Original Article

Thrombospondin-1 aggravates colonic mucosal inflammatory injuries via promoting the differentiation of CD11c$^+$ macrophages with lysosomal activity limited in colitis

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Background: Increased CD11c$^+$ Mφ aggravates colonic mucosal injuries in ulcerative colitis (UC) with TSP1 protein increased. The thrombospondin-1 (TSP1) protein which could activate Mφ is closely related to the colonic mucosal damage in UC. Here, we investigated the role of TSP1 in the differentiation of CD11c$^+$ Mφ and the mechanism.

Methods: We analyzed the population characteristics of *TSP1* genes using the Genotype-Tissue Expression (GTEx) database, and human serum TSP1 protein was detected with ELISA. DSS-induced colitis rats were used to explore the effects of TSP1 on colonic mucosal inflammation. We analyzed the serum cytokines and tissue histopathology to evaluate the severity of UC. Furthermore, we analysed the main source of TSP1 in colon tissue. In vitro, lamina propria mononuclear cells (LPMC) and CD11c$^+$ lamina propria macrophages (LPMP) was isolated from model rats *in vivo*. The target of TSP1 protein was assessed by LSKL, *CD36* and *CD47* interfering plasmids. The proteins, the lysosome, lysosomal activity and Cathepsin E activity, and the migration were detected by western blotting, test kits and Transwell.

Results: The expression of TSP1 was significantly higher in younger, male, and in the rectum and sigmoid than that in older, females, and colon tissues, and was closely related to the severity of UC. Compared with normal rats, the worse disease activity index (DAI) score, more histological damage, CD11c$^+$ Mφ infiltration, and increased expression of several proinflammatory cytokines was displayed in colitis rats with the elevation of serum TSP1 protein. *In vitro*, TSP1 protein derived from cmMφ and endothelial cells promoted the migration and the differentiation of CD11c$^+$ Mφ via binding on CD36, rather than the cell proliferation. Furthermore, PRKCQ/NF-κB signaling pathway was activated by CD36. However, the effect of TSP1 protein could be reversed by LSKL *in vivo*, and LSKL and anti-TSP1 antibody *in vitro*.

Conclusions: TSP1 promotes the migration and the differentiation of CD11c$^+$ LPMP with lysosomal activity limited via activating the CD36-PRKCQ/NF-κB signaling pathway, which aggravates the colonic mucosal inflammatory injuries in UC.

Keywords: Thrombospondin1 (TSP1); CD11c$^+$ macrophages; CD36; lysosomal activity

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Introduction

The mononuclear-phagocyte system (MPS), including macrophages (Mφ), monocytes, and naive monocytes scattered in various organs and tissues, is a cellular system with strong phagocytosis and defense functions in the body (1,2). Among them, colonic mucosal monocytes (cmMC) and colonic mucosal CD11c$^+$ macrophages (cmCD11c$^+$ Mφ), as 2 important cell types of MPS distributed in colon mucosa, play a central role in discriminating harmful from harmless antigens (3,4). They initiate and sustain protective immune responses mounted towards pathogenic organisms, but also ensure that local and systemic tolerance is generated in response to innocuous antigens (4,5). The homeostasis of cmMC and CD11c$^+$ Mφ is essential for maintaining colonic immune homeostasis which is also essential for protection against colonic mucosal inflammatory injuries (6). When the homeostasis is disrupted, it can lead to chronic inflammatory disorders in colon such as ulcerative colitis (UC) and Crohn's disease (CD) (6,7).

Large numbers of cmMC and CD11c$^+$ Mφ infiltrating the colonic mucosa are not only an important pathological characteristic, but also an important pathological factor that aggravates the colonic mucosal injuries in UC (4,8). However, reducing the infiltration of cmMC and CD11c$^+$ Mφ can effectively ameliorate colonic mucosal inflammatory injuries in UC, and then promote the repair of colonic mucosal damage (9). Although some results have suggested that the intestinal macrophage pool requires continual renewal from circulating blood monocytes (cirMC), unlike most other tissue Mφ, which appear to derive from primitive precursors that subsequently self-renew, cmMC and CD11c$^+$ Mφ infiltrated in the colonic mucosa have significantly different immune properties from cirMC (5,10). Therefore, understanding the immune characteristics and the effect of cmMC and CD11c$^+$ Mφ on colonic mucosal inflammatory injuries is important for the development of new therapies for the treatment of UC.

Large amounts of CD11c$^+$ Mφ infiltration in colonic mucosa can be differentiated from cmMC, and has been shown to promote colonic mucosal inflammation and inflammatory injuries in UC (8). However, the increase of Mφ infiltration is not completely proportional to the severity of colonic mucosal inflammation and mucosal injuries in UC. Even when the infiltration of Mφ is increased significantly, the inflammatory mediators in colonic mucosa do not increase exponentially like Mφ in colonic mucosa (11,12). Furthermore, the differentiation of Mφ plays a key role in regulating the biological function of Mφ, such as autophagy, phagocytosis, and synthesis and release of inflammatory cytokines, which is accompanied by changes in intracellular lysosomal activity. For example, the polarization of M1 phenotype Mφ induced by Mycobacterium tuberculosis (Mtb) has been shown to be related to the increased trafficking of Mtb to lysosomes (13,14). Besides, Mφ into M2 phenotype is attributable to the restoration of lysosomal function and autophagy activity (15). Furthermore, the transcriptional and post-transcriptional mechanisms that govern the balance between M1 and M2 polarization is also reliant on the activity of lysosome in Mφ (16,17). In addition, the lysosome and lysosomal activity in CD11c$^+$ Mφ is significantly inhibited compared with monocyte (18). Taken together, we speculated that the lysosome and lysosomal activity are important and critical for regulating polarization of CD11c$^+$ Mφ that aggravates colonic mucosal inflammation.

Lysosomal activity in macrophages could be regulated by some endogenous or exogenous stimulating factor, such as lipopolysaccharide (LPS), interleukin (IL), thrombospondin-1 (TSP1), and so on (19). Among them, the TSP1 protein plays a critical role in immunomodulation (20), but has a controversial effect on regulating MPS-mediated immunity in the colon. Some results in previous studies have suggested that TSP1 could reduce Mφ infiltration and the release of inflammatory cytokines, thereby limiting inflammation (21); however, some studies have found that increased TSP1 could aggravate colonic mucosal injuries though promoting the migration and activation of monocytes (22). Interestingly, our previous study found that the expression of TSP1 in colon was significantly increased in UC, which could increase the inflammation and vascular injuries in colonic mucosa (23). Conversely, colonic mucosal damage and micro-vascular injury could be impaired with the decreased expression of TSP1 in UC (24,25). Some other results have suggested that TSP1 protein could activate selective autophagy via promoting the autophagy-lysosome pathway

in Mφ (26). Therefore, we hypothesized that TSP1 protein could promote the differentiation of $CD11c^+$ Mφ in colonic mucosa in colitis, and the mechanism may be related to the regulation of lysosomal activity. The present study was designed to examine whether TSP1 had the effect on the differentiation of $CD11c^+$ Mφ and lysosomal activity, and if so, to investigate the potential mechanism involved. We present the following article in accordance with the ARRIVE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-6034).

Methods

Animals

Sprague-Dawley (SD) rats (male, 4–5 weeks old, 120–140 g) were purchased from Shanghai Laboratory animal Co. Ltd. (SLAC; Shanghai, China). All animals used in the present study were housed in a pathogen-free environment. Animal experiments were performed under a project license (No. SZY201612006) granted by the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine, in compliance with the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine's guidelines for the care and use of animals. A protocol was prepared before the study without registration. The study began after 1 week of adaptive feeding. Rats were divided into the following groups: Control group [treated with normal saline (NS), n=6], Leucine-serine-lysine-leucine (LSKL) group (treated with LSKL, n=6), Model group [dextran sulfate sodium (DSS)-induced colitis treated with NS, n=6], DSS + LSKL group (DSS-induced colitis treated with LSKL, n=6).

Reagents

We purchased DSS (D6924) was purchased from Sigma–Aldrich (St. Louis, MO, USA); recombinant rats thrombospondin-1 protein (LS-G12484), TSP1 enzyme-linked immunosorbent assay (ELISA) kit (LS-F37746) and lysosomal intracellular activity kit (LS-K491) were purchased from LifeSpan BioSciences, Inc. (Seattle, WA, USA); ELISA kits for human TSP1 (DTSP10), rat IL-6 (R6000B), IL-1β (RLB00), IL-4 (R4000), IL-10 (R1000), and tumor necrosis factor (TNF)-α (RTA00) proteins were purchased form R&D Systems (Minneapolis, MN, USA); LSKL (HY-P0299, an inhibitor of TSP1) was purchased from Med Chem Express (MCE, Princeton, NJ, USA). The lysosome staining kit was obtained from Abnova (KA4111, Taibei City, Taiwan). We purchased TSP1 (#37879) and β-actin (#4970) antibody from Cell Signaling Technology (Boston, MA, USA); anti-F4/80 (sc-71085) was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Cathepsin E Activity Assay Kit (ab211081), anti-CD36 (ab252922), CD11c (ab264107), PRKCQ (Protein Kinase C Theta, ab52494), phospho-PRKCQ [Thr-538 (ab203565), Ser-676 (ab131479) and Ser-695 (ab194745)], NF-κB (ab16502), and phospho-NF-κB (ab194726) antibodies, goat anti-rabbit antibodies, and rabbit anti-mouse antibodies were purchased from Abcam (Cambridge, UK). Rats with *CD47* gene silencing and over-expression plasmid (designed by targeting NM_019195 gene) and rats with *CD36* gene silencing and over-expression plasmid (designed by targeting NM_007643 gene) were constructed and synthesized by GK Gene (Shanghai, China); Lipofectamine 2000 Transfection Reagent (#11668019) was purchased from Thermo Fisher (Waltham, MA, USA); BD BioCoat™ BD Matrigel™ Invasion Chamber (40480) was purchased from BD Biosciences (Becton, Dickinson, and Co., Franklin Lakes, NJ, USA).

DSS-induced colitis and intervention in vivo

In this study, we used DSS-induced colitis rats. Rats were allowed free access to purified water containing 5% DSS (w/v) for 7 d. The DSS solution was prepared daily. For the intervention groups, peptide LSKL was dissolved in saline and injected intraperitoneally at 1.0 mg/kg body weight to normal rats and DSS-induced rats once daily for 7 consecutive days. Meanwhile, NS was administrated to rats in the control group by gavage. The initial administration times were on day 3 after establishing the colitis models; After that, the rats were anesthetized with 180 μL 3% sodium pentobarbital per 100 g, and, the colonic tissues and blood samples were collected.

Disease activity index (DAI), histological assays, ELISA, cell viability, cell proliferation, and Western blotting

During and after treatment, the severity of colitis was evaluated with DAI and histological assays. The serum concentrations of TSP1, IL-4, IL-6, IL-10, and TNF-α were detected using the appropriate ELISA kits; the expressions of related proteins were measured by WB; the cell viability and cell proliferation were detected with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT) and trypan blue. All assays were carried out as described previously (23).

