Article

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PTIP Deficiency in B Lymphocytes Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice

Jiaxuan Liu[1], Yaqin Xu[2], Yong Q. Chen[1,*], Dan Su[1,*]

[1]Wuxi School of Medicine, Jiangnan University, 214122 Wuxi, Jiangsu, China
[2]School of Food Science and Technology, Jiangnan University, 214122 Wuxi, Jiangsu, China
*Correspondence: yqc_lab@126.com (Yong Q. Chen); dan.su@jiangnan.edu.cn (Dan Su)
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Background: Immune dysregulation contributes to the development of ulcerative colitis (UC). The research on the inflammatory response of UC is mainly focused on T cells, with less understanding of the role of B cells. Pax transactivation domain-interacting protein (PTIP) is essential for the development of B cell subpopulations and humoral immunity. The purpose of this study was to elucidate the role of PTIP in B cells of mice with dextran sodium sulfate (DSS)-induced colitis.

Methods: The B-cell-specific PTIP knockout ($PTIP^{-/-}$) mice were established by crossbreeding cluster of differentiation (CD)$19^{cre/cre}$ mice with $PTIP^{flox/flox}$ mice. The UC mice were induced by drinking water supplemented with 3.8% Dextran Sulfate Sodium (DSS) ($PTIP^{-/-}$ + DSS). The histological analysis was performed using hematoxylin and eosin staining. The immune cells were isolated using a fluorescence-activated cell sorter. The serum antibodies (immunoglobulin M (IgM) or immunoglobulin G (IgG)) and tumor necrosis factor (TNF)-α were determined by Enzyme linked immunosorbent assay (ELISA).

Results: Interestingly, our findings demonstrate that PTIP deficiency in B cells significantly ameliorates UC. In contrast to $PTIP^{-/-}$ + DSS, the wild type (WT) + DSS group showed a more robust increase in disease activity index (DAI) scores ($p < 0.05$), a substantially shortened colon ($p < 0.001$) and a decrease of mucous-producing goblet cells and the complete destruction of crypts. Moreover, PTIP-deficient mice manifested markedly altered neutrophil and T-cell distribution in UC ($p < 0.05$). Although anti-commensal IgG exacerbates UC, we demonstrated, for the first time, that serum natural IgG does not aggravate the pathology of UC. Furthermore, PTIP regulates UC by controlling B-2 cells independently from T cells.

Conclusions: Transplantation of splenic B-2 cells from PTIP-deficient mice protected recipient NOD/ShiltJGpt-Prkdcem26Cd52Il2rgem26Cd22/Gpt (NCG) mice from severe UC.

Keywords: Pax transactivation domain-interacting protein (PTIP); B lymphocytes; ulcerative colitis; IgG; B-2 cells

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are cardinal forms of inflammatory bowel disease (IBD), with high prevalence and rising incidence [1]. Of these, UC is incurable because of the multiple pathogenic factors concerning epithelial barrier deficiency, immune dysregulation, and genetic and environmental components [2]. Due to the accessibility of the intestinal commensal flora as stimulatory antigens, UC approximates to autoimmune disease [3]. The establishment of the distinction between CD and UC was based on the discovery of T helper (Th) 1 and Th2 cells. The T cells are deeply involved in the pathogenesis of UC, among which T regulatory cells (Tregs) resist inflammation, whereas Th17 and Th2 exacerbate UC [4,5]. By contrast, the function of B cells in UC is uncertain. Recent studies have revealed that both the absence of B cells and the increase of immunoglobulin G (IgG) can exacerbate UC in mice [6], whereas B-1 cells protect against UC by secreting IL-10 [7].

Pax transactivation domain-interacting protein (PTIP) consists of six tandem BRCA1 C-terminal (BRCT) domains and a polyglutamine (PolyQ) region, which are implicated in DNA repair and replication [8]. It promotes sterile transcription at the IgH locus, and N-terminal BRCT-1-2 domains are required in IgH class switching [9]; PTIP deficiency impairs the establishment and maintenance of B-1 cells. In addition, PTIP plays a crucial role in licensing humoral immunity [10]. However, the effects of PTIP on B cell-associated inflammatory diseases are still largely unknown.

Here, we used B-cell PTIP-deficient mice to investigate the involvement of PTIP in UC. In dextran sodium sulfate (DSS)-induced colitis, PTIP deficiency in B cells attenuated the severity of diseases, but the decrease in the steady-state serum IgG levels was not linked to a resistance of DSS in $PTIP^{-/-}$ mice. By transplanting $PTIP^{-/-}$ B-2 cells, recipient mice acquired resistance to UC, demonstrating that PTIP regulates UC by controlling B-2 cells.

Materials and Methods

Mice

The B-cell-specific PTIP knockout mice ($CD19^{cre/+}PTIP^{flox/flox}$, $PTIP^{-/-}$) were obtained by crossbreeding $CD19^{cre/cre}$ mice (Jackson Laboratory, Bar Harbor, ME, USA) with $PTIP^{flox/flox}$ mice [10]. The NOD/ShiltJGpt-Prkdcem26Cd52Il2rgem26Cd22/Gpt (NCG) mice (Strain NO. T001475) were purchased from GemPharmatech. All female mice used in the experiments are 8 to 12 weeks old.

DSS-Induced Colitis

The drinking water of the mice was supplemented with 3.8% DSS (MP Biomedicals, USA) for 7 days, and then with sterile water for 2 days. Fecal samples and body weights were collected and measured every day, and the weights were recorded as a percentage relative to the initial weight. The disease activity index (DAI) was defined by cumulatively scoring the body weight loss (0: none; 1: 1%–5% weight loss; 2: 5%–10% weight loss; 3: 10%–20% weight loss; 4: >20% weight loss), the fecal characteristics (0: average; 2: loose stool; 4: diarrhea), and the fecal occult blood or hematochezia (0: no blood; 1: weak positive reaction of fecal occult blood test (FOBT); 2: strong positive reaction of FOBT; 3: visual pellet bleeding; 4: gross bleeding) [11]. The FOBT was determined using a urine fecal occult blood test kit (C027-1-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Euthanasia

Mice were anesthetized with intraperitoneally injected 1% sodium pentobarbital solution according to 0.5 mL/10 g before euthanasia by cervical dislocation. At the endpoint of the experiment, the spleen, colon, peritoneal cavity and blood were collected for subsequent analysis. The length of the colon from the cecum to the rectum was measured to determine the length. The colon tissue was processed and then used for histology.

