



# Autotaxin-Lysophosphatidic Acid Axis Blockade Improves Inflammation by Regulating Th17 Cell Differentiation in DSS-Induced Chronic Colitis Mice

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**Abstract**—Autotaxin-lysophosphatidic acid (ATX-LPA) axis is closely associated with several inflammation-related diseases. In the colonic mucosa of patients with chronic ulcerative colitis (UC), the expression of ATX and the percentage of Th17 cells are found to increase. However, it is unclear whether ATX-LPA axis affects the differentiation of Th17 cells in chronic UC. To investigate whether ATX-LPA axis contributes to Th17 cell differentiation, a mouse model of chronic UC was established by drinking water with DSS at intervals. ATX inhibitor was used as an intervention. The disease active index (DAI), colonic weight to length ratio, colon length, colon histopathology, and MAdCAM-1 were observed. Additionally, the expression of ATX, LPA receptor, CD34, IL-17A, IL-21, IL-6, ROR- $\gamma$ t, STAT3 in colonic tissue, and the percentage of Th17 cells in spleens and mesenteric lymph nodes (MLNs) were measured using different methods. ATX blockade was able to relieve symptoms and inflammatory response of DSS-induced chronic colitis. The DAI and colonic weight to length ratio were apparently decreased, while the colon length was increased. The pathological damage and colitis severity were lighter in the inhibitor group than that in the DSS group. Inhibiting ATX reduced the expression of ATX, LPA receptor, and CD34 and also decreased the percentages of Th17 cells in spleens and MLNs and the expressions of IL-17A and IL-21, as well as the factors in Th17 cell signaling pathway including IL-6, ROR- $\gamma$ t, and STAT3 in colonic tissue. ATX-LPA axis blockade could alleviate inflammation by suppressing Th17 cell differentiation in chronic UC.

**KEY WORDS:** ulcerative colitis (UC); autotaxin (ATX); lysophosphatidic acid (LPA); Th17 cell differentiation.

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## INTRODUCTION

Ulcerative colitis (UC), also known as nonspecific chronic colonic disease, is a recurrent mucosal ailment that is clinically characterized by abdominal pain and bloody diarrhea. The main pathological changes of UC are multiple ulcerative lesions and inflammation in the rectum and colon, which is diagnosed using colonoscopy and histological findings [1]. The incidence rate of colorectal cancer among patients with UC is significantly increased, which is positively correlated with the time of illness. In recent years, the morbidity of UC-induced colorectal cancer is

obviously increased when compared with that of sporadic colorectal cancer among the Asian population who used to be at a low risk of UC, which again indicates a growing incidence of UC [2, 3]. Now, the exact etiology of UC is still not known completely, but accumulating evidences suggest that environmental factors, genetic susceptibility, and dysregulation of immune responses are all closely related to the pathology of UC, and the most important factor is intestinal mucosal immune disorder [1, 4, 5].

Our previous research has shown that Th17 cells conduce to intestinal immune disorder in mice with dextran sodium sulfate (DSS)-induced acute colitis [6]. Th17 cells, a subset of CD4+ T lymphocytes, play a pivotal role in the induction and persistence of chronic inflammation by secreting interleukin 17A (IL-17A) [7–9]. A growing number of studies reveal that Th17 cells play an important role in the pathogenesis of several inflammatory diseases, including autoimmune arthritis [10] and experimental autoimmune myocarditis [11]. Th17 cells are differentiated from naive T cells under the condition of several cytokines and transcription factors *via* the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, particularly STAT3 [12]. In the process of Th17 cell differentiation, the retinoid-related orphan receptor gamma-t (ROR- $\gamma$ t) is an essential transcription factor [13] and IL-6 is the most potent cytokine [14]. Many studies have suggested that IL-6 accompanied by TGF- $\beta$  are necessary for the induction of Th17 cells *in vitro* [6, 15], while another report indicates that IL-6 is the only requirement [16].