Isolation of colonic mucosal cells, culture, and intervention

Lamina propria mononuclear cells (LPMC) and CD11c$^+$ lamina propria macrophages (CD11c$^+$ LPMP) were isolated and cultured as previously described (27,28). Colonic mucosal epithelial cells (cmECs) and colonic mucosal micro-vascular endothelial cells (cmVEC) were also isolated as described in previous studies (29,30). Then, LPMC characterized with F4/80, CD11c$^+$ LPMP characterized with F4/80 and CD11c, cmECs characterized with cytokeratin-18, and cmVEC characterized with CD31 were sequentially detected with flow cytometry, and sorted by double selection using magnetically activated cell sorting (MACS). Cells were cultured in complete growth medium [89% Dulbecco's Modified Eagle's Medium; DMEM, 30-2002, American Type Culture Collection (ATCC), Baltimore, MD, USA] +10% fetal bovine serum (FBS; 10099, Gibco, Waltham, MA, USA) +1% Penicillin-Streptomycin Solution (15070063, Gibco, Waltham, MA, USA) at 37 ℃ in a humidified atmosphere of 95% O_2 and 5% CO_2.

The LPMC was divided into 6 groups (n=3) with different concentrations of LSKL (0, 0.5, 5, 25, 50, and 100 μM) and cultured for 24 and 48 h, respectively.

Plasmid construction and cell transfection

To clarify the mechanism that TSP1 is involved in regulating the polarization of Mφ, both the over-expression and silencing plasmids of *CD47* and *CD36* gene were used. Empty plasmids were used as negative control (E-p). The cells were seeded into 24-well plates (Corning, Shanghai, China). According to the manufacturer's instructions, transfection was performed with Lipofectamine 2000 Transfection Reagent when the cells reached 50% to 80% confluence. Culture medium was changed after 4–6 h, and the cells continued to be incubated at 37 ℃ in a CO_2 incubator for 24 h. Then, gene expression was detected with WB. The cells were divided into following groups: control group (untransfected), E-p group (transfected with empty plasmid), $CD36^{over}$ group (transfected with *CD36* over-expression plasmid), $CD36^-$ group (transfected with *CD36* silencing plasmids), $CD47^{over}$ group (transfected with *CD47* over-expression plasmid), and $CD47^-$ group (transfected with *CD47* silencing plasmids).

Motility and Matrigel invasion assays

The motility and migration ability were evaluated with motility and Matrigel invasion assays as described previously (31,32).

The LPMC (1.0×10^5) were seeded into 8 mm pore size 24-well inserts (BD Biosciences, USA) in TSP1-free medium, and the inserts were placed into growth medium containing 100 pg/mL TSP1, 300 pg/mL IL-1β or/and 50 μM LSKL for 12 h before fixation in 4% paraformaldehyde. The number of migrated cells was counted and normalized to the number of cells loaded. Assays were performed in triplicate in at least 3 independent experiments, and the results were normalized to the control cells.

For Matrigel invasion assays, LPMC were seeded at 1×10^5 cells/insert on Matrigel invasion chambers in 24-well culture dishes in a TSP1, IL-1β or/and LSKL gradient with no TSP1, IL-1β or/and LSKL in the chamber insert, and cultured for 24 h before fixation in 4% para-formaldehyde. The membranes were counted as described above to calculate the number of cells that had invaded through the Matrigel.

Lysosome staining

The LPMC (100 μL/well, 1.0×10^4/mL) were cultured in 96-well plates for 12 h respectively. When cells were properly fused, they were divided into different groups and treated for 24 h. After that, 100 μL of Lyso Green working solution (20 μL of 500× Lyso Green stock solution in 10 mL of live cell staining buffer) were added according to the instructions of the lysosomal staining kit. The cells were incubated at 37 ℃ in an atmosphere containing 5% CO_2 for 1 h. Finally, the cells were visualized under a fluorescence microscope with a fluorescein isothiocyanate (FITC) filter set (excitation and emission at 490 and 525 nm).

Lysosome activity and Cathepsin E (CTSE) activity

CTSE activity were assayed according to the manufacturer's instructions. Briefly, LPMC were solubilized in 25 μL of 0.1% Triton X-100. Next, the lysates were incubated with 150 μL of 10 mM p-nitrophenyl phosphate for 1 h at 37 ℃. The reaction was stopped by adding 50 μL of 0.2 M borate buffer, and the absorbance of the mixture at 405 nm was determined using a spectrophotometer. Then lysosome enzyme activity (%) was calculated as the ratio of the absorbance at 405 nm to that of control cells multiplied by 100%.

For CTSE activity, LPMC (1.0×10^6) were cultured in 6-well plates and treated for 24 h respectively. After that, cells were lysed with 100 µL CTSE lysis buffer on ice for 5 min. The lysate was collected and a sample was centrifuged at 4 ℃ at 16,000 ×g for 10 min using a cold microcentrifuge. The supernatant was then collected, the amount of protein in the lysate was measured using a bicinchoninic acid (BCA) protein assay kit, and 50 µL of reaction mix was added into each sample and positive control well. The reaction mix was not added to standard wells or background control wells. Finally, CTSE activity was quantified using a fluorimeter or fluorescence microplate reader at Ex/Em =320/420 nm.

Immunofluorescence (IF)

Colonic tissue or treated cells were fixed with 4% (w/v) paraformaldehyde, blocked, and incubated with anti-CD36 (1:100) or anti-CD11c (1:100) + anti-F4/80 (1:100) antibodies overnight at 4 ℃. Then, cells were washed in phosphate-buffered saline (PBS). After incubation with a secondary fluorescein-conjugated antibody and 4',6-diamidino-2-phenylindole (DAPI), the cells were washed with PBS again, mounted in anti-fade reagent, and observed under an Olympus (Tokyo, Japan) microscope as described previously (33).

Statistical analysis

Data were presented as the mean ± SD. One-way analysis of variance (ANOVA) or general linear model with repeated measures was used to analyze the data sets with 3 or more groups and least significant difference post hoc test for multiple comparisons. Student's *t*-test was used to analyze data sets with two groups. A P value <0.05 was considered significant. Only the corresponding author who performed the data statistics was aware of the group allocation at the different stages of the experiment.

Results

The expression characteristics of TSP1 were found in different colorectal tissues, ages, and genders

Clinically, age and gender are considered as 2 independent factors which are closely related with the incidence of UC (34-37). Here, in order to bridge the gaps between gene expression and disease, we analyzed the data on the expression of the *TSP1* gene in human colorectal tissues from the Genotype Tissue Expression (GTEx) database (https://www.gtexportal.org/home/datasets) (38) which contains data from 507 people. Our results revealed that the expression level of the *TSP1* gene was significantly higher in the younger than that in the elder people (*Figure 1A*). In addition, the expression level of the *TSP1* gene in males was significantly higher than that in females (*Figure 1B*), and significantly higher in rectum and sigmoid tissue than that in colon tissue (*Figure 1C*).

Furthermore, the colonic mucosa and serum of 24 patients diagnosed with UC, including 12 patients with inactive UC and 12 patients with active (mild-moderate) UC, and 12 age- and gender-matched healthy controls were collected at the Second Affiliated Hospital of Guangzhou University of Chinese Medicine according to the Second European Evidence-based Consensus on Diagnosis and Management of UC (2015). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (No. B2017-068) and informed consent was taken from all the patients. The demographic and clinical data are documented in *Table 1*. As shown in *Figure 1D-1H* and *Table 1*, *TSP1* expression was significantly elevated in colonic tissues and serum from patients with active UC than that from patients diagnosed with inactive UC. Moreover, the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) and Mayo score showed that exaggerated colonic mucosal inflammation and mucosal injuries were associated with increased infiltration of *TSP1*. No difference of TSP1 protein was observed between patients diagnosed with inactive UC and healthy controls [non-irritable bowel disease (IBD) patients].

Increased TSP1 protein aggravated colonic mucosal inflammatory injuries in colitis

To illuminate the role of *TSP1* in the injured mucosa during colitis, rats were challenged with 5% DSS. The DSS-induced model rats showed more severe colitis as measured by histological analysis (*Figure 2A*), cytokines production in the serum (*Figure 2B*), and DAI (*Figure 2C*). Meanwhile, serum TSP1 was markedly increased in model rats. When treated with LSKL (1 mg/kg), a competitive inhibitor of *TSP1*, the serum TSP1 was decreased, together with the DAI score, and colonic mucosal injuries were significantly attenuated (*Figure 2A-2D*). Taken together, *TSP1* could play

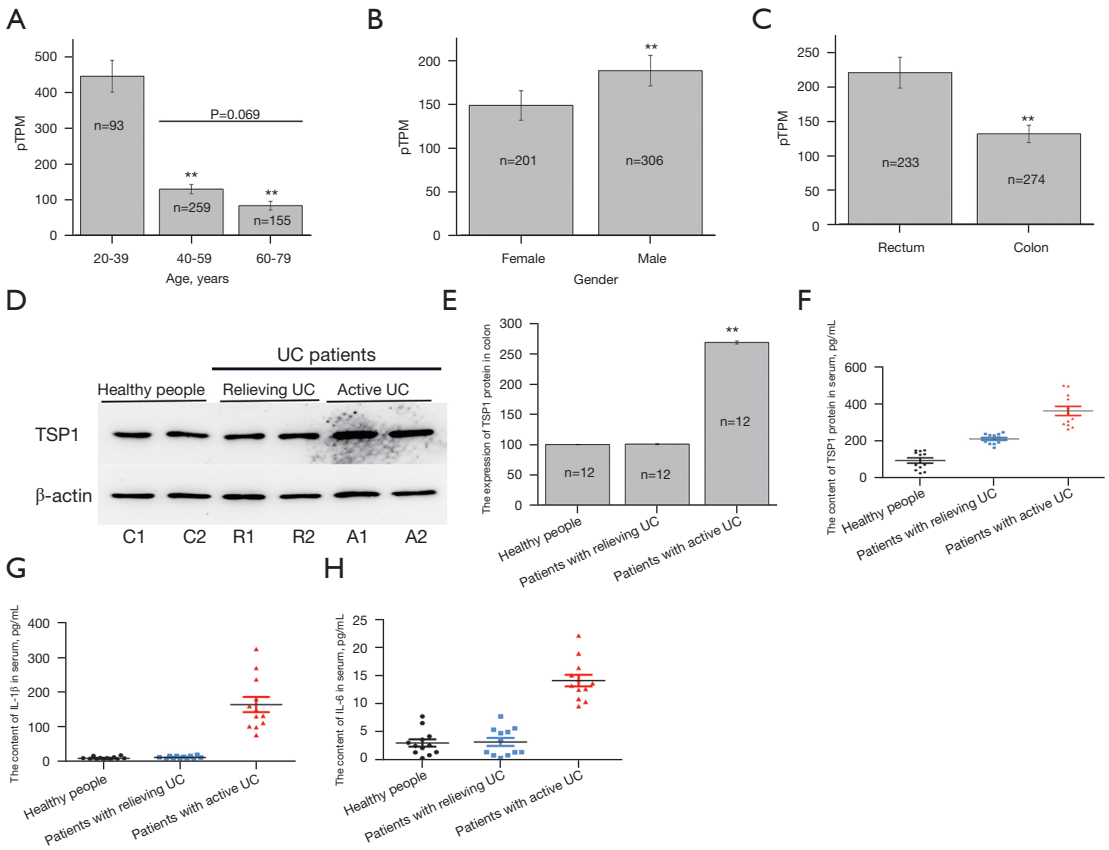


Figure 1 The expression of TSP1 in different colorectal tissues, ages, and genders. (A-C) The relationship between the expression of *TSP1* gene and ages, genders, and different colorectal tissues. (C-E) The serum TSP1 protein and the expression of TSP1 protein in patients with UC. (F-H) The increasing TSP1 protein was accompanied by increased colonic mucosal inflammation and mucosal and mucosal injuries (UCEIS and Mayo score) in patients with UC. (**, P<0.001). UC, ulcerative colitis; UCEIS, ulcerative colitis endoscopic severity index.