Histological Analysis

Distal colon tissues were preserved in 4% paraformaldehyde for 24 h. The fixed tissues were cut using an ultramicrotome and stained with hematoxylin and eosin (H&E) (R20570, Shanghai Yuanye BioTechnology, Shanghai, China).

Preparation of Cell Suspensions from the Spleen, Peritoneal Cavity, and Colon

The spleen was suspended in red blood cell (RBC) lysis buffer (555899, BD Biosciences, Franklin Lakes, NJ, USA) and then in ice-cold phosphate buffer saline (PBS). Peritoneal cavity cells were collected directly by peritoneal lavage with 5 mL of PBS. Colonic cells were isolated following previous studies [6]. Briefly, the colon was isolated from fat and cut into 0.5 cm^3 pieces. After vortex shaking, tissue pieces of mice intestines were incubated with RPMI-1640 (11875093, ThermoFisher Scientific, Center Valley, PA, USA) with 2% fetal bovine serum (FBS, 04-007-1A, Biological Industries, Beit Haemek, Israel), 10 mM of 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 1 mM of DL-Dithiothreitol (DTT) (DTT-RO, Sigma-Aldrich, Rockville, MD, USA), and 5 mM of Ethylene diaminetetra acetic acid tetrasodium salt (EDTA) for 20 min, followed by enzymatic digestion with 1 mg/mL of collagenase IV (A004186-0100, Sangon Biotech, Shanghai, China) and 60 mg/mL of DNase I (11284932001, Roche, Basel, Switzerland) in RPMI-1640 (collagenase solution). The digested tissue was resuspended in ice-cold PBS and then used for further analysis.

FACS (Fluorescence-Activated Cell Sorter) Experiments

A suspension containing 1×10^6 isolated cells was used for the FACS experiment. All monoclonal antibodies, including CD16/32 (clone 93), CD3 (clone okt3), CD4 (clone okt4), CD25 (clone 4C9), IFN-γ (clone XMG1.2), B220 (clone RA3-6B2), immunoglobulin M (IgM) (clone II/41), F4/80 (clone CI: A3-1), Foxp3 (clone FJK-16s), TNF-α (clone MP6-XT22), CD11b (clone M1/70), CD38 (clone LS-C46310), Ly-6G (clone RB6-8C5), CD43 (clone S7), and IL-6 (clone MP5-20F3), were purchased from BioLegend and BD Biosciences. Cells were acquired on BD LSRFortessa, and FACS data were analyzed using FlowJo_V10 (BD Biosciences, San Diego, CA, USA).

ELISA (Enzyme Linked Immunosorbent Assay)

The concentrations of serum antibodies and cytokines were determined by ELISA according to our previous protocol [10]. The ELISA plates were incubated with 1% bovine serum albumin (BSA) for 1 h after being coated with anti-mouse IgM or IgG antibodies. Then, diluted serum samples were added and bound to the antibodies at 37 °C for 1.5 h, followed by washing and incubation with HRP-conjugated IgM (1020–05), IgG_1 (1070–05), IgG_{2b} (1090–05), IgG_{2c} (1079-04), or IgG_3 (1100–05) (Southern Biotechnology, Birmingham, AL, USA). The absorbance of the ELISA experiments was measured at 450 nm using an Ultra TMB-Blotting substrate system (Thermo Fisher Scientific Inc., Walkersville, MD, USA). The serum TNF-α assay kit and the standards of serum antibodies were purchased from BioLegend (430201, San Diego, CA, USA).

Serum Injection

According to our previous study [12], the serum was obtained from wild-type (WT) mice aged 8–12 weeks and injected intraperitoneally (150 μL per mouse) into the $PTIP^{-/-}$ mice every 3 days. The injection lasted for 3 weeks, and the induction of DSS started in the second week.

Transplantation

Splenic B-2 cells were isolated from the mice by immunomagnetic depletion with a-CD43 beads (Miltenyi Biotech, 130-091-333, Bergisch Gladbach, Germany). One day after DSS administration, 1×10^7 B cells from the WT and PTIP$^{-/-}$ mice were transplanted intravenously into the NCG recipient mice [13].

Statistical Analysis

GraphPad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA) was used for data analyses. All data are expressed as mean $\pm$ standard error of the mean (SEM). The statistics from the independent mouse groups were obtained by using the two-tailed unpaired *t*-test, applying Welch's correction to analyze the comparisons between groups. The comparison of the before-after study in the same mouse group was conducted by using the two-tailed paired *t*-test.

Results

PTIP Deficiency in B Cells Significantly Protects Mice from DSS-Induced Colitis

The body weight data and fecal samples from the mice were daily recorded. The weight loss started at 6 days after DSS administration in the two groups, but only the WT mice continued to lose weight rapidly. On the 10th day, the overall weight loss percentages of WT mice were about 2-fold of PTIP$^{-/-}$ mice ($p < 0.01$) (Fig. 1a). Hematochezia is an important indication of gastrointestinal (GI) bleeding. We performed the FOBT to determine the slight GI bleeding before the hematochezia was visible. Significant FOBT was observed in the WT group on the third day after DSS administration but not in the PTIP$^{-/-}$ group until the sixth day of DSS induction (Fig. 1b). With the aggravation of the disease, the WT + DSS mice showed the most intense increase in the fecal scores, which was higher than that in PTIP$^{-/-}$ + DSS mice before euthanasia ($p < 0.05$) (Fig. 1c). Only the WT + DSS mice displayed severe anemia after DSS inducement. As expected, the DAI scores representing the severity of the disease increased in both DSS-induced groups, but the WT + DSS mice showed a more robust increase ($p < 0.05$) (Fig. 1d). Together, these findings reveal that PTIP-deficient B-cell significantly protects mice from DSS-induced colony inflammation.

Ten days after DSS administration, the WT mice displayed a substantially shortened colon in contrast to the PTIP$^{-/-}$ mice ($p < 0.01$) (Fig. 1e). To confirm the microscopic alterations, the colon of DSS- and water-fed mice were stained with H&E. The colons isolated from both DSS-fed groups exhibited increased cellular infiltration and deformation of the crypts. However, these traits were more serious in the WT + DSS mice, which are characterized by the decrease of mucous-producing goblet cells and the destruction of crypts (Fig. 1f). A pronounced enhancement in serum glutamate aminotransferase (ALT) levels was only observed in the WT mice after DSS administration ($p < 0.01$) (Fig. 1g), indicating that the WT mice had more severe colitis.