Lysophosphatidic acid (LPA) is a small and naturally occurring active lipid, which is involved in several cellular functions by binding to its receptors [17]. The main approach of LPA production *in vivo* is associated with autotaxin (ATX), which is the unique enzyme with lysophospholipase D activity among nucleotide pyrophosphatases/phosphodiesterases (NPPs) family [18]. Therefore, ATX and LPA are generally regarded as a biological axis, ATX-LPA axis. There are seven LPA receptors (LPA<sub>1–7</sub>) distributed on different cell surfaces and their downstream signaling pathways are also different [19, 20]. A body of evidences suggests that ATX-LPA signaling is involved in a variety of physiological processes of cells, such as migration, differentiation, and proliferation [21], and pathological processes including cancer, chronic inflammation, and fibrosis [22–24]. In pathological conditions, the expressions of ATX and LPA receptors are significantly upregulated in the tissue [20]. Although ATX-LPA axis has been widely studied in several diseases, its role in UC remains to be elucidated. A recent study shows that in colonic tissue of patients with chronic inflammatory

bowel disease (IBD) and DSS-induced chronic colitis mice, ATX is aberrantly expressed along with high endothelial venules (HEVs) and HEV-like vessels, which are also the source of ATX in the gut, while they are not significantly increased in the tissue of acute colitis model of mice [25]. However, the mechanism of ATX-LPA axis in chronic colitis is not fully revealed yet. Considering the role of ATX-LPA axis and Th17 cells in the disease-causing process of UC, we hypothesized that ATX-LPA axis might be able to modulate the differentiation of Th17 cells.

In this study, we aim to investigate the relationship between ATX-LPA axis and Th17 cell development in the pathological process of DSS-induced chronic colitis in mice. Our data showed that ATX-LPA axis did aggravate inflammation responses, and inhibition of ATX-LPA axis could alleviate chronic colitis by restraining the differentiation of Th17 cells as well as decreasing the source of ATX.

## METHODS

### Experimental Animals

7~8-week-old male Balb/c mice were purchased from the experimental animal center of Huazhong University of Science and Technology (HUST, Wuhan, China) and raised under specific pathogen-free (SPF) conditions with free access to food and water, 12-h light-dark cycles, as well as relatively constant humidity and temperature. All the procedures and care of the laboratory animals were strictly in accordance with the guidelines of the Animal Research Institute Committee of HUST and approved by the Institutional Animal Care and Use Committee (IACUC) of HUST.

### Model of Chronic UC

Three-cycle DSS (molecular weight of 36,000–50,000; MP Biomedicals, Illkirch, France) treatment was used to induce chronic UC of mice according to the description of Wirtz et al. [26] with a minor modification. During each cycle, 2.5% (wt/vol) DSS was supplemented in drinking water for 7 days, followed by a 7-day interval of drinking purified water. From the beginning of treatment with DSS, the clinical features including body weight, stool consistency, and occult blood were daily monitored to evaluate the disease activity index (DAI) as described previously [27].

### Animal Treatment

Thirty mice were randomly assigned to three groups ( $n = 10$  per group): control group, DSS group, and inhibitor group. After 1 week of adaptive feeding, mice in DSS group and inhibitor group were disposed with three-cycle DSS, while mice in the normal group were given purified water. Bithionol (Selleck chem, Beijing, China), a selective inhibitor of ATX, was chosen as an intervention on ATX-LPA axis according to the previous description [25, 28]. More specifically, 1 mg (per body) of bithionol was dissolved in 25- $\mu$ L DMSO that was finally diluted to a concentration of 5% with PBS solution for eliminating toxic and side effects. Mice of the inhibitor group were injected with 0.5-ml bithionol solution intraperitoneally every other day from the beginning of colitis induction, while mice of the DSS group were injected with 0.5 ml DMSO solution (5%) intraperitoneally at the same time.

### Assessment of Inflammation

At the end of the third cycle treatment of DSS, all colons of the mice were detached after they were euthanized through cervical dislocation. And the lengths of the colons were measured, then all the colons were rinsed with PBS to remove feces, and weighted after draining with filter paper. Parts of colonic tissue were resected and fixed in buffered formalin, then embedded in paraffin, sectioned, and stained with hematoxylin-eosin (HE). Histological evaluation was performed according to the criteria described by Teruji Totsuka et al. [29], which is graded from 0 to 3 in every aspect depending on mucosa damage, submucosal damage, and myenteric damage.

### Western Blotting

RIPA lysis buffer supplemented with a protease inhibitor cocktail (Goodbio Technology, Wuhan, China) was used to extract protein from colon samples. Then, the protein concentrations were detected by BCA protein assay kit (Goodbio Technology). An equal amount of protein from each sample was separated on SDS-PAGE gels and transferred to PVDF membranes under proper voltage. Each membrane was blocked with 5% skimmed milk and incubated with anti-ATX antibody (1:1000; Abcam, Cambridge, UK), anti-MAdCAM-1 antibody (1:1000; Abcam), respectively, overnight at 4 °C, then washed with TBST thrice and incubated with peroxidase-conjugated secondary antibody at room temperature for 1 h. After being washed, the bands were visualized and analyzed, and  $\beta$ -

actin and GAPDH were used as controls to standardize the protein expression, respectively.