Table 1 The demographic and clinical data of UC patients

Characteristics	Healthy people	Patients with inactive UC	Patients with active UC	P value
Age (mean ± SD) (minimum/maximum)	45.23±3.11 (27/63)	42.83±3.76 (26/61)	38.33±3.04 (23/52)	0.339
Gender (female/male)	8/4	6/6	5/7	0.728
UCEIS score (mean ± SD)	–	4.92±0.19	7.83±0.72	<0.001
Mayo score (mean ± SD)	–	1.42±0.14	5.75±0.35	<0.001
Lesion				
Rectum	–	12	12	
Sigmoid colon	–	8	4	
Left colon	–	0	2	

UC, ulcerative colitis; UCEIS, The Ulcerative Colitis Endoscopic Index of Severity. "–": no obvious pathological changes were found.

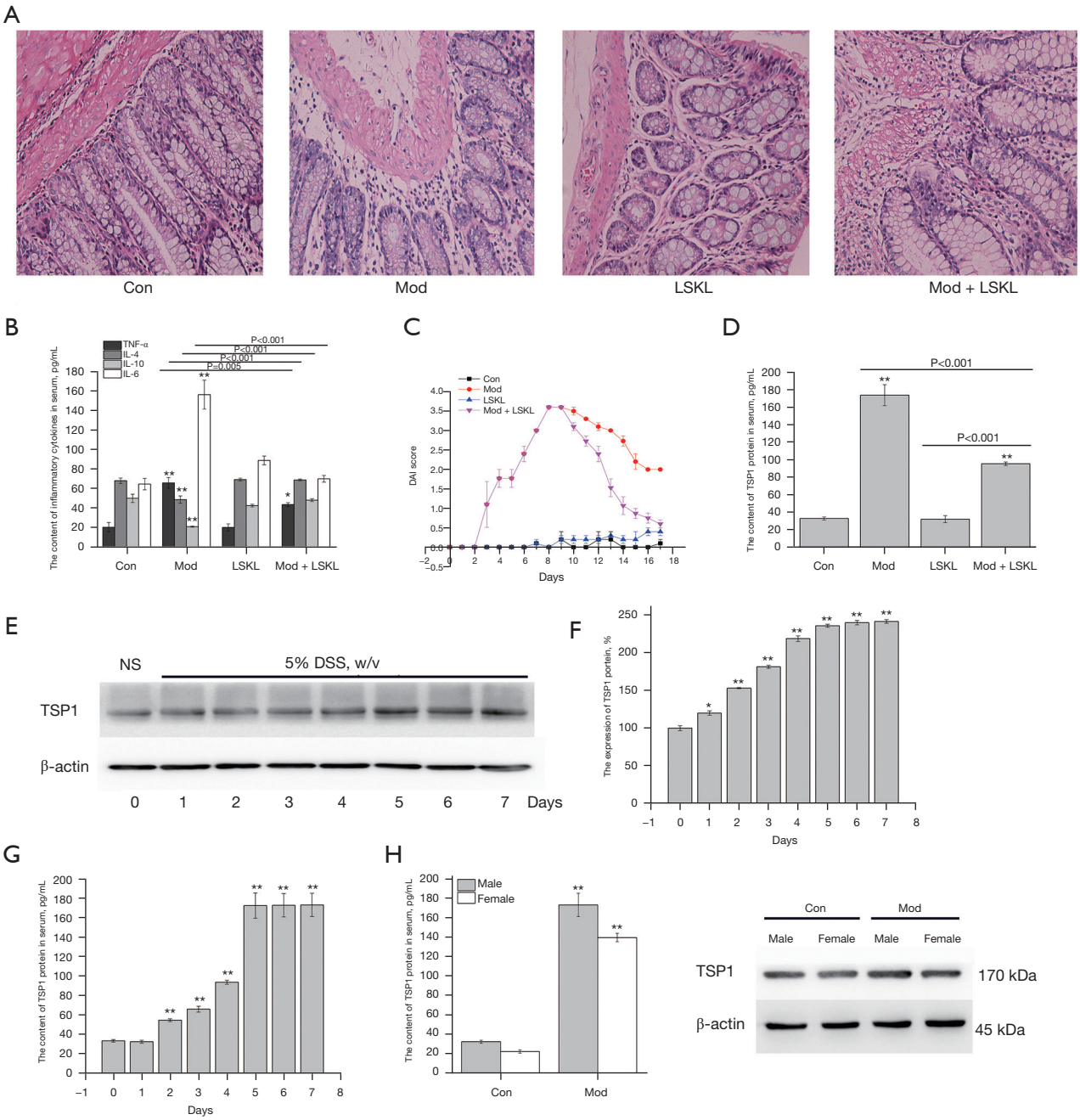


Figure 2 TSP1 protein was significantly increased in colonic mucosa in colitis. (A-C) The DAI score, colonic inflammation, and colonic mucosal injuries (n=6, HE, 400×). (D) Serum TSP1 protein in rats. (E-G) The TSP1 protein was also significantly increasing with the worsening of DSS-induced experimental colitis. (H) TSP1 protein was significantly higher in female rats than in males. (*, P<0.05, **, P<0.001). Con, Control group; Mod, Model group; LSKL, an inhibitor of Thrombospondin (TSP-1); DAI, disease activity index; NS, normal saline; DSS, Dextran Sulfate Sodium Salt.

an important role in colonic mucosal injuries during colitis.

Next, we focused on the expressions of *TSP1* in colonic tissues in the development of colitis. Through WB, the expression of TSP1 protein was shown to be significantly increased at day 1, and levels of TSP1 protein peaked stabilized at day 5 (*Figure 2E,2F*). Meanwhile, serum TSP1 was increased at day 2, and stabilized at day 5 (*Figure 2G*). Interestingly, the expression of *TSP1* was higher in male rats than in female rats (*Figure 2H*), which was consistent with the clinical conditions in clinic. Therefore, male rats were selected for the following experiments.

Increased lysosomal activity limited CD11c$^+$ Mφ infiltrating in the colonic mucosa promoted the inflammation

It is well known that the infiltrated macrophages in colonic mucosa play central role in mucosal inflammation. In humans, the infiltration of cmMC and CD11c$^+$ Mφ in colonic mucosa is aggravated significantly in patients with active UC (Figure S1A, *Figure 3A*). Similarly, the infiltration of cmMC and CD11c$^+$ Mφ in colonic mucosa was also greatly aggravated in the model group compared with that in control group, which was positively correlated with the content of serum TSP1 in colitis rats (*Figure 3B,3C*).

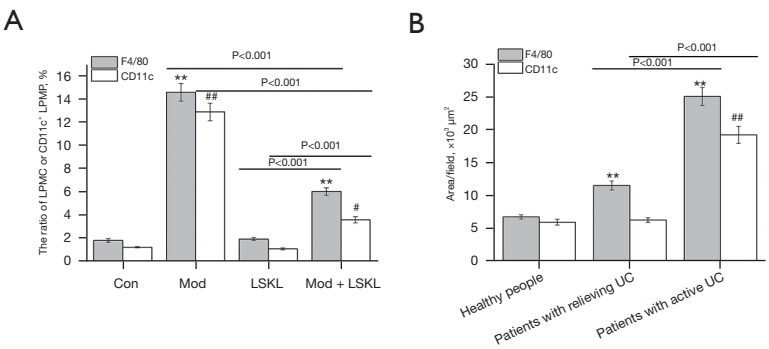
As the lysosome and lysosomal activity are important internal regulatory factors which affect the function of Mφ, LPMC and CD11c$^+$ LPMP were isolated from colonic tissues, the quantity of lysosome was measured, as well as the lysosome activity and CTSE activity of Mφ. The results showed that lysosome, lysosomal activity, and CTSE activity of CD11c$^+$ LPMP in colitis rats were significantly decreased compared with LPMC and CD11c$^+$ LPMP isolated from the control group (Figure S1B, *Figure 3D-3F*). However, the expressions of IL-6 and IL-1β were significantly increased in the serum of colitis rats (*Figure 3G,3H*).

On the contrary, the number of lysosome, lysosome activity, and CTSE activity were significantly increased in LSKL group compared with control group. In addition, the decreased lysosome, lysosome activity, and CTSE activity were reversed in CD11c$^+$ LPMP isolated from colonic mucosa of rats in the DSS + LSKL group. Meanwhile, both the infiltration of cmMC and CD11c$^+$ Mφ in colonic mucosa and serum IL-6 and IL-1β were significantly decreased in the DSS + LSKL group compared with those in Model group (*Figure 3A-3H*).

TSP1 promoted the differentiation and migration of cmMC and CD11c$^+$ Mφ

Although CD11c$^+$ Mφ in colonic mucosa was significantly increased in colitis rats, the cell proliferation of CD11c$^+$ LPMP was greatly decreased compared with that of LPMC for 24 and 48 h *in vitro* (Figure S2A). The cell proliferation of both LPMC and CD11c$^+$ LPMP could be significantly inhibited at 24 h by mucosal lysis supernatant (MLS) from colitis rats [MLS (Mod)], but not from normal rats [MLS (Con)] (Figure S2B). Meanwhile, the differentiation of CD11c$^+$ LPMP from LPMC treated with MLS(Mod) was significantly increased compared with that treated with MLS(Con) (*Figure 4A-4C*).

The content of TSP1 protein in MLS was significantly higher than that in MLS(Con) (*Figure 4D*). With TSP1 protein neutralized by the anti-TSP1 antibody, the cell proliferation of LPMC, not CD11c$^+$ LPMP was partially restored (Figure S2B), and the differentiation of CD11c$^+$ LPMP from LPMC treated with MLS(Mod) was significantly decreased (*Figure 4B-4D*). *In vitro*, TSP1 protein significantly induced the differentiation of LPMC to CD11c$^+$ LPMP and inhibited the cell proliferation of LPMC and CD11c$^+$ LPMP at 24 h (*Figure 4C-4E*).