PTIP Deficiency in B Cells Significantly Alters the Distribution of Immune Cells in Mice with DSS-Induced Colitis

To investigate the underlying cause of B cells lacking PTIP protecting against UC in mice, we analyzed the splenic immune cells by using multicolor flow cytometry. The frequencies of spleen B cells, T cells, and macrophages in PTIP$^{-/-}$ mice were comparable to those in WT mice ($p > 0.05$) (Fig. 2a). In addition, the significant difference of spleen B cells in the frequency and number were not observed in the two genotypes after DSS induced ($p > 0.05$) (Fig. 2b,c). However, in WT + DSS mice, a 2.5-fold decrease (20% vs. 50%) was observed in T-cell frequency as compared with WT mice, whereas that in the PTIP$^{-/-}$ and PTIP$^{-/-}$ + DSS mice was unchanged (Fig. 2a,c).

Given the high content of spleen T cells in the PTIP-deficient mice with UC, we examined the subsets of T cells. The PTIP$^{-/-}$ + DSS mice showed a markedly elevated frequency of CD4-T cells versus the WT + DSS mice ($p < 0.0001$) (Fig. 2c). As expected, the splenic CD4$^+$/CD4$^-$ ratio was significantly increased in the WT + DSS mice as compared with the PTIP$^{-/-}$ + DSS mice ($p < 0.05$) (Fig. 2d). In addition, compared with WT + DSS mice, a significant enhancement of the splenic Treg frequency was observed in the PTIP$^{-/-}$ + DSS mice ($p < 0.05$) (Fig. 2e).

The frequency of neutrophils and macrophages in spleens of WT + DSS mice decreased versus the PTIP$^{-/-}$ + DSS mice ($p < 0.05$), whereas that of M1-polarized macrophages (F4/80+CD11b+CD38+cells) was significantly increased ($p < 0.001$) (Fig. 2f,g). Moreover, CD38 is an essential marker of the splenic M1-polarized macrophages [14]. The CD38 increase was validated at the protein levels by FACS on macrophages from the WT + DSS mice ($p < 0.0001$) (Fig. 2h).

In UC, the damaged intestinal barrier destroys the balance between the immune cells and the commensal flora, and the intestinal mucosa becomes a site of intense immune activity. The PTIP$^{-/-}$ + DSS mice showed an increase in the frequency of colon B cells than the WT + DSS mice ($p < 0.001$), but no difference was observed between colon T cells and macrophages ($p > 0.05$) (Fig. 2i,j). In contrast to Tregs distribution in the spleen, Tregs in the colon increased in the WT + DSS mice as compared with PTIP$^{-/-}$ + DSS mice ($p < 0.01$) (Fig. 2k).

PTIP Deficiency in B Cells Significantly Alters Cytokine Levels in Mice with DSS-Induced Colitis

To investigate whether PTIP affects cytokine production in DSS-induced colitis through the regulation of B cells, we examined the secretion of TNF-α, IFN-γ, and IL-6 in colitis mice. After DSS inducement, the WT geno-

