.

B. Quantitative analysis confirmed that PSL treatment significantly reduced the proportion of peripheral neutrophils. *$P < 0.05$.

C. PSL markedly decreased the abundance of peripheral monocytes ($CD11b^+Ly6C^+Ly6G^-$). **$P < 0.01$.

D. Representative flow cytometry profiles of monocyte subsets ($Ly6C^{high}$, $Ly6C^{int}$, $Ly6C^{low}$) in in mouse peripheral blood.

E. PSL treatment significantly reduced the proportion of pro-inflammatory Ly6C monocytes. **$P < 0.01$.

F. The proportion of $Ly6C^{int}$ monocytes remained unchanged following PSL intervention.

G. PSL significantly increased the proportion of immunosuppressive $Ly6C^{low}$ monocyte. *$P < 0.05$.

H. Representative flow cytometry plots of splenic Treg cells ($CD4^+Foxp3^+$).

I. PSL treatment significantly elevated the frequency of Treg cells ($CD4^+Foxp3^+$). *$P < 0.05$.

J. PSL markedly decreased the proportion of pro-inflammatory Th17 cells ($CD4^+IL\text{-}17\alpha^+$). *$P < 0.05$.

K. PSL exerted no significant effect on the overall proportion of CD4 T cells.

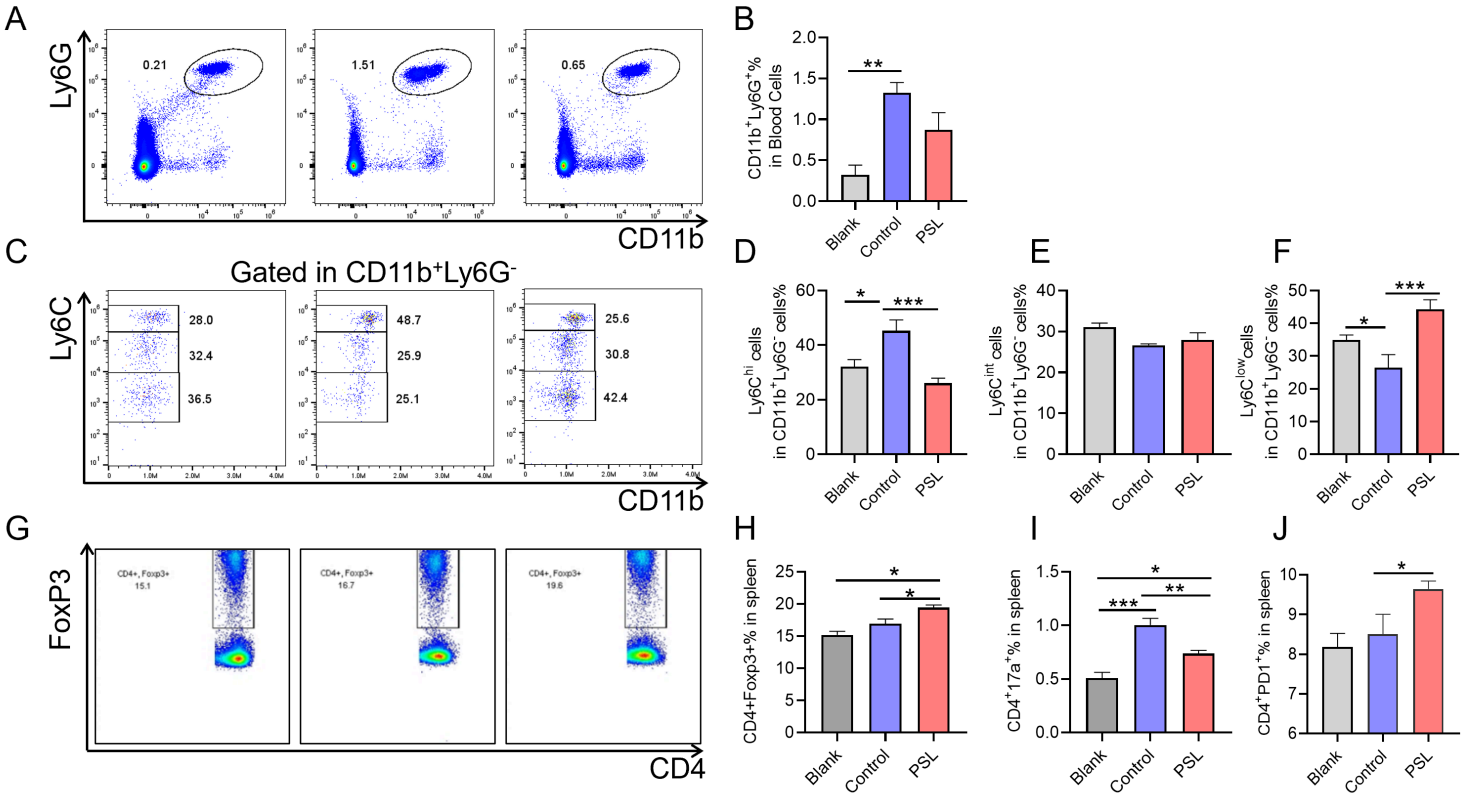


Figure 5

PSL modulates innate and adaptive immune homeostasis in TNBS-induced chronic colitis.