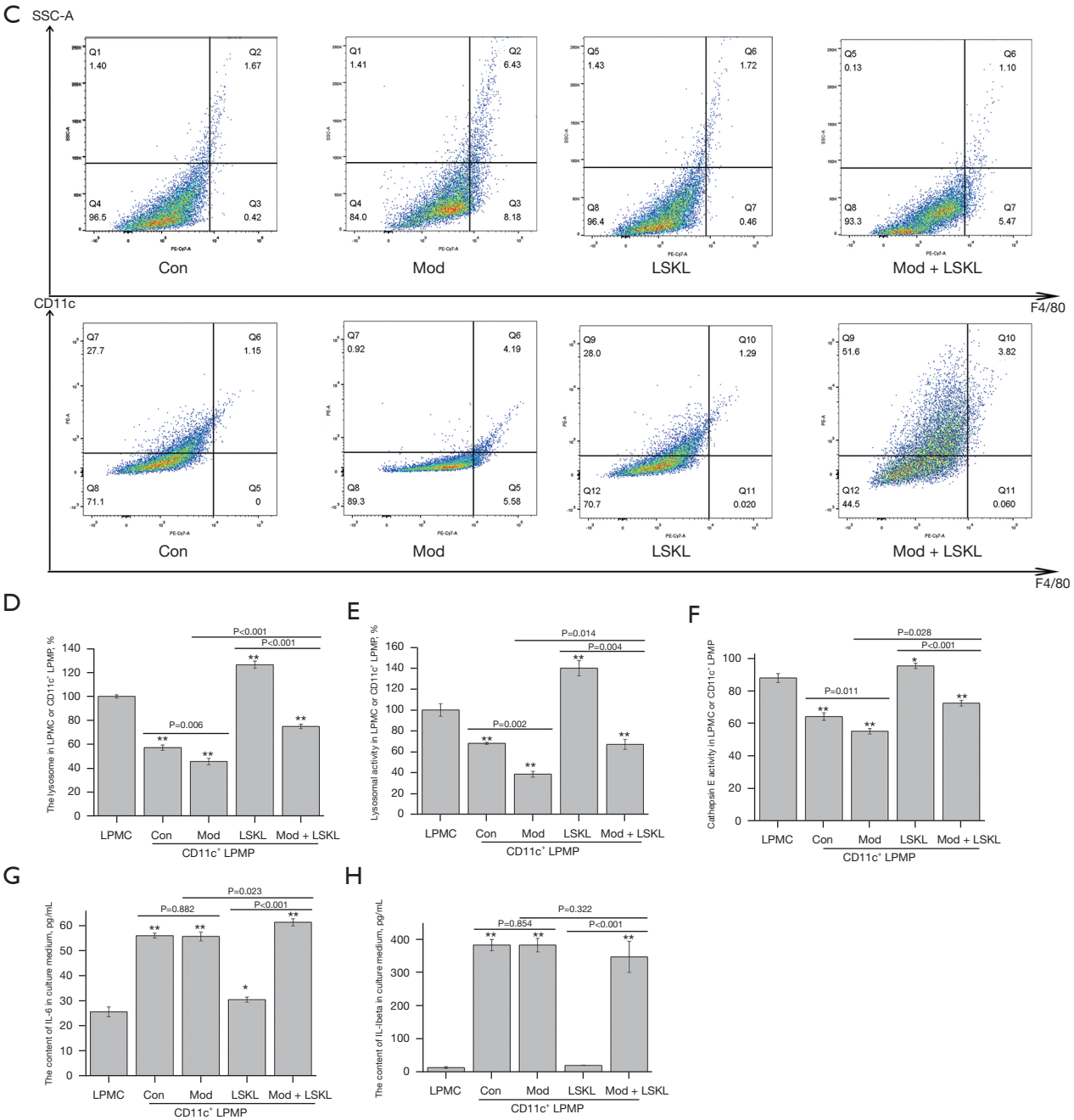


Figure 3 The lysosomal activity of increased CD11c$^+$ Mφ was limited. (A) The infiltration of CD11c$^+$ Mφ in colonic mucosa of UC patients. (B,C) The infiltration of cmMC and CD11c$^+$ Mφ in colonic mucosa of rats. (D-F) The lysosome, lysosomal activity, and Cathepsin E activity in CD11c$^+$ LPMP. (G-H) The expression of IL-6 and IL-1β were significantly increased in the serum of colitis rats. (*, P<0.05, **, P<0.001, *vs.* Con or LPMC; $^{\#}$, P<0.05, $^{\#\#}$, P<0.001, *vs.* Con). UC, ulcerative colitis; Con, Control group; Mod, Model group; LSKL, an inhibitor of Thrombospondin (TSP-1); IL, interleukin; LPMC, Lamina propria mononuclear cells; LPMP, Lamina propria macrophages; Mφ, macrophage.

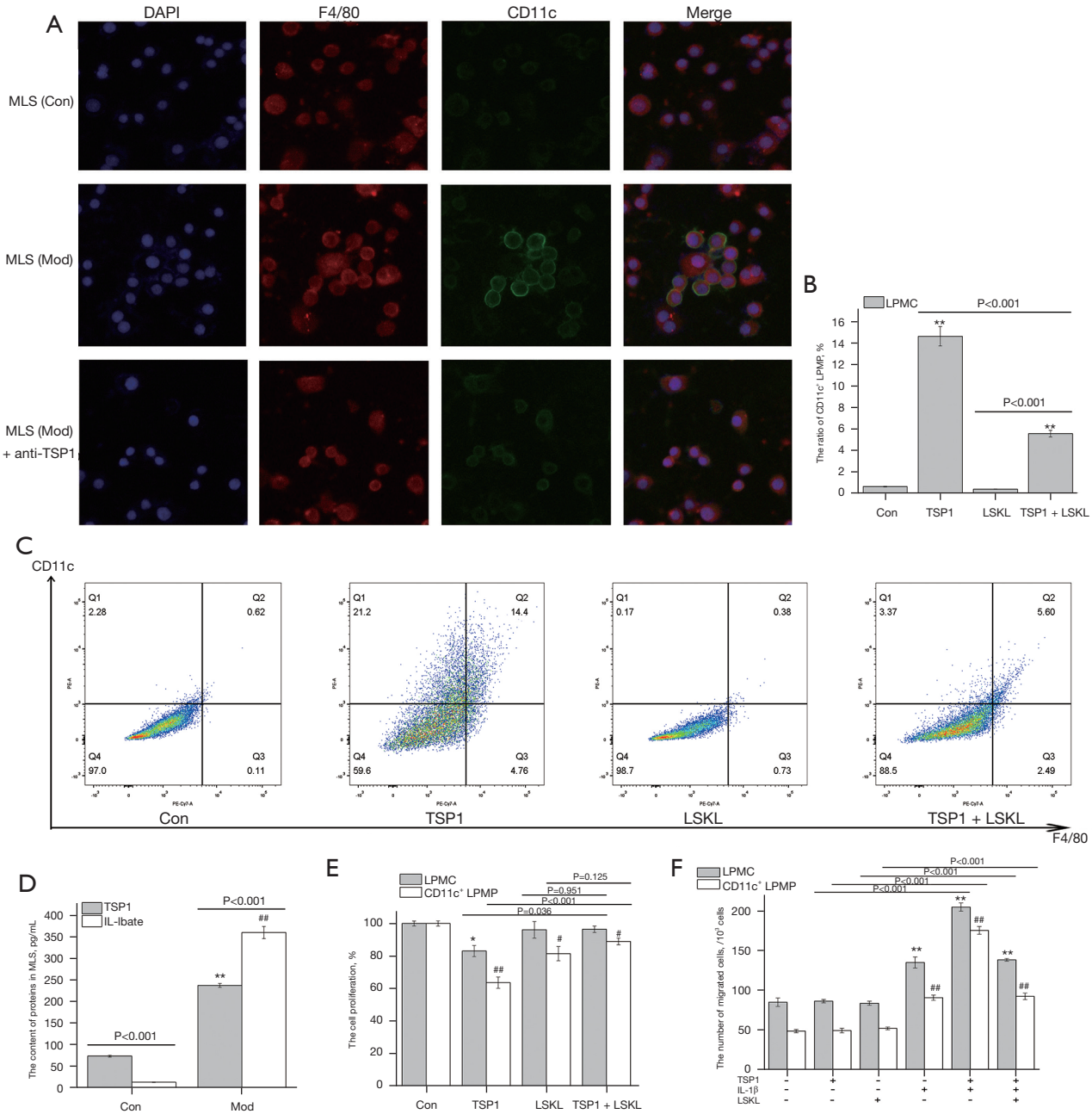


Figure 4 TSP1 promoted the differentiation of cmMφ, and enhanced migration. (A-C) *In vitro*, TSP1 protein promoted the differentiation of LPMC to CD11c$^+$ LPMP and inhibited the cell proliferation of LPMC and CD11c$^+$ LPMP (IF, 200×). (D) The number of CD11c$^+$ LPMP in LPMC treated with MLS. (E) *In vitro*, TSP1 protein inhibited the cell proliferation of LPMC and CD11c$^+$ LPMP. (F) The migration of LPMC and CD11c$^+$ LPMP induced by IL-1β. MLS, mucosal lysis supernatant; LPMC, lamina propria mononuclear cells. (*, P<0.05, **, P<0.001, $^{\#}$, P<0.05, $^{\#\#}$, P<0.001, *vs.* Con). MLS, mucosal lysis supernatant; IL, interleukin; anti-TSP1, anti-TSP1 antibody; Con, Control group; Mod, Model group; LSKL, an inhibitor of Thrombospondin (TSP-1); LPMC, Lamina propria mononuclear cells; LPMP, Lamina propria macrophages; Mφ, macrophage.

However, the effect of TSP1 protein on the differentiation and cell proliferation of LPMC and CD11c$^+$ LPMP was inhibited by LSKL (50 μM) (*Figure 4C-4E*).

In addition, IL-1β was shown to significantly increase the cell migration of LPMC and CD11c$^+$ LPMP. However, the cell migration of LPMC and CD11c$^+$ LPMP was not increased by TSP1 protein, but the migration induced by IL-1β (300 pg/mL) was greatly enhanced by increasing the TSP1 protein in culture medium. Furthermore, the effect of TSP1 protein on enhancing the chemotactic migration could be also limited by LSKL (*Figure 4F*, Figure S2C,S2D).

TSP1 inhibited the lysosome activity via binding on CD36, but not CD47

In vitro, when LPMC was treated with TSP1 protein for 12 h, the lysosome, lysosomal activity, and CTSE activity was significantly decreased in the TSP1 group compared with that in control group (*Figure 5A-5C*). However, the expression of IL-6 and IL-1β were significantly increased in the TSP1 group (*Figure 5D,5E*). Moreover, the effect of TSP1 protein on lysosome was reversed by LSKL (*Figure 5A-5E*). Above all, the TSP1 protein c induced the polarization of lysosomal activity-limited cmCD11c$^+$ LPMP; however, the mechanism is still unclear.