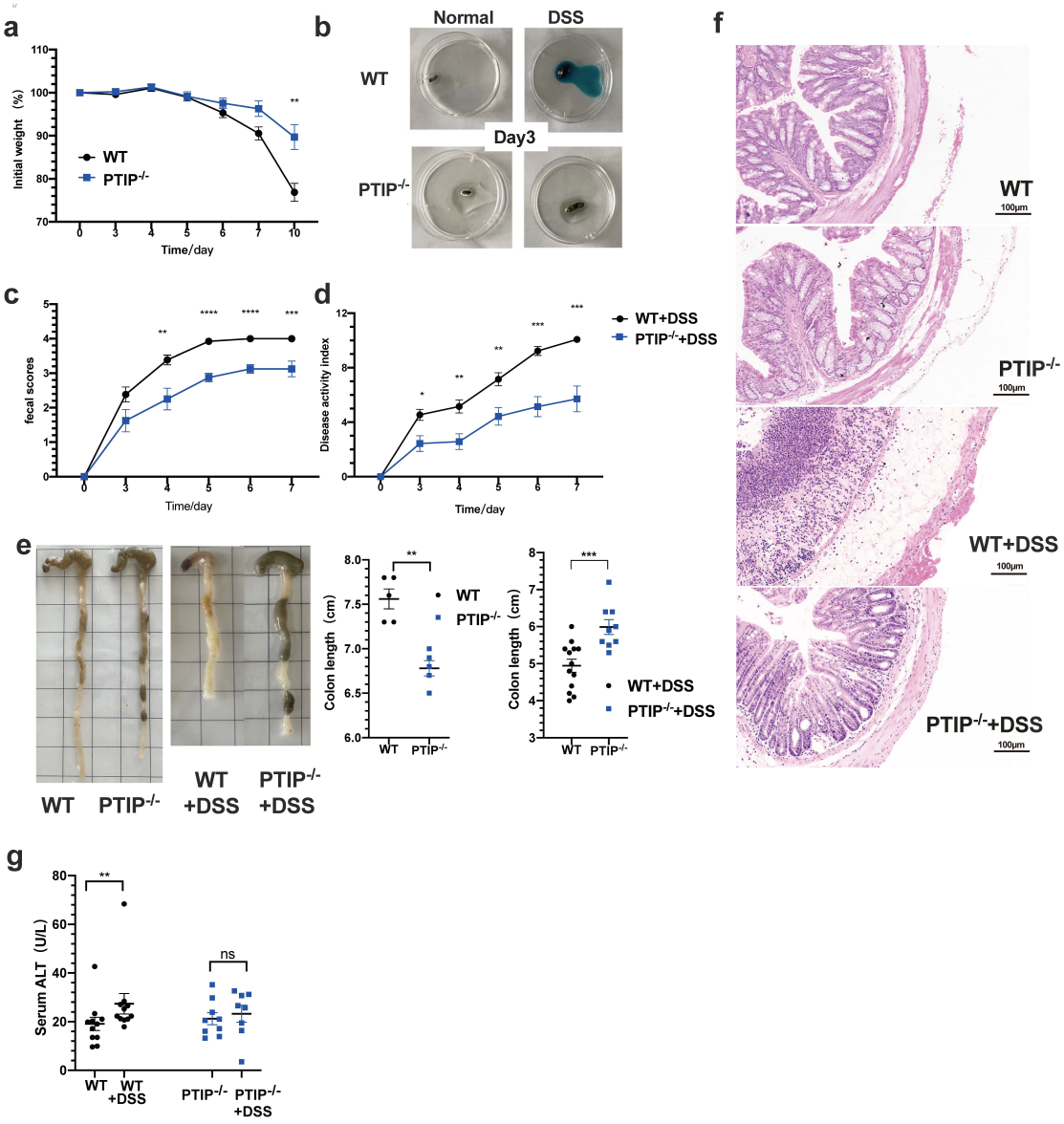

Fig. 1. PTIP deficiency in B cells significantly ameliorate DSS-induced colitis in mice. 3.8% DSS or sterile water was provided for 7 days (at least 9 mice per genotype). (a) Body weight. (b) FOBT on the third day of DSS-induced mice. (c) Fecal scores. (d) DAI scores. (e) Colon length. (f) Representative images of H&E-stained colon sections were captured at 17.9× magnification (Scale bar = 100 μm). (g) Serum ALT levels (paired *t*-test). Data are presented as mean ± SEM (n ≥5); a two-tail unpaired *t*-test was performed per group except in the comparison of serum ALT levels (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, no significance).

type mice exhibited remarkably higher serum TNF-α levels than the PTIP$^{-/-}$ mice ($p < 0.05$) (Fig. 3a). The B cells are mainly divided into B-1 cells (including B-1a and B-1b cells) and B-2 cells (including marginal zone-MZ B cells and follicular-FO B cells). We also detected the expression of TNF-α in organs and the expression in spleen macrophages and colon T cells from PTIP$^{-/-}$ + DSS mice decreased significantly as compared with WT + DSS mice ($p < 0.01$). By contrast, the colon macrophages and peritoneal cells (B-1a, B-1b, B2, T cells, large peritoneal macrophages-LPM) from PTIP$^{-/-}$ + DSS mice had higher levels of TNF-α than WT + DSS mice ($p < 0.05$) (Fig. 3b–d). Besides, IL-6 was only increased in colon T cells of WT + DSS mice ($p < 0.05$) (Fig. 3e and **Supplementary Fig. 1a**). Compared to WT + DSS mice, colon macrophages and peritoneal B-1b cells from PTIP$^{-/-}$ + DSS mice expressed higher levels of IFN-γ ($p < 0.01$), whereas colon T cells from PTIP$^{-/-}$ + DSS mice expressed lower levels ($p < 0.05$) (Fig. 3f,g). The difference in the expression of IFN-γ in splenic immune cells was not observed between groups (**Supplementary Fig. 1b**).