A. Representative flow cytometry plots illustrating the proportions of peripheral blood neutrophils ($CD11b^+Ly6G^+$) across experimental groups.

B. Quantitative analysis showed that PSL treatment significantly reduced peripheral neutrophil abundance. **$P < 0.01$.

C. Representative flow cytometry profiles of circulating monocyte subsets ($Ly6C^{high}$, $Ly6C^{int}$, $Ly6C^{low}$) in mice with chronic colitis.

D. PSL intervention markedly decreased the proportion of pro-inflammatory $Ly6C^{high}$ monocytes. ***$P < 0.001$.

E. PSL treatment had no significant effect on the frequency of $Ly6C^{int}$ monocytes.

F. PSL PSL significantly increased the proportion of immunosuppressive $Ly6C^{low}$ monocytes. ***$P < 0.001$.

G. Representative flow cytometry plots of splenic Treg cells ($CD4^+Foxp3^+$).

H. PSL treatment significantly elevated the abundance of Treg cells ($CD4^+Foxp3^+$).

I. PSL markedly reduced the proportion of pro-inflammatory Th17 cells ($CD4^+IL\text{-}17A^+$), $^{**}P < 0.01$.

J. The frequency of immunosuppressive $CD4^+PD\text{-}1^+$T cells was significantly upregulated following PSL treatment. $^{*}P < 0.05$.