### Real-Time PCR for mRNA

Trizol (TaKaRa, Shiga, Japan) was used to isolate total RNA from colonic tissue. All primer sequences are listed in Table 1. Complementary DNAs (cDNAs) were generated by reverse transcription using PrimeScript™ RT Master Mix (TaKaRa). Then, the SYBR Premix Ex Taq™ (TaKaRa) was used for real-time quantitative PCR (RT-PCR) analysis. The expression of mRNAs was normalized to  $\beta$ -actin and the relative expression of target genes was calculated with the  $2^{-\Delta\Delta C_t}$  method.

### Immunohistochemistry

Slices of paraffin-embedded colonic tissue were immersed in dimethylbenzene and different densities of alcohol to elute paraffin, then performed with repairing antigens, blocking endogenous peroxidase, and reducing non-specific staining sequentially. Slices were incubated with primary antibody: anti-CD34 (1:100; Abcam) at 4 °C overnight, followed by a secondary antibody for 20 min. After washing, slices were added with fresh diaminobenzidine (DAB) color liquid and then counterstained with hematoxylin. Optical microscope was used to observe and analyze the images of immunohistochemistry (IHC).

### Enzyme-Linked Immunosorbent Assay

Colonic tissue was homogenized in saline solution at the proportion of 0.1 g/1 mL. Then, the supernatants were collected for concentration detection of IL-6, IL-17A, and IL-21. Sandwich enzyme-linked immunosorbent assay (ELISA) was performed according to the protocol of the manufacturer with the corresponding ELISA kit (NeoBioscience, Shenzhen, China) as described previously [27].

### Flow Cytometry

To measure the percentage of Th17 cells in CD4<sup>+</sup>T cells derived from spleens and MLNs, all spleens and MLNs were isolated from mice to make cell suspension as described previously [30]. First, cells were treated with ionomycin (Abcam, Cambridge, UK) and phorbol myristate acetate (PMA) (Abcam) in a dark place for 1 h, then treated with GolgiPlug protein transport inhibition (BD Biosciences, San Diego, USA) and kept in 5% CO<sub>2</sub> at 37 °C for 6 h. Next, cells were stained with FITC-anti-CD4 antibody (BD Biosciences) for 15 min, treated with fixation and permeabilization (BD Biosciences) for 20 min,

**Table 1.** Primer Sequences for RT-PCR

Gene		Primer sequences (5'-3')
β-actin	Sense	GTGACGTTGACATCCGTAAAGA
	Anti-sense	GTAACAGTCCGCCTAGAAGCAC
ATX	Sense	TTCCTGTATGGACGACCTGC
	Anti-sense	GAGACCTCAGCCTGCTTAGAAA
LPA <sub>2</sub>	Sense	TTCTGCGGGAAGGGAGTATG
	Anti-sense	CACGGTGGAGCAGCTAGACA
MAdCAM-1	Sense	AACGCTGGCGGTTACCCT
	Anti-sense	GTGATGTTGAGCCCAGTGGGA
IL-17	Sense	TCAGACTACCTCAACCGTTCCA
	Anti-sense	CAGCTTTCCCTCCGCATT
IL-21	Sense	AGACTTCGTCACCTTATTGACATTG
	Anti-sense	GGTTTGATGGCTTGAGTTTGG
IL-6	Sense	ACAACCACGGCCTTCCCTA
	Anti-sense	TCAGAATTGCCATTGCACAAC
STAT3	Sense	AGGAGGGTCACTTTCACCTG
	Anti-sense	GGAATGTCGGGGTAGAGGTA
ROR-γt	Sense	TCAGGAGTGCTTACTGTCCGGTC
	Anti-sense	AGTTCTTCGGGCTGGAAT

and stained with PE-anti-IL-17A antibody (BD Biosciences) for 30 min. All the operations were performed in dark. After being washed, stained cells were used for analysis.

### Statistical Analysis

SPSS 22.0 software was used to analyze the data. All data were presented as mean ± standard deviation (SD). The statistical significance was determined using one-way ANOVA followed by Dunnett's test.  $P < 0.05$  was considered to be statistically significant.