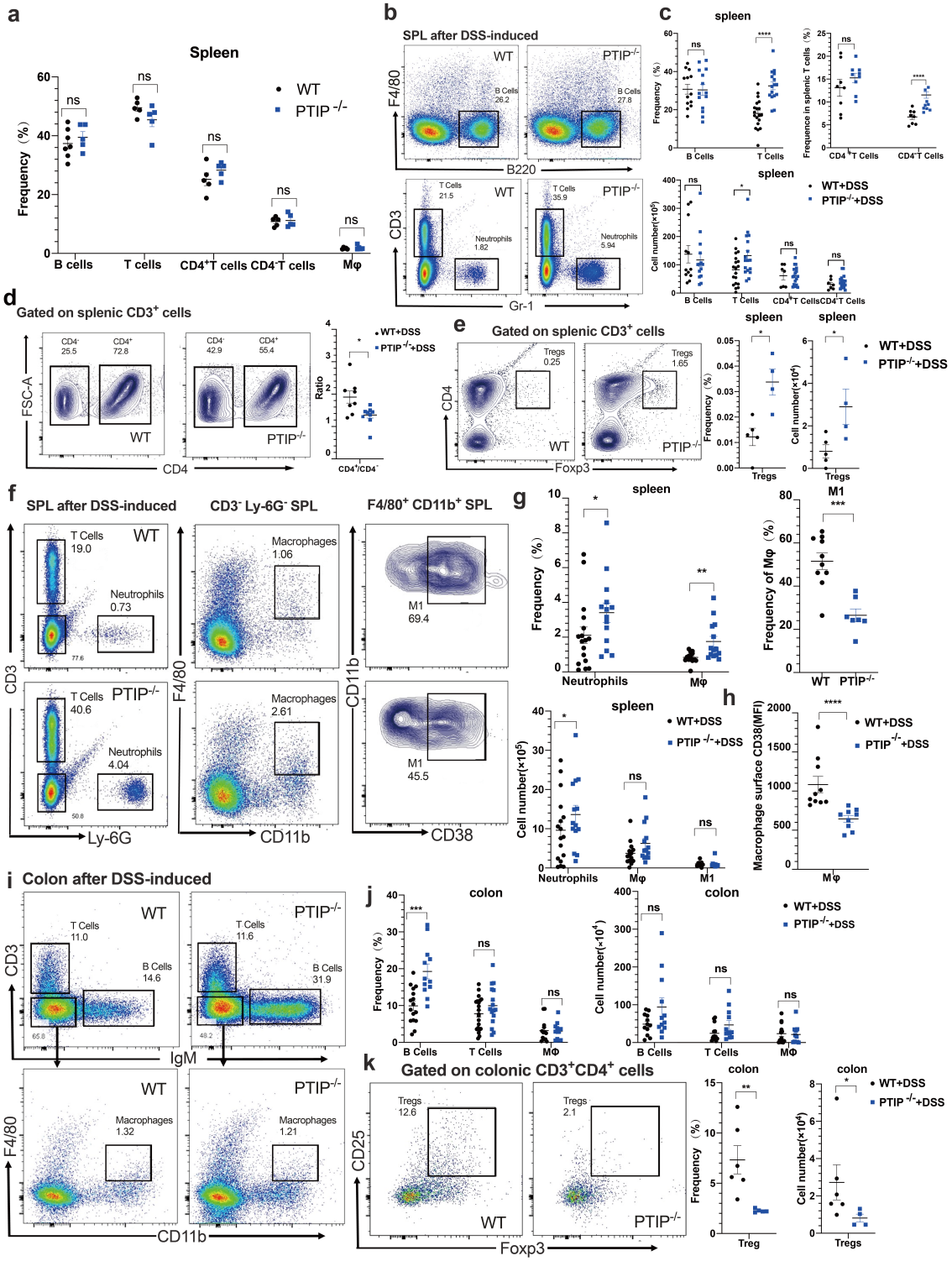

Fig. 2. PTIP deficiency in B cells significantly alters the distribution of immune cells in DSS-induced colitis mice. (a) Frequency of immune cells from spleens of normal mice. (b) Frequency and numbers of splenic B, T cells. (c) Flow cytometry analysis images of splenocytes. (d) Flow cytometry analysis of splenic T cells ($CD4^+$ and $CD4^-$), and the ratio of splenic $CD4^+/CD4^-$ T cells. (e) Frequency, numbers, and flow cytometry analysis of splenic Tregs. (f,g) Frequency, numbers, and flow cytometry analysis of splenic neutrophils and macrophages. (h) Surface expression of CD38 on macrophages. (i) Flow cytometry analysis of colonic cells. (j) Frequency and cell numbers of colonic B cells, T cells, and macrophages. (k) Frequency, cell numbers, and flow cytometry analysis images of colonic Tregs. Data are presented as mean ± SEM of at least 5 mice per genotype (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, no significance).

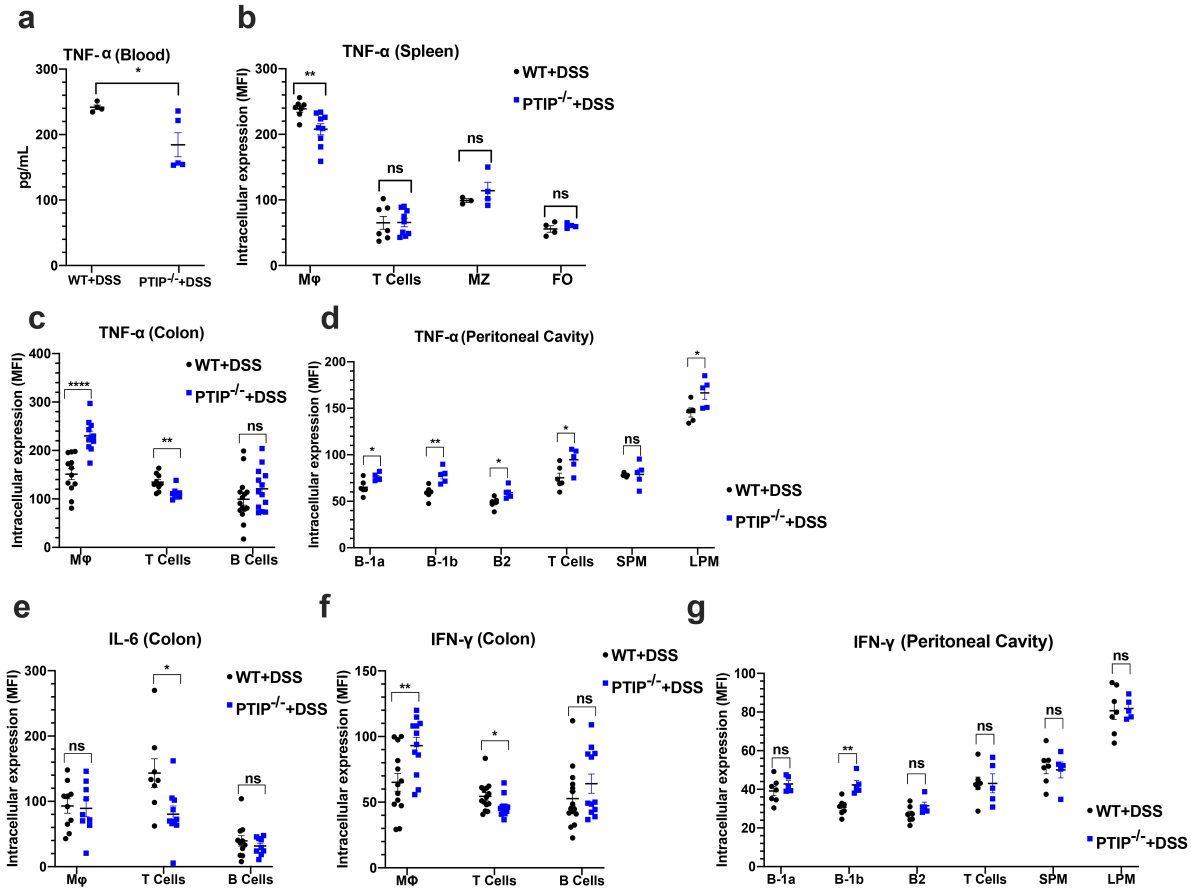

Fig. 3. PTIP deficiency in B cells significantly alters cytokine levels in colitis mice. (a) Serum TNF-α levels after DSS administration (paired *t*-test). (b–d) Intracellular expression of TNF-α in B cells (marginal zone [MZ] B, follicular [FO] B, B-1a, B-1b, and B-2 cells), T cells, and macrophage (small peritoneal macrophage-SPM and LPM). (e) Intracellular expression of IL-6 in colonic B, T cells, and macrophages. (f,g) Intracellular expression of IFN-γ in B cells (B-1a, B-1b, and B-2 cells), T cells, and macrophage (SPM and LPM). Data are expressed as mean ± SEM of at least 4 mice per group (*$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$; ns, no significance).

The Reinfusion of Serum Natural Antibodies does not Exacerbate DSS-Induced Colitis in PTIP$^{-/-}$ Mice

PTIP is required for the production of natural antibodies, which is a crucial part of innate immunity. In this study, PTIP$^{-/-}$ mice showed impaired IgG and IgM levels in steady-state [10]. IgG is thought to combine with symbiotic bacteria to activate the complement after epithelial barrier disruption occurs and interacts with FcγR to induce the production of proinflammatory factors [15]. We did not observe the change of serum IgM levels in mice after DSS administration ($p > 0.05$) (Fig. 4a). By contrast, IgG (IgG$_1$, IgG$_{2b}$, IgG$_{2c}$, and IgG$_3$) decreased significantly in the DSS-induced group ($p < 0.05$) (IgG$_{2a}$ serum levels were below the detection limit). The most profound decline was observed in WT colitis mice, with a 3-fold decrease in IgG$_1$ ($p < 0.001$) (Fig. 4b), suggesting that IgG antibodies are associated with the inflammatory immune response in experimental colitis.