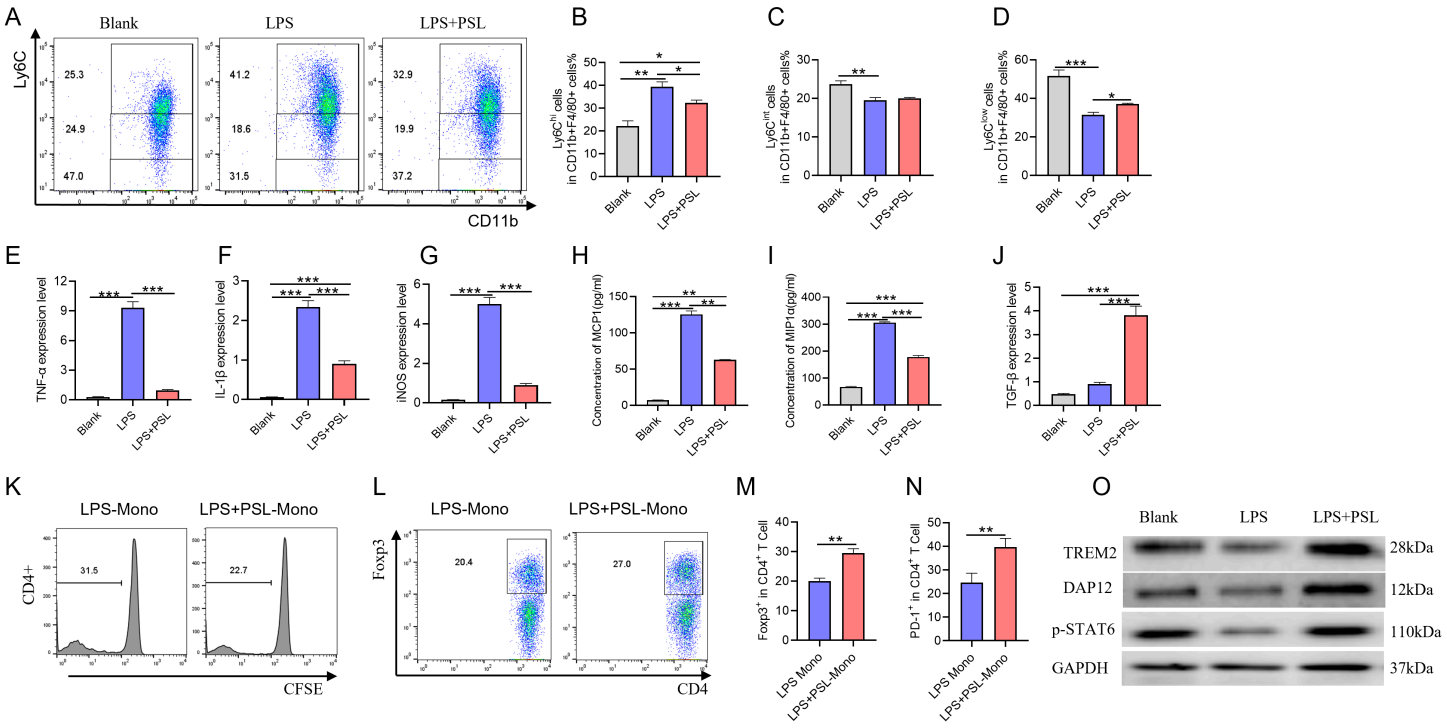


Figure 6

PSL promotes anti-inflammatory monocyte polarization and immunoregulatory T cell responses.

A. Representative flow cytometry plots showing in vitro monocyte subsets ($Ly6C^{high}$, $Ly6C^{int}$, $Ly6C^{low}$) with different treatments.

B. PSL treatment significantly reduced the proportion of pro-inflammatory $Ly6C^{high}$ monocytes. $^{*}P < 0.05$.

C. The proportion of $Ly6C^{int}$ monocytes remained unchanged after PSL intervention.

D. PSL significantly increased the proportion of immunosuppressive $Ly6C^{low}$ monocyte. $^{*}P < 0.05$.

E-G. Quantitative real-time PCR analysis of inflammatory gene expression in cultured monocytes. PSL markedly suppressed the mRNA levels ofTNF-α (E), IL-1β (F), and iNOS (G). $^{***}P < 0.001$ for all three markers.

H-I. ELISA measurement of chemokine concentrations in monocyte culture supernatants. PSL significantly reduced the secretion of MCP1 (H, $^{**}P < 0.01$) and MIP1α (I, $^{***}P < 0.001$).

J. PSL treatment significantly upregulated the expression of the anti-inflammatory cytokine TGF-β. $^{***}P < 0.001$.

K. Representative profiles of CFSE-labeled T cells co-cultured with LPS-primed or LPS plus PSL-primed monocytes.

L–N. Flow cytometry analysis of T cell function after co-culture. PSL-educated monocytes inhibited T cell proliferation (L) and significantly increased the expression of immunosuppressive Foxp3 (M, $^{**}P < 0.01$) and PD-1 (N, $^{**}P < 0.01$) in T cells.

O. PSL-induced activation of the TREM2/DAP12/p-STAT6 signaling pathway was detected by Western blotting.