## RESULTS

### ATX Blockade Attenuated the Symptoms of DSS-Induced Chronic Colitis

The damage of colon caused by DSS is analogous to pathological change of human colitis. To evaluate the effect of ATX blockade on chronic UC, we observed and recorded weight loss, diarrhea, and bloody stools of mice in all groups daily from the beginning of model establishment. When compared with the control group, the DAI scores were significantly increased in mice treated with DSS, but they were significantly decreased by the intervention of ATX inhibitor (Fig. 1a). Mice administrated with DSS at intervals turn to the model of chronic colitis characterized by shortened and fibrotic colons, and the changes of the colons eventually lead to a rise in colonic weight to length

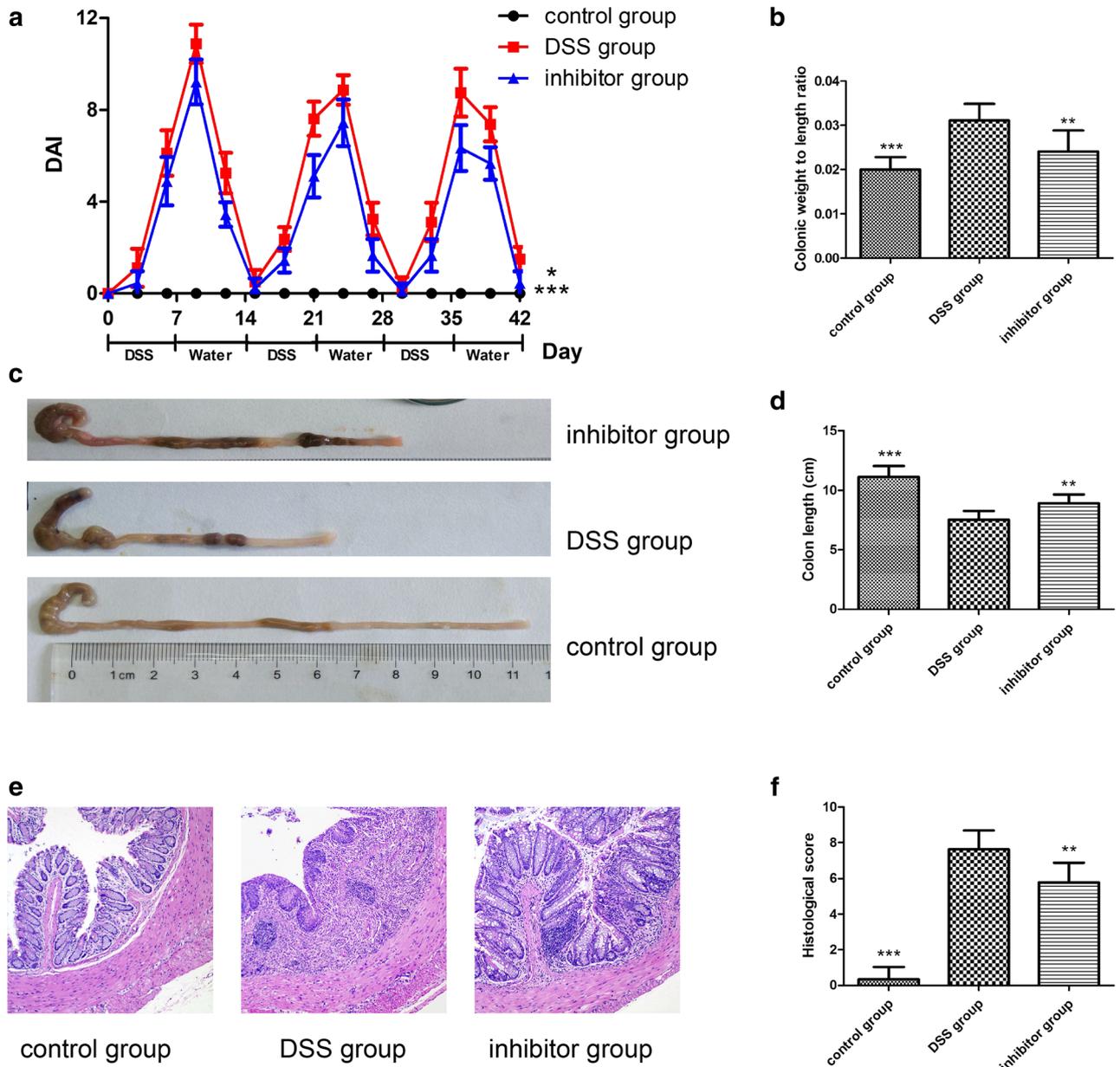
ratio, which is also a parameter to assess the severity of chronic colitis. The colonic weight to length ratio of the DSS group was the highest but dramatically declined in the inhibitor group (Fig. 1b). Furthermore, the reduction of colon length caused by DSS was alleviated by the ATX inhibitor (Fig. 1c, d).