Some results in previous studies have suggested that both CD36 and CD47 are important receptors of TSP1 protein, which play an important role in regulating the polarization, synthesis, and release of inflammatory cytokines in Mφ (39,40). To explore the mechanism that TSP1 protein inhibited the lysosomal activity, *CD36* over-expressed plasmid (*CD36*^{over} plasmid) and *CD36* silencing plasmids1/2/3 (*CD36*$^-$ plasmid), *CD47* over-expressed plasmid (*CD47*^{over} plasmid) and *CD47* silencing plasmids1/2/3 (*CD47*$^-$ plasmid) were used to regulate the expression of *CD36* and *CD47* in LPMC.

The expression of CD36 protein was significantly up-regulated by *CD36*^{over} plasmid (*CD36*^{over} group) (Figure S3A), but obviously down-regulated by *CD36*$^-$ plasmid (siRNA 2) which was selected in the following tests (*CD36*$^-$ group) (Figure S3B). The differentiation of CD11c$^+$ LPMP was significantly increased in the *CD36*^{over} group, but inhibited in the *CD36*$^-$ group. Furthermore, the differentiation of CD11c$^+$ LPMP induced by TSP1 protein was significantly inhibited in the *CD36*$^-$ group or LSKL group (LPMC treated with LSKL) compared with the TSP1 group, while it was obviously enhanced in the *CD36*^{over} group (*Figure 5F-5G*).

In addition, the lysosome, lysosomal activity, and CTSE activity were also significantly increased in the *CD36*$^-$ group and *CD36*$^-$ + TSP1 group (LPMC treated with *CD36*$^-$ plasmid + TSP1 protein), but decreased in the *CD36*^{over} group and *CD36*^{over}+TSP1 group (LPMC treated with *CD36*^{over} plasmid + TSP1 protein) compared with that in TSP1 group. Meanwhile, the content of IL-6 and IL-1β in culture medium was significantly increased in the *CD36*^{over} group and *CD36*^{over} + TSP1 group, but decreased in the *CD36*$^-$ group and *CD36*$^-$ + TSP1 group. Furthermore, there was no obvious difference in the cells differentiation, lysosome, lysosomal activity, CTSE activity, and content of IL-6 and IL-1β in culture medium found between the *CD36*$^-$ group and *CD36*$^-$ + TSP1 group, and between the *CD36*^{over} group and *CD36*^{over} + TSP1 group (*Figure 5H-5L*).

Similarly, the expression of CD47 protein was significantly up-regulated by the *CD47*^{over} plasmid (*CD47*^{over} group) (Figure S3C), but obviously down-regulated by *CD47*$^-$ plasmid (siRNA 2) and *CD47*$^-$ plasmid (siRNA 3) which was selected as the silencing plasmid in the following experiments (*CD47*$^-$ group) (Figure S3D). However, the *CD47*^{over} plasmid and *CD47*$^-$ plasmid almost had no effect on the lysosome, lysosomal activity, and CTSE activity in LPMC with or without TSP1 protein treatment (Figure S3E-S3G).

The PRKCQ/NF-κB signaling pathway played an important role in regulating the effect of TSP1 protein

The PRKCQ/NF-κB signaling pathway was revealed to be an important signal pathway in regulating the cell proliferation and the polarization of Mφ (41). *In vitro*, the expression of CD36, phospho-PRKCQ (Thr-538, Ser-695), NF-κB, and phospho-65 NF-κB were all significantly up-regulated in LPMC treated with TSP1, CD11c$^+$ LPMP, and CD11c$^+$ LPMP treated with TSP1 compared with control group. However, there was no obvious difference in the expression of PRKCQ and phospho-PRKCQ (Ser-676) found between CD11c$^+$ LPMP and LPMC (*Figure 6A-6C*).

Furthermore, the expression of CD36, phospho-PRKCQ (Thr-538, Ser-695), NF-κB, and phospho-65 NF-κB were also significantly up-regulated in the TSP1 group, *CD36*^{over} group, and *CD36*^{over}+TSP1 group, but greatly down-regulated in the *CD36*$^-$ group, *CD36*$^-$+TSP1 group, LSKL group, and LSKL+TSP1 group. However, there were no significant differences in the expressions of CD36, phospho-PRKCQ (Thr-538, Ser-695), NF-κB, and phospho-65 NF-κB proteins found between the *CD36*$^-$ group and *CD36*$^-$+TSP1 group, LSKL group and LSKL+TSP1 group,

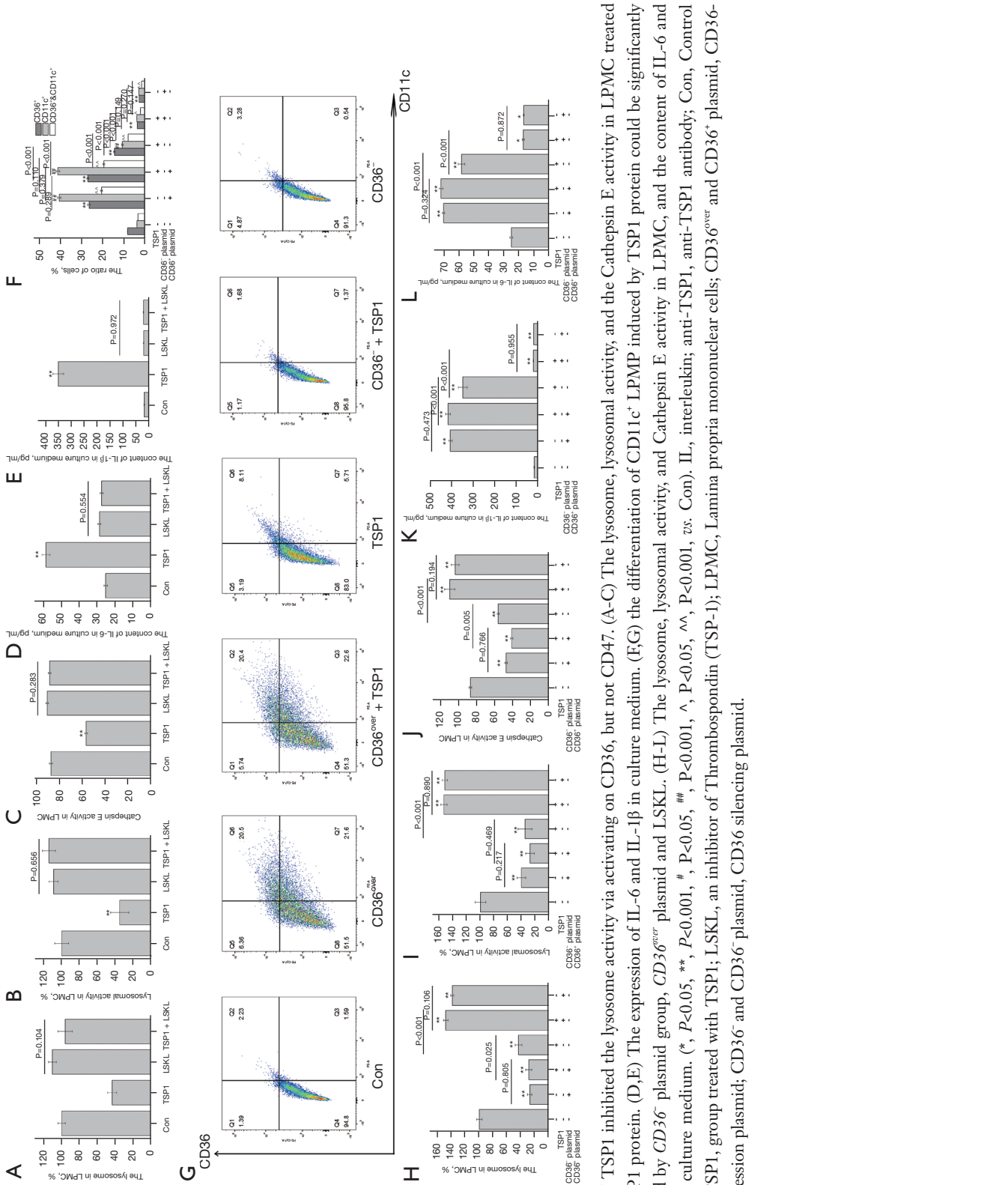


Figure 5 TSP1 inhibited the lysosome activity via activating on CD36, but not CD47. (A-C) The lysosome, lysosomal activity, and the Cathepsin E activity in LPMC treated with TSP1 protein. (D,E) The expression of IL-6 and IL-1β in culture medium. (F,G) the differentiation of CD11c^{+} LPMP induced by TSP1 protein could be significantly regulated by *CD36*$^{-}$ plasmid group, *CD36*^{over} plasmid and LSKL. (H-L) The lysosome, lysosomal activity, and Cathepsin E activity in LPMC, and the content of IL-6 and IL-1β in culture medium. (*, *P*<0.05, **, *P*<0.001, #, P<0.05, ##, P<0.001, ^, P<0.05, ^^, P<0.001, *vs.* Con). IL, interleukin; anti-TSP1, anti-TSP1 antibody; Con, Control group; TSP1, group treated with TSP1; LSKL, an inhibitor of Thrombospondin (TSP-1); LPMC, Lamina propria mononuclear cells; CD36^{over} and CD36^{+} plasmid, CD36-overexpression plasmid; CD36^{-} and CD36^{-} plasmid, CD36 silencing plasmid.