To investigate whether the low levels of natural IgG in the PTIP$^{-/-}$ mice are responsible for the resistance to DSS-induced colitis, we intraperitoneally injected WT serum into PTIP$^{-/-}$ mice to increase the steady-state IgM and IgG. After DSS induction, IgM levels tend to increase in the WT group, but they did not reach statistical significance ($p > 0.05$), which was possibly due to the short half-life of IgM antibodies (Fig. 4c). The induction of DSS significantly decreased the serum IgG$_1$ levels of all groups ($p < 0.05$, Fig. 4d). In contrast to PTIP$^{-/-}$ mice, the significant increase of serum IgG$_1$ in PTIP$^{-/-}$ + WT serum mice demonstrated the effectiveness of serum reinfusion ($p < 0.05$) (Fig. 4d). However, WT serum injection did not aggravate DSS-induced colitis. A similar trend of weight loss was observed in both PTIP$^{-/-}$ + WT serum and PTIP$^{-/-}$ mice after DSS inducement, whereas WT mice showed increased weight loss (Fig. 4e). The increased DAI scores in the PTIP$^{-/-}$ + WT serum and the PTIP$^{-/-}$ mice were also smaller than those in WT mice after DSS inducement ($p < 0.01$) (Fig. 4f). Moreover, the colon lengths of the colitis mice were shortened in the WT mice compared with PTIP$^{-/-}$ + WT serum and the PTIP$^{-/-}$ mice ($p <$

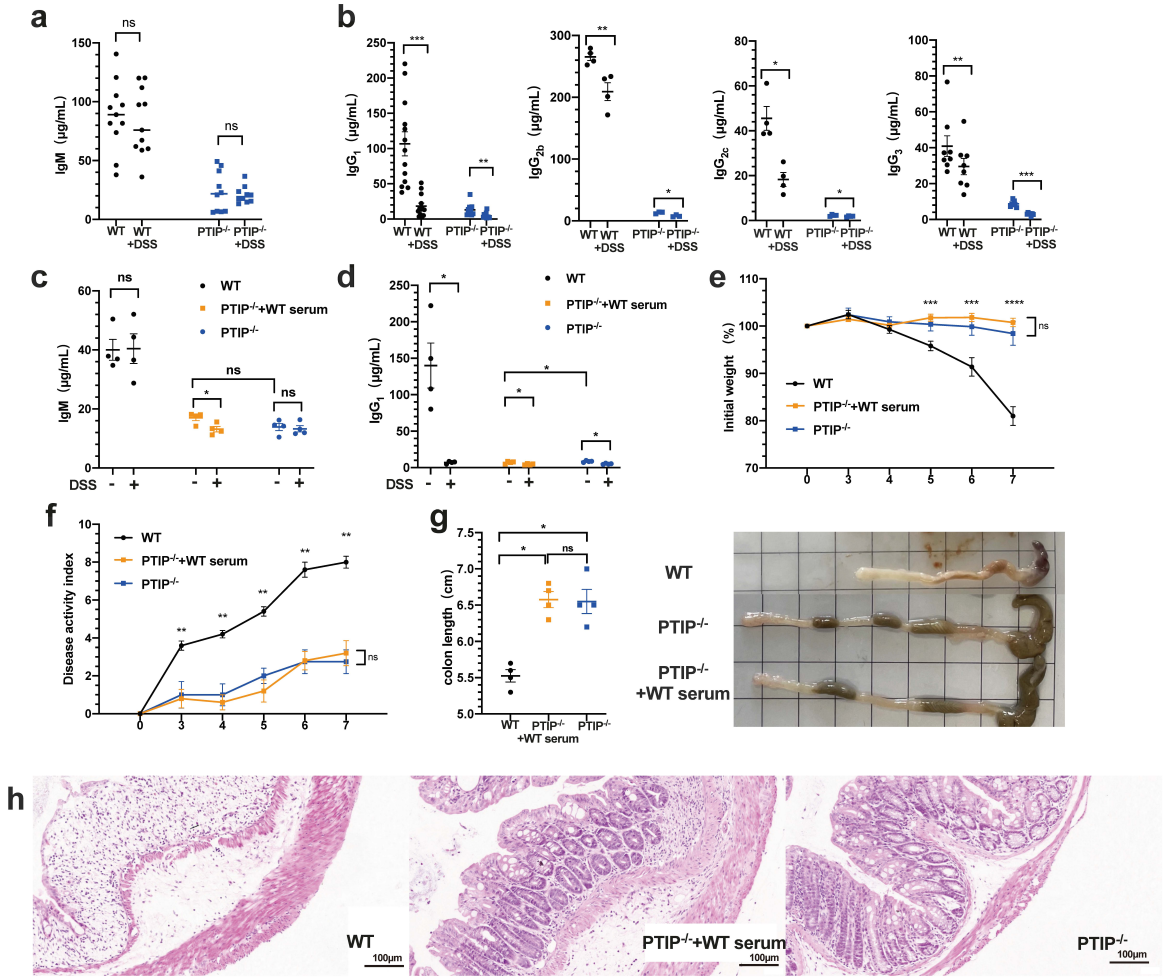

Fig. 4. The reinfusion of serum natural antibodies does not exacerbate DSS-induced colitis in PTIP$^{-/-}$ mice. (a) Serum IgM and (b) serum IgG alterations during the DSS-induced period (paired *t*-test). (c) The alters of serum IgM and (d) serum IgG_1 before and after DSS administration during the injection period (paired *t*-test). (e) Weight changes and (f) DAI scores after DSS-induced. (g) Colon length of mice. (h) Representative images of H&E-stained colon samples were captured at 17.9× magnification (unpaired *t*-test) (Scale bar = 100 μm). Data are presented as mean ± SEM of at least 4 mice per group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, no significance).

0.05) (Fig. 4g). Furthermore, the microscopic changes of the colon in the PTIP$^{-/-}$ + WT serum mice were similar to those in the PTIP$^{-/-}$ mice, showing partial loss of goblet cells and morphological abnormalities of the crypts (Fig. 4h).