### Suppression of ATX Ameliorated Inflammatory Response of Chronic Colitis

Histological analysis was used to evaluate inflammatory conditions of colons in all groups. As shown in Fig. 1e, there was no damage in colon tissue of the control group, and the slices of DSS group showed thickening of the colonic wall, infiltration of the inflammatory cell, and reduction of the crypts, while all those traits in slices were alleviated in the inhibitor group. Correspondingly, the DSS group showed the highest histological score followed by the inhibitor group (Fig. 1f). In chronic colitis, the main adhesion between intestinal epithelial cells is closely related to mucosal address cell adhesion molecule-1 (MAdCAM-1), which is decreased when the inflammation is relieved [25, 31, 32]; so, MAdCAM-1 could reflect the severity of inflammation. In the DSS group, the protein level of MAdCAM-1 in colon was obviously increased but decreased in the inhibitor group (Fig. 2a, c). We also detected the expression of MAdCAM-1 with RT-PCR and the level of mRNA in the DSS group was 1.89-fold of that in the inhibitor group and 39.68-fold of that in the control group (Fig. 2f). In short, these outcomes indicate that inhibition of ATX could prevent intestinal mucosal inflammatory response in chronic UC.

### Effect of ATX Inhibition on the Expression of ATX, LPA Receptor, and HEV-Like Vessel

To analyze the expression of ATX in chronic colitis mice, we detected ATX using Western blotting and RT-PCR. As shown in Fig. 2a, b, and d, the ATX expression was significantly increased in the DSS group when compared with that in the control group, whereas it was lower in the inhibitor group than that in the DSS group. Subsequently, because the hydrolysis of LPA is so rapid that it is difficult to detect LPA directly [25, 33], we explored the expression of LPA receptor, LPA<sub>2</sub>, and the main LPA receptor in the intestine, by RT-PCR. And the outcomes were the same as that of ATX (Fig. 2e). ATX is constitutively expressed in the endothelial cells of HEV-like vessels [25]. In colonic tissue of chronic colitis, a large number of HEV-like vessels appear; so, the generation of ATX increases as well. The biomarker of HEV-like vessels is



**Fig. 1.** The effect of ATX-LPA axis on the damage of colon in DSS-induced colitis mice. **a** The DAI score. **b** Colonic weight to length ratio. **c** Representative photos of colons. **d** Colon length. **e** Histological analysis ( $\times 100$ ) of colonic tissue. **f** Histological scores of colonic tissues. Each bar represents mean  $\pm$  SD ( $n = 8$ ).  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ , vs. DSS group.

CD34. Therefore, we detected the expression of CD34 by IHC. As depicted in Fig. 2g, the expressions of CD34 were lower in the inhibitor group than that in the DSS group. Taken together, inhibition of ATX could decrease the LPA receptor, improve inflammation, and reduce HEV-like vessels, thereby decreasing the production of ATX in return.

#### Inhibition of ATX Reduced the Proportion of Th17 Cells in Spleens and MLNs

To investigate the effect of ATX-LPA axis on the differentiation of Th17 cells, we used flow cytometry to examine the percentage of Th17 cells in  $CD4^+$ T cells in

spleens and MLNs isolated from mice of all groups (Fig. 3a, b). The proportions of Th17 cells were all increased in both spleens and MLNs of two DSS-treated groups, but the changes in DSS group were more significant than that in the inhibitor group.