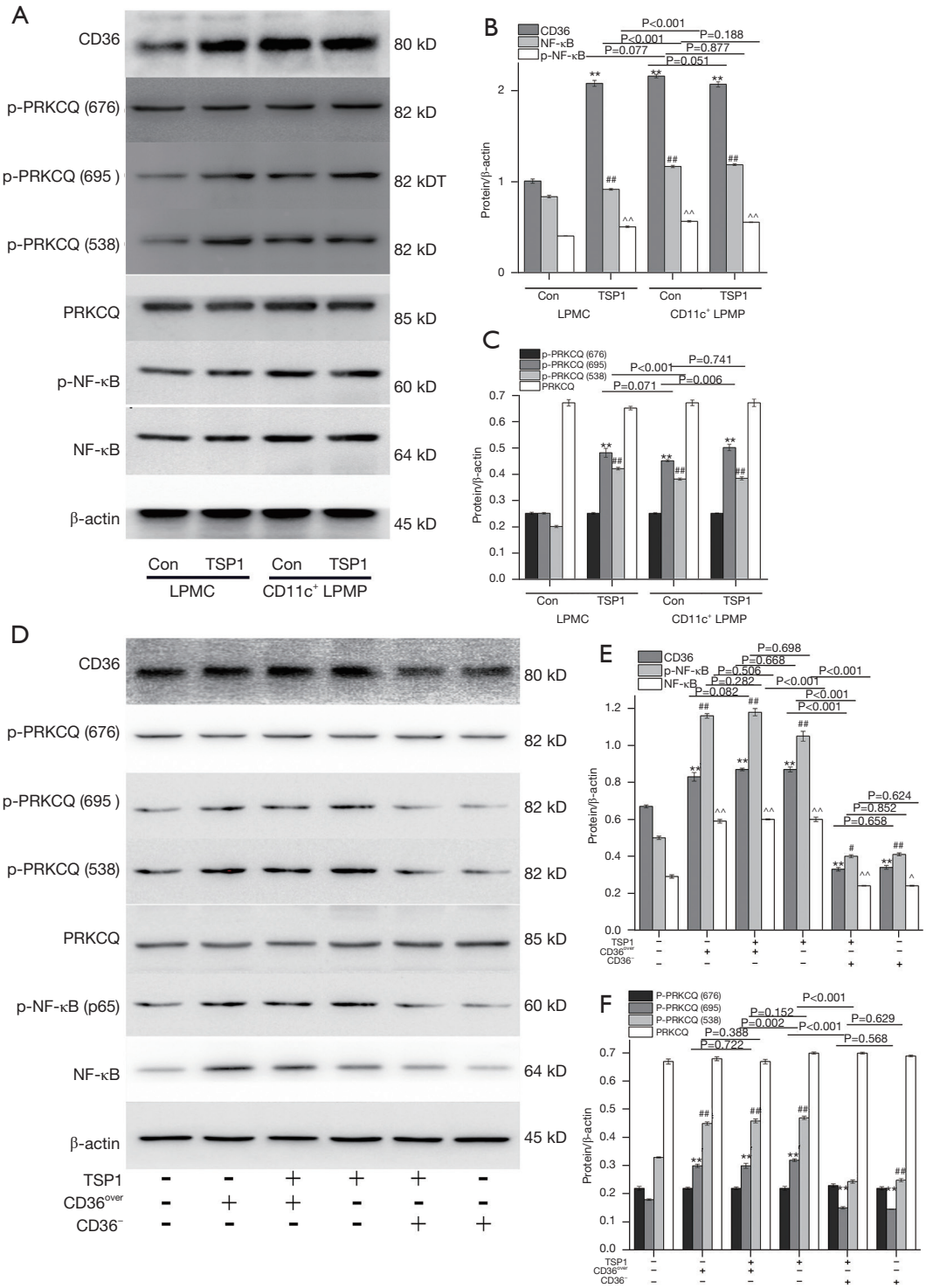


Figure 6 The PRKCQ/NF-κB signaling pathway played an important role in regulating the effect of TSP1 protein. (A-C) The expression of CD36, PRKCQ, phospho-PRKCQ (Ser-676), phospho-PRKCQ (Thr-538), phospho-PRKCQ (Ser-695), NF-κB, and phospho-65 NF-κB in LPMC and CD11c^{+} LPMP. (D-F) The expression of CD36, PRKCQ, phospho-PRKCQ (Ser-676), phospho-PRKCQ (Thr-538), phospho-PRKCQ (Ser-695), NF-κB, and phospho-65 NF-κB in group stimulated with *CD36*^{over} plasmid, *CD36*$^{-}$ plasmid, and TSP1 protein. (**, P<0.001, $^{\#}$, P<0.05, $^{\#\#}$, P<0.001, ^, P<0.05, ^^, P<0.001, *vs.* Con). Con, Control group; TSP1, group treated with TSP1 protein; LPMC, Lamina propria mononuclear cells; LPMP, Lamina propria macrophages; CD36^{over}, CD36-overexpression plasmid; CD36^{-}, CD36 silencing plasmid.

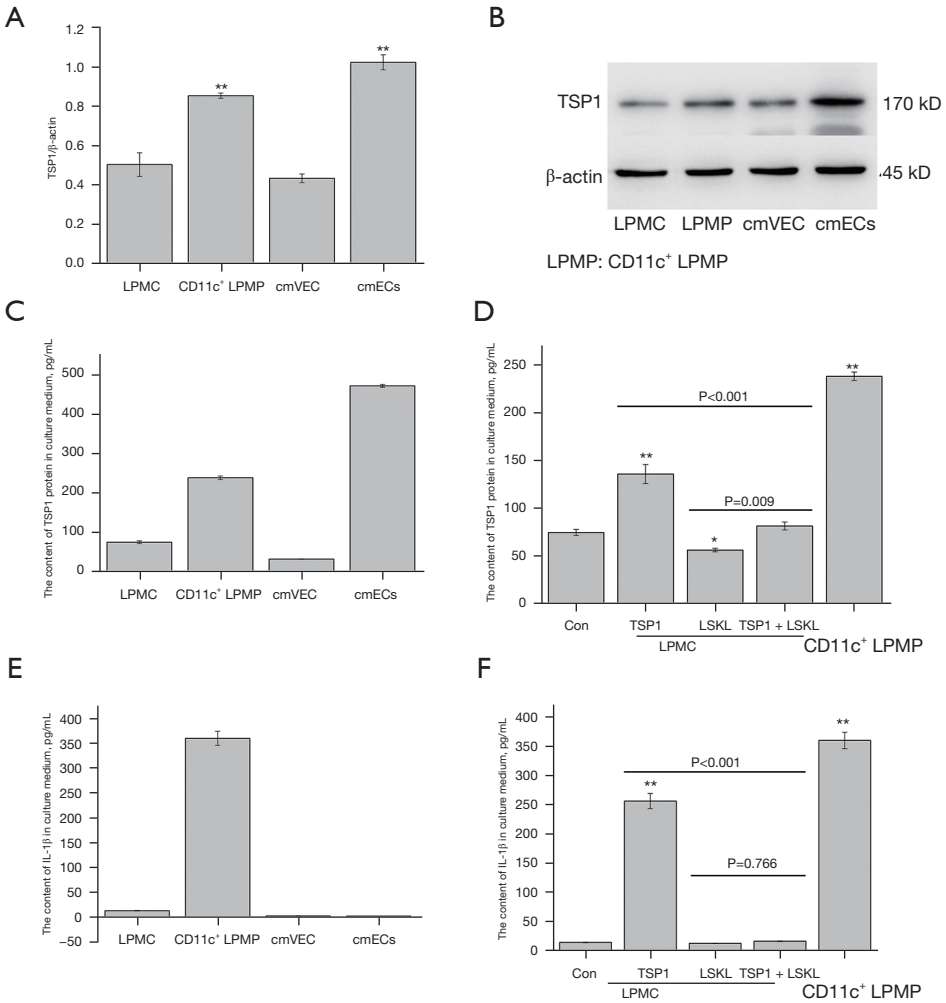


Figure 7 TSP1 protein was mainly derived from cmMφ and cmECs. (A,B) The content of TSP1 and IL-1β protein in MLS. (B-D) The expression of intracellular TSP1 protein and TSP1 protein in culture medium. (E) CD11c+ LPMP secrete more TSP1 protein. (F) The content of IL-1β in culture medium. MLS, mucosal lysis supernatant. (*, P<0.05, **, P<0.001, *vs.* LPMC). IL, interleukin; Con, Control group; TSP1, group treated with TSP1 protein; LSKL, an inhibitor of Thrombospondin (TSP-1); LPMC, Lamina propria mononuclear cells; LPMP, Lamina propria macrophages; cmECs, colonic mucosal epithelial cells; cmVEC, colonic mucosal micro-vascular endothelial cells.

and the *CD36*^{over} group and *CD36*^{over}+TSP1 group. In addition, the expression of PRKCQ and phospho-PRKCQ (Ser-676) had not significantly changed in each group (*Figure 6D-6F*).

TSP1 protein was mainly derived from cmMφ and cmECs

We had shown that TSP1 played a central role in regulating the lysosome activity, differentiation, and migration of cmMC and CD11c+ Mφ. Next, we tried to explore the source of TSP1 protein. We isolated LPMC, CD11c+ LPMP, cmECs, and cmVEC from colon of colitis rats. The results showed that both the expression of intracellular *TSP1* and TSP1 protein in culture medium were the most obviously increased in cmECs and CD11c+ LPMP, followed by cmVEC cells, and the least in LPMC (*Figure 7A-7C*).

Furthermore, TSP1 protein induced the differentiation of CD11c+ LPMP which could secrete more TSP1 protein in turn (*Figure 4C,4D,7D*). Meanwhile, the content of IL-1β in culture medium was only significantly increased in the CD11c+ LPMP group and TSP1 group, which was inhibited by LSKL (*Figure 7E,7F*). However, IL-1β did not promote the differentiation of CD11c+ LPMP and the cell proliferation of LPMC, and the expression of TSP1 and

IL-1β protein was not increased in LPMC which had been treated with IL-1β (Figure S4A-S4E).

Discussion

The MPS plays a critical role in colonic mucosal inflammation in colitis, which is a cellular system with strong phagocytosis and defense functions (42,43). Among them, cmMC can discriminate harmful from harmless antigens, and initiate and sustain protective immune responses mounted towards pathogenic organisms (5). When cmMC is stimulated by pathogenic factors, it can differentiate into activated CD11c$^+$ Mφ in colonic mucosa which do not only synthesize and secrete inflammatory cytokines, but also have high phagocytic activity (44). Some results in previous studies have suggested that cmMC and CD11c$^+$ Mφ are essential for maintaining immune homeostasis which is important for maintaining the normal structure and function of the colonic mucosa (8,45).

In addition, reducing the infiltration of cmMC and CD11c$^+$ Mφ can effectively ameliorate the colonic mucosal inflammation in UC, which promotes the repair of colonic mucosal injuries (9,46). The results of our study also showed that there were large numbers of cmMC and CD11c$^+$ Mφ infiltrating the colonic mucosa in UC patients and colitis rats, which closely relate to colonic mucosal injuries. However, the cell proliferation of LPMC and CD11c$^+$ LPMP was obviously limited in colitis rats compared with that in normal rats. Therefore, not all cmMC and CD11c$^+$ Mφ increased in UC rats come from self-renewal of Mφ.

There are abundant monocytes in the blood. Under inflammatory conditions, inflammatory cells in tissues can synthesize and secrete cytokines, such as IL-1β and colony stimulating factor (CSF), which promote the migration of cirMC (5). While the cirMC is migrating across blood vessels and differentiating, the inflammation is greatly aggravated in tissue (47). Consistent with previous studies (5,10), we also found that the cmMC needed to be supplemented by cirMC, unlike most other tissue Mφ which appear to derive from primitive precursors that subsequently self-renew. There are some intact and functional capillaries in normal colon mucosa which is an important factor to block the trans-vascular migration of cirMC in UC (5). For the structure and function of microvessels in colonic mucosa to have been severely damaged, the trans-vascular migration of cirMC needs to be significantly facilitated (5,47). In this case, there may be theoretically 2 ways of trans-vascular migration of monocytes. The first is the leakage of monocytes from blood vessels, which will cause the monocytes to be mainly distributed around the damaged blood vessel. Obviously, this is not in line with the distribution characteristics of cmMC and CD11c$^+$ Mφ in colonic mucosa in UC patients. The second is that monocytes undergo a directional migration when treated with cytokines, which will cause the cells to be concentrated in the lesion. This is much more in line with the distribution characteristics in UC. Therefore, we speculate that cmMC and CD11c$^+$ Mφ infiltrating in colonic mucosa in UC are mainly derived from cirMC that could undergo directional trans-vascular migration induced by cytokines in colonic mucosa.