PTIP Regulates DSS-Induced Colitis in Mice by Controlling B-2 Cells

According to a previous study, PTIP deficiency impairs B-1 cell establishment [10], but PTIP$^{-/-}$ mice showed strong protection against DSS-induced colitis. This leads us to infer that PTIP might regulate UC by controlling B-2 cells. To establish the direct function of B-2 cells in UC pathogenesis, we isolated B-2 cells from spleens by CD43 magnetic beads (B-1 cells are CD43^{+}). The 1×10^7 B-2 cells from PTIP$^{-/-}$ and control mice were transplanted intravenously into NCG recipient mice. The transplantation of CD43^{-} B-2 cells was performed 1 day after DSS administration. Similar to the PTIP$^{-/-}$ + DSS mice, the NCG mice transplanted with PTIP$^{-/-}$ B-2 cells showed significant resistance to UC. The onset of body weight loss was consistent in WT donor group after DSS administration, but PTIP$^{-/-}$ donor group displayed a significant weight recovery on day 6 ($p < 0.01$) (Fig. 5a). Furthermore, the WT donor group displayed stronger positive FOBT reactions and higher fecal scores than the PTIP$^{-/-}$ donor group, with massive hemorrhages in the gastrointestinal tract (Fig. 5b). Therefore, the DAI scores were dramatically increased in the WT donor group, with nearly twice that of the PTIP$^{-/-}$ donor group ($p < 0.05$) (Fig. 5c). As expected, the WT donor group displayed a significantly shortened colon compared to PTIP$^{-/-}$ donor group ($p < 0.05$) (Fig. 5d) and the loss of goblet cells and crypts in the H&E-stained colon sections (Fig. 5e).

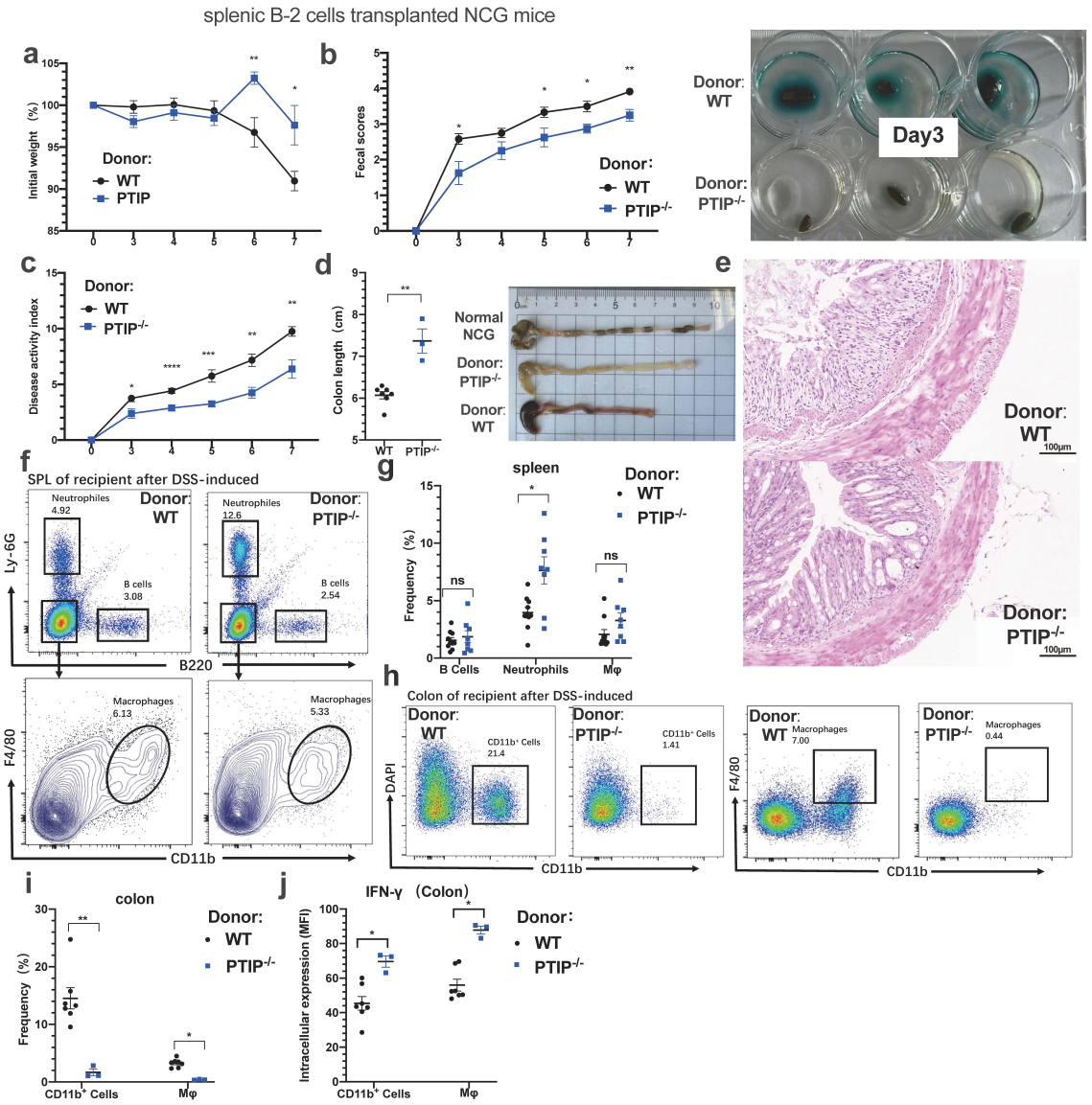

Fig. 5. PTIP regulates DSS-induced colitis in mice through B-2 cells. A day after DSS administration, NCG mice were intravenously administered 1×10^7 splenic WT or PTIP$^{-/-}$ B-2 cells. (a) Body weight. (b) Fecal scores and FOBT on the third day. (c) DAI scores. (d) Colon length. (e) Representative images of H&E-stained colon samples were captured at 17.9× magnification (Scale bar = 100 μm). (f) Flow cytometry analysis of splenocytes. (g) Frequency of splenic B cells, neutrophils, and macrophages. (h) Flow cytometry analysis of colonic cells. (i) Frequency of colonic CD11b$^+$ cells and macrophages. (j) The intracellular expression of IFN-γ in colonic CD11b$^+$ cells and macrophages. Data are presented as mean ± SEM, n ≥3. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, no significance).