### Effect of ATX Blockade on Th17 Cell Function and Signaling Pathway

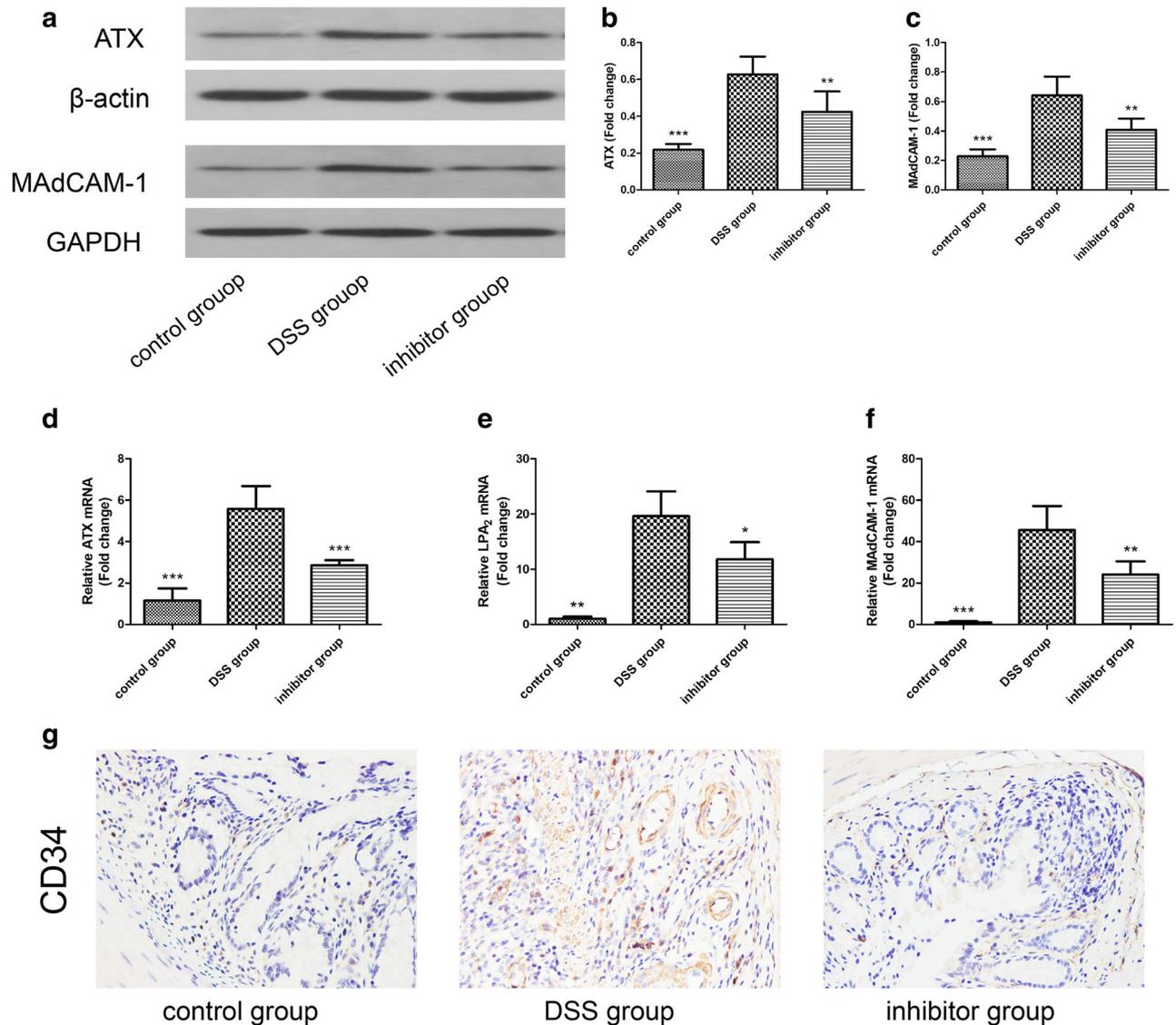
To explore the function of Th17 cells, we measured symbolic cytokines secreted by them. In many pathological processes, IL-17A induces and maintains the inflammation, while IL-21 amplifies it [34]. We detected the expressions of IL-17A and IL-21 with RT-PCR and ELISA. The treatment of DSS increased the mRNA expressions of both IL-17A and IL-21 in colonic tissue, but they were obviously decreased in the inhibitor group when compared with those in the DSS group (Fig. 4a, b). The concentrations of IL-17A and IL-21 in colonic tissue homogenate supernatants showed the same tendency as that revealed by RT-PCR (Fig. 4c, d). Subsequently, to investigate whether ATX-LPA axis acts on Th17 signaling pathway in chronic colitis mice, we chose to examine the expressions of IL-6, STAT3, and ROR- $\gamma$ t in colon. As shown in Fig. 4e, the expression of IL-6 mRNA was significantly increased in the DSS group and the inhibitor group, while the level of IL-6 mRNA in the DSS group was 1.77-fold of that in the inhibitor group, and so was the trend of IL-6 concentration in colonic tissue, which was measured by ELISA (Fig. 