Some studies have confirmed that IL-1β is an important cytokine that clearly has the function of promoting cell migration of MPS (48,49). The results of our study showed that the content of IL-1β in serum and colonic mucosa in colitis rats was significantly increased compared with that in normal rats. Further IL-1β obviously promoted the migration of LPMC and CD11c$^+$ LPMP *in vitro*. The migration ability of CD11c$^+$ LPMP was obviously stronger than that of LPMC, but LPMC had higher responsiveness to IL-1β. Interestingly, IL-1β was synthesized and secreted by CD11c$^+$ LPMP, but not LPMC. Therefore, the massive infiltration of cmMC in colonic mucosa in UC may mainly come from the migration of cirMC induced by IL-1β that could be synthesized and secreted by CD11c$^+$ Mφ.

In addition, the results of previous studies have shown that CD11c$^+$ Mφ can be differentiated from cmMC treated with lipopolysaccharide (LPS) or commensal microbiota, which has high phagocytic activity and can secrete inflammatory cytokines, such as IL-6 and IL-1β (50,51). In the present study, we found that large amounts of CD11c$^+$ Mφ infiltrated in the colonic mucosa, accompanied by IL-1β and IL-6 in serum, and MLS significantly increased both in UC patients and DSS-induced colitis rats. The MLS(Mod) that had higher concentration of TSP1 protein significantly promoted the differentiation of LPMC into CD11c$^+$ LPMP, which could be limited by anti-TSP1 antibody and LSKL. Furthermore, TSP1 protein significantly promoted the differentiation of CD11c$^+$ LPMP and increased the release of IL-6 and IL-1β. Overall, the increased TSP1 regulated the differentiation of cmMC into CD11c$^+$ Mφ to increase the release of IL-6 and IL-1β, which subsequently prompted the migration of Mφ. The *TSP1* gene is not only an inhibitory factor for angiogenesis, but also an important immune regulatory factor (52). Our previous research found that colonic mucosal micro-vascular injury

was significantly aggravated when TSP1 protein was increased (23). However, there is controversy regarding the regulation of inflammation. Most researchers believe that the TSP1 protein promotes the migration of mononuclear cells, which subsequently increases inflammatory Mφ to infiltrate vascular tissues (53). However, TSP1 protein could also inhibit pathogenic inflammation by stabilizing monocytes (21,54). In this study, we found that the TSP1 protein tended to increase obviously with the aggravation of colonic mucosal inflammation in colitis rats. In addition, the colonic mucosal inflammation could be significantly reduced with the decrease of TSP1 (23). Here, we found that TSP1 protein promoted the differentiation of LPMC to CD11c$^+$ LPMP and increased the synthesis and secretion of IL-1β and IL-6. However, the number of lysosomes, important organelles that are closely related to the phagocytosis and autophagy of Mφ, was significantly decreased in CD11c$^+$ LPMP, and both the lysosomal activity and CTSE activity were also significantly decreased in CD11c$^+$ LPMP induced by TSP1. Moreover, these effects of TSP1 protein were limited by LSKL. Overall, TSP1 promoted the differentiation of CD11c$^+$ LPMP, increased the synthesis and secretion of inflammatory factors, and the tropic migration of LPMC and CD11c$^+$ LPMP. However, the phagocytic and autophagy functions of CD11c$^+$ LPMP are obviously limited due to the reduction of lysosomes and lysosome activity. This may explain to some extent the controversy present in previous studies regarding the effect of TSP1 protein on inflammation. Besides, it is well known that CD11c$^+$ Mφ can be further differentiated into the M1 type Mφ *in vivo* that has complete secretion and phagocytic functions (55,56). So, CD11c$^+$ Mφ induced by TSP1 protein may only be an intermediate subtype of immature monocyte that differentiates into mature Mφ (M1 or M2 type Mφ), which implies that the differentiation of Mφ may be an irreversible complex regulatory process.

The expression of TSP1 protein is consistent with the clinical characteristics of the UC patients. Multiple clinical studies have found that age and gender should be considered as 2 independent factors and are closely related to the occurrence of UC. The incidence of UC mostly increased significantly with increasing age, and was also significantly higher in males than that in females (34-37). According to the data from the GTEx database, we found that the expression of TSP1 in young, male, and colon tissues was significantly higher than that in old, female, and rectal tissues, which is consistent with the clinical characteristics of the active UC. Besides, we found that the expression of TSP1 protein was significantly increased in cmECs and CD11c$^+$ LPMP, followed by LPMC, and least in cmVEC. Besides, IL-1β could be rarely synthesized and secreted by CD11c$^+$ LPMP, but not others; however, IL-1β did not promote the differentiation of CD11c$^+$ LPMP. These findings suggested that the TSP1 protein was mainly secreted by cmECs and CD11c$^+$ Mφ, which could in turn promote the differentiation of CD11c$^+$ Mφ. Interestingly, although TSP1 protein does not have the effect of promoting the tropic migration of LPMC and CD11c$^+$ LPMP like IL-1β, it further enhanced the effect of IL-1β on the migration.

The receptors CD47 and CD36 are the cell surface receptors of TSP1 protein, which is involved in regulating the polarization of Mφ, the synthesis and secretion of inflammatory cytokines, and so on (39,54). They have both commonality and individuality in the functional regulation of macrophages. For example, TSP1 protein can promote the polarization of Mφ and the synthesis and secretion of inflammatory cytokines via activating the CD36-TLR4 signal pathway (40). Furthermore, CD36 activated by TSP1 has a limiting effect on the lysosomal activity in Mφ (57). However, activated CD47 induced by TSP1 protein could significantly enhance the pinocytosis of Mφ to increase the foam cells in tissues, as implicated in cardiovascular diseases (58); there was no clear evidence to support that CD47 is involved in the regulation of lysosomal activity of Mφ. Our results also showed that the differentiation of CD11c$^+$ Mφ and lysosomal activity in Mφ was mainly regulated by CD36, but not CD47. In addition, the PRKCQ/NF-κB signaling pathway is an important signaling pathway involved in various cellular processes, such as cell proliferation, differentiation, lysosomal activity, and so on (50,59), which could activate CD36 in Mφ (59). The PRKCQ is a serine- and threonine-specific protein kinase which has multiple serine-threonine sites, such as Thr-538, Ser-695, and Ser-676 (60). The phosphorylation at different sites of PRKCQ protein could mediate different signal transduction (61). In this study, we found that the CD36-TSP1 interaction played an important role in activating the PRKCQ/NF-κB signaling pathway, which subsequently promoted the differentiation of CD11c$^+$ LPMP and the synthesis and release of pro-inflammatory cytokines. Furthermore, the phosphorylation of PRKCQ (Thr-538 and Ser-695) plays a critical role in the signal transduction. In summary, *TSP1* could promote the migration and the differentiation of CD11c$^+$ LPMP with limited lysosomal activity via binding on CD36 to activate the PRKCQ/NF-

κB signaling pathway, which aggravates colonic mucosal inflammatory injuries in UC. However, there were still some unresolved problems in the present study. For example, the mechanism by which $CD11c^+$ Mφ differentiates into M1 type Mφ and the mechanism by which IL-1β induces migration of Mφ are still unclear. It has been reported that TSP1 could participate in the regulation of lysosomal activity-dependent autophagy and pinocytosis of Mφ by binding to CD47 (26,58), but the results in this experiment did not find that CD47 has the effect on lysosomal activity in Mφ. Therefore, we suspect that the changes of lysosomal activity in Mφ stimulated by TSP1 binding to CD47 may be achieved through the CD36-CD47 interaction. Although we know that TSP1 could inhibit the lysosomal activity in $CD11c^+$ Mφ, the mechanism is still unknown. Besides, we found that the Mφ induced by TSP1 *in vivo* and colonic mucosal macrophages of UC patients is not completely consistent, suggesting that there might be some other contributing factors which remained to be elucidated via further study. The $CD11c^+$ LPMP induced by TSP1 did not have an effect on phagocytosis *in vitro* like M1 type Mφ (62) that is a kind of terminally differentiated $CD11c^+$ Mφ in colonic mucosa *in vivo*. However, the mechanism is still unknown. All of these issues mentioned above need to be addressed through further study on UC.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. SZY201612006) granted by the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine, in compliance with the Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine's guidelines for the care and use of animals. The study on human was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (No. B2017-068) and informed consent was taken from all the patients.

References

1. Hume DA, Irvine KM, Pridans C. The Mononuclear Phagocyte System: The Relationship between Monocytes and Macrophages. Trends Immunol 2019;40:98-112.
2. Weng TY, Huang SS, Yen MC, et al. A novel cancer therapeutic using thrombospondin 1 in dendritic cells. Mol Ther 2014;22:292-302.
3. Moriwaki K, Balaji S, Bertin J, et al. Distinct Kinase-Independent Role of RIPK3 in CD11c+ Mononuclear Phagocytes in Cytokine-Induced Tissue Repair. Cell Rep 2017;18:2441-51.
4. Arnold IC, Mathisen S, Schulthess J, et al. CD11c(+) monocyte/macrophages promote chronic Helicobacter hepaticus-induced intestinal inflammation through the production of IL-23. Mucosal Immunol 2016;9:352-63.
5. Bain CC, Mowat AM. The monocyte-macrophage axis in the intestine. Cell Immunol 2014;291:41-8.