To examine the role of PTIP in the regulation of B-2 in UC, we analyzed the immune cells of the spleen from the NCG recipient mice. Ten days after transplantation, the WT donor group and PTIP$^{-/-}$ donor group displayed a similar frequency in B cells and macrophages ($p > 0.05$). However, markedly increased neutrophils were observed in the PTIP$^{-/-}$ donor group compared with the WT donor group ($p < 0.05$) (Fig. 5f,g). Moreover, significantly fewer CD11b$^+$ cells and macrophages were observed in the colon obtained from the PTIP$^{-/-}$ donor group ($p < 0.05$) (Fig. 5h,i). The distribution of splenic and colonic macrophages in the B-2 cell recipient mice differed from that of the donor, whereas it was consistent in splenic neutrophils. Furthermore, we observed an enhancement of IFN-γ expression in colonic CD11b$^+$ cells and macrophages obtained from the PTIP$^{-/-}$ donor group ($p < 0.05$) (Fig. 5j), which was consistent with findings for the PTIP$^{-/-}$ mice. Thus, this regulatory capacity of PTIP in cytokine was also independent of the T cells.

Discussion

Dysregulated immune responses are among the major causes of UC [16]. In the last decade, attention has been focused on the function of T cells and macrophages in UC, but the role of B cells is still unclear. Previous studies have found that PTIP regulates B cell development and function [10]. However, whether the function of B cells in UC is associated with PTIP or not is uncertain. Therefore, we established a DSS-induced colitis mouse model in PTIP$^{-/-}$ mice to investigate the role of PTIP in UC. Mice received 3.8% DSS administration for 7 days; similar to UC patients, mice exhibited severe diarrhea, blood loss and colonic peristaltic dysfunction [17]. In this study, we demonstrate that PTIP deficiency in B cells protects mice from DSS-induced colitis. The PTIP-deficient mice displayed an altered distribution of neutrophils and T cells in UC; the transplantation of splenic B-2 cells from PTIP-deficient mice protected the recipient mice from severe UC. Moreover, we demonstrate, for the first time, that natural IgGs did not aggravate the pathology of UC. It has been provided that no sex predominance exists in UC [18]. Our findings in female mice are equally applicable to male mice.

In UC, the damaged colonic mucosa leads to a mass of intestinal immune cells to bind to multiple microbial antigens and activates the body's defense mechanism. We found a significant decline in splenic CD3$^+$CD4$^-$ T cells and a downward trend (no statistical significance) in colonic CD3$^+$ T cells in WT colitis mice, in contrast to a significant increase in colonic Tregs, versus PTIP$^{-/-}$ colitis mice. The data obtained from the WT mice are similar to those obtained from UC patients [5]. Accordingly, we speculate that activated spleen T cells leave the spleen through the peripheral blood, and massive CD3$^+$CD4$^-$ T cells die when the colon is injured. Compared with WT mice, PTIP$^{-/-}$ colitis mice displayed a markedly increased frequency of splenic macrophages and a significant decline in M1-type pro-inflammatory macrophages. Besides, B cells can cooperate with Tregs to protect mice from DSS-induced UC and can also regulate macrophage phenotype in some autoimmune diseases and cancers [13,19]. Therefore, the effect of PTIP on colitis may involve the cooperation between B cells, T cells and macrophages. These assumptions should be further investigated.

According to our previous study, PTIP contributes to the establishment of steady-state IgG and IgM [10]. Anticommensal IgG activates the FcγR receptor, leading to IL-1b production and aggravation of UC [6]; an increase in IgG levels was observed in the intestinal mucosa from UC patients [20]. However, the function of natural IgM and IgG antibodies in UC is unclear. The development of DSS-induced acute UC is related to innate immune activation [21]. Natural antibodies, especially IgM, as an important part of innate immunity, plays an established role in immune surveillance. Surprisingly, the serum levels of IgM did not change after DSS inducement, while the IgG levels decreased both in our WT and PTIP$^{-/-}$ mice. Indeed, patients with fulminant UC might experience a transient decrease in plasma IgG, which was considered to be linked to severe diarrhea [22]. The PTIP$^{-/-}$ mice impair the establishment of the steady-state IgG and IgM. By transfusing the WT serum into PTIP$^{-/-}$ mice to increase their serum levels of IgG, we found that the natural IgG levels did not exacerbate UC. The decreased serum levels of IgG in WT mice induced by DSS might be associated with severe diarrhea, which is consistent with the patients with fulminant UC.

According to previous findings, B-1 cells protect mice against colitis [23], whereas PTIP deficiency impairs B-1 cell establishment [10]; strong resistance to UC was observed in PTIP$^{-/-}$ mice. We speculate that B cell subpopulations have distinct ramifications in UC. The B-2 cells, especially their FO B cell subset, are the largest B cell populations in the body. By transplanting PTIP$^{-/-}$ B-2 cells, recipient mice acquired resistance to UC, NCG recipient mice were lack of B, T, and NK cells, which reveals that PTIP regulates the splenic neutrophils by controlling B-2 cells independently from T and natural killer (NK) cells, demonstrating the regulatory mechanism of PTIP on the UC by controlling B-2 cells. The distribution of neutrophils in the spleen of recipient mice was consistent with that of the donors, indicating that PTIP regulates splenic neutrophils by controlling B-2 cells. In another study, B cells regulated colitis by cooperating with Tregs [13], and the involvement of neutrophils in colitis seems to be T-cell-dependent [24]. However, our recipient NCG mice had no T cells, and therefore, PTIP regulated UC by controlling B-2 independently from T cells. The underlying mechanism is, however, still unclear, and requires further investigation.

Conclusions

In conclusion, this study demonstrates the crucial role of PTIP in UC that PTIP deficiency could protect mice from DSS-induced colitis. The mechanism may be related to the altered immune cell distribution and serum cytokine levels by PTIP through controlling B-2 cells independently from T cells. Our study may bring new insights and therapeutic targets for the clinical treatment of UC.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

JXL: Investigation, Methodology, Data curation, Writing-Original draft preparation. YQX: Investigation, Methodology. YQC: Resources, Funding acquisition, Conception and Design. DS: Writing-Reviewing and Editing,

Conceptualization, Methodology, Funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal procedures in this study were approved by the Animal Ethics Committee of Jiangnan University (permission JN. No 20220330t10007[111]).

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202335176.35.

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