6. Mencarelli A, Khameneh HJ, Fric J, et al. Calcineurin-mediated IL-2 production by CD11chighMHCII+ myeloid cells is crucial for intestinal immune homeostasis. Nat Commun 2018;9:1102.
7. Ihara S, Hirata Y, Hikiba Y, et al. Adhesive Interactions between Mononuclear Phagocytes and Intestinal Epithelium Perturb Normal Epithelial Differentiation and Serve as a Therapeutic Target in Inflammatory Bowel Disease. J Crohns Colitis 2018;12:1219-31.
8. Bernardo D, Marin AC, Fernández-Tomé S, et al. Human intestinal pro-inflammatory CD11chighCCR2+CX3CR1+ macrophages, but not their tolerogenic CD11c-CCR2-CX3CR1- counterparts, are expanded in inflammatory bowel disease. Mucosal Immunol 2018;11:1114-26.
9. Fuke N, Takagi T, Higashimura Y, et al. Lactobacillus brevis KB290 With Vitamin A Ameliorates Murine Intestinal Inflammation Associated With the Increase of CD11c+ Macrophage/CD103- Dendritic Cell Ratio. Inflamm Bowel Dis 2018;24:317-31.
10. Mortha A, Chudnovskiy A, Hashimoto D, et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. Science 2014;343:1249288.
11. Boros É, Prontvai B, Kellermayer Z, et al. Transcriptome Based Profiling of the Immune Cell Gene Signature in Rat Experimental Colitis and Human IBD Tissue Samples. Biomolecules 2020;10:974.
12. Villanacci V, Baert F, Cornillie F, et al. Challenges Faced by Cross-sectional Imaging and Histological Endpoints in Clinical Trials. J Crohns Colitis 2017;11:S586-92.
13. Sahu SK, Kumar M, Chakraborty S, et al. MicroRNA 26a (miR-26a)/KLF4 and CREB-C/EBPβ regulate innate immune signaling, the polarization of macrophages and the trafficking of Mycobacterium tuberculosis to lysosomes during infection. PLoS Pathog 2017;13:e1006410.
14. Chandra V, Mahajan S, Saini A, et al. Human IL10 gene repression by Rev-erbα ameliorates Mycobacterium tuberculosis clearance. J Biol Chem 2013;288:10692-702.
15. Yuan Y, Li L, Zhu L, et al. Mesenchymal stem cells elicit macrophages into M2 phenotype via improving transcription factor EB-mediated autophagy to alleviate diabetic nephropathy. Stem Cells 2020;38:639-52.
16. Li Y, Cao F, Li M, et al. Hydroxychloroquine induced lung cancer suppression by enhancing chemo-sensitization and promoting the transition of M2-TAMs to M1-like macrophages. J Exp Clin Cancer Res 2018;37:259.
17. Canton J, Khezri R, Glogauer M, et al. Contrasting phagosome pH regulation and maturation in human M1 and M2 macrophages. Mol Biol Cell 2014;25:3330-41.
18. Coria LM, Ibañez AE, Tkach M, et al. A Brucella spp. Protease Inhibitor Limits Antigen Lysosomal Proteolysis, Increases Cross-Presentation, and Enhances CD8+ T Cell Responses. J Immunol 2016;196:4014-29.
19. Memetimin H, Li D, Tan K, et al. Myeloid-specific deletion of thrombospondin 1 protects against inflammation and insulin resistance in long-term diet-induced obese male mice. Am J Physiol Endocrinol Metab 2018;315:E1194-203.
20. Tang X, Miao Y, Luo Y, et al. Suppression of Endothelial AGO1 Promotes Adipose Tissue Browning and Improves Metabolic Dysfunction. Circulation 2020;142:365-79.
21. Fang LL, Yu HQ, Wu RJ, et al. Thrombospondin 1 Modulates Monocyte Properties to Suppress Intestinal Mucosal Inflammation. J Innate Immun 2015;7:601-11.
22. Yip-Schneider MT, Wu H, Schmidt CM. Novel expression of vascular endothelial growth factor isoforms in the pancreas and pancreatic cystic lesions. Biochimie 2021;181:234-9.
23. Shiying W, Boyun S, Jianye Y, et al. The Different Effects of VEGFA121 and VEGFA165 on Regulating Angiogenesis Depend on Phosphorylation Sites of VEGFR2. Inflamm Bowel Dis 2017;23:603-16.
24. Esteban S, Clemente C, Koziol A, et al. Endothelial MT1-MMP targeting limits intussusceptive angiogenesis and colitis via TSP1/nitric oxide axis. EMBO Mol Med 2020;12:e10862.
25. Saadi A, Shannon NB, Lao-Sirieix P, et al. Stromal genes discriminate preinvasive from invasive disease, predict outcome, and highlight inflammatory pathways in digestive cancers. Proc Natl Acad Sci U S A 2010;107:2177-82.
26. Chen CY, Chao YM, Lin HF, et al. miR-195 reduces age-related blood-brain barrier leakage caused by thrombospondin-1-mediated selective autophagy. Aging Cell 2020;19:e13236.
27. Ren YJ, Zhang L, Bai T, et al. Transfer of CD11c+ lamina propria mononuclear phagocytes from post-infectious irritable bowel syndrome causes mucosal barrier dysfunction and visceral hypersensitivity in recipient mice. Int J Mol Med 2017;39:1555-63.
28. Weigmann B, Tubbe I, Seidel D, et al. Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue. Nat Protoc 2007;2:2307-11.
29. Ungaro F, Tacconi C, Massimino L, et al. MFSD2A Promotes Endothelial Generation of Inflammation-Resolving Lipid Mediators and Reduces Colitis in Mice. Gastroenterology 2017;153:1363-1377.e6.
30. Degagné E, Turgeon N, Moore-Gagné J, et al. P2Y(2)

receptor expression is regulated by C/EBPβ during inflammation in intestinal epithelial cells. FEBS J 2012;279:2957-65.
31. Silva LM, Lum AG, Tran C, et al. Plasmin-mediated fibrinolysis enables macrophage migration in a murine model of inflammation. Blood 2019;134:291-303.
32. Dwyer AR, Mouchemore KA, Steer JH, et al. Src family kinase expression and subcellular localization in macrophages: implications for their role in CSF-1-induced macrophage migration. J Leukoc Biol 2016;100:163-75.
33. Zhang W, He KF, Yang JG, et al. Infiltration of M2-polarized macrophages in infected lymphatic malformations: possible role in disease progression. Br J Dermatol 2016;175:102-12.
34. King D, Reulen RC, Thomas T, et al. Changing patterns in the epidemiology and outcomes of inflammatory bowel disease in the United Kingdom: 2000-2018. Aliment Pharmacol Ther 2020;51:922-34.
35. Shah SC, Khalili H, Chen CY, et al. Sex-based differences in the incidence of inflammatory bowel diseases-pooled analysis of population-based studies from the Asia-Pacific region. Aliment Pharmacol Ther 2019;49:904-11.
36. Tong J, Zheng Q, Zhang C, et al. Incidence, prevalence, and temporal trends of microscopic colitis: a systematic review and meta-analysis. Am J Gastroenterol 2015;110:265-76; quiz 277.
37. Gentile NM, Khanna S, Loftus EV Jr, et al. The epidemiology of microscopic colitis in Olmsted County from 2002 to 2010: a population-based study. Clin Gastroenterol Hepatol 2014;12:838-42.
38. eGTEx Project. Enhancing GTEx by bridging the gaps between genotype, gene expression, and disease. Nat Genet 2017;49:1664-70.
39. Koduru SV, Sun BH, Walker JM, et al. The contribution of cross-talk between the cell-surface proteins CD36 and CD47-TSP-1 in osteoclast formation and function. J Biol Chem 2018;293:15055-69.
40. Li Y, Qi X, Tong X, et al. Thrombospondin 1 activates the macrophage Toll-like receptor 4 pathway. Cell Mol Immunol 2013;10:506-12.
41. Pfeifhofer-Obermair C, Albrecht-Schgoer K, Peer S, et al. Role of PKCtheta in macrophage-mediated immune response to Salmonella typhimurium infection in mice. Cell Commun Signal 2016;14:14.
42. Chapuy L, Bsat M, Rubio M, et al. Transcriptomic Analysis and High-dimensional Phenotypic Mapping of Mononuclear Phagocytes in Mesenteric Lymph Nodes Reveal Differences Between Ulcerative Colitis and Crohn's Disease. J Crohns Colitis 2020;14:393-405.
43. Hoshi N, Schenten D, Nish SA, et al. MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice. Nat Commun 2012;3:1120.
44. Zimmermann HW, Bruns T, Weston CJ, et al. Bidirectional transendothelial migration of monocytes across hepatic sinusoidal endothelium shapes monocyte differentiation and regulates the balance between immunity and tolerance in liver. Hepatology 2016;63:233-46.
45. Kang B, Alvarado LJ, Kim T, et al. Commensal microbiota drive the functional diversification of colon macrophages. Mucosal Immunol 2020;13:216-29.
46. Qualls JE, Tuna H, Kaplan AM, et al. Suppression of experimental colitis in mice by CD11c+ dendritic cells. Inflamm Bowel Dis 2009;15:236-47.
47. Zucker SD, Vogel ME, Kindel TL, et al. Bilirubin prevents acute DSS-induced colitis by inhibiting leukocyte infiltration and suppressing upregulation of inducible nitric oxide synthase. Am J Physiol Gastrointest Liver Physiol 2015;309:G841-54.
48. Zhang Q, Wang H, Mao C, et al. Fatty acid oxidation contributes to IL-1β secretion in M2 macrophages and promotes macrophage-mediated tumor cell migration. Mol Immunol 2018;94:27-35.
49. Wang Y, Galli M, Shade Silver A, et al. IL1β and TNFα promote RANKL-dependent adseverin expression and osteoclastogenesis. J Cell Sci 2018;131:jcs213967.
50. Wang T, Pan D, Zhou Z, et al. Dectin-3 Deficiency Promotes Colitis Development due to Impaired Antifungal Innate Immune Responses in the Gut. PLoS Pathog 2016;12:e1005662.
51. Kim JK, Lee KE, Lee SA, et al. Interplay Between Human Gut Bacteria Escherichia coli and Lactobacillus mucosae in the Occurrence of Neuropsychiatric Disorders in Mice. Front Immunol 2020;11:273.
52. Yang HD, Kim HS, Kim SY, et al. HDAC6 Suppresses Let-7i-5p to Elicit TSP1/CD47-Mediated Anti-Tumorigenesis and Phagocytosis of Hepatocellular Carcinoma. Hepatology 2019;70:1262-79.
53. Liu Z, Morgan S, Ren J, et al. Thrombospondin-1 (TSP1) contributes to the development of vascular inflammation by regulating monocytic cell motility in mouse models of abdominal aortic aneurysm. Circ Res 2015;117:129-41.
54. Beguier F, Housset M, Roubeix C, et al. The 10q26 Risk Haplotype of Age-Related Macular Degeneration Aggravates Subretinal Inflammation by Impairing Monocyte Elimination. Immunity 2020;53:429-441.e8.
55. Jang HM, Park KT, Noh HD, et al. Kakkalide and

irisolidone alleviate 2,4,6-trinitrobenzenesulfonic acid-induced colitis in mice by inhibiting lipopolysaccharide binding to toll-like receptor-4 and proteobacteria population. Int Immunopharmacol 2019;73:246-53.
56. Macias-Ceja DC, Cosín-Roger J, Ortiz-Masiá D, et al. Stimulation of autophagy prevents intestinal mucosal inflammation and ameliorates murine colitis. Br J Pharmacol 2017;174:2501-11.
57. Crombie R, Silverstein R. Lysosomal integral membrane protein II binds thrombospondin-1. Structure-function homology with the cell adhesion molecule CD36 defines a conserved recognition motif. J Biol Chem 1998;273:4855-63.
58. Csányi G, Feck DM, Ghoshal P, et al. CD47 and Nox1 Mediate Dynamic Fluid-Phase Macropinocytosis of Native LDL. Antioxid Redox Signal 2017;26:886-901.
59. Raghavan S, Singh NK, Gali S, et al. Protein Kinase Cθ Via Activating Transcription Factor 2-Mediated CD36 Expression and Foam Cell Formation of Ly6Chi Cells Contributes to Atherosclerosis. Circulation 2018;138:2395-412.
60. Liu Y, Graham C, Li A, et al. Phosphorylation of the protein kinase C-theta activation loop and hydrophobic motif regulates its kinase activity, but only activation loop phosphorylation is critical to in vivo nuclear-factor-kappaB induction. Biochem J 2002;361:255-65.
61. Xu ZB, Chaudhary D, Olland S, et al. Catalytic domain crystal structure of protein kinase C-theta (PKCtheta). J Biol Chem 2004;279:50401-9.
62. Krysko O, Holtappels G, Zhang N, et al. Alternatively activated macrophages and impaired phagocytosis of S. aureus in chronic rhinosinusitis. Allergy 2011;66:396-403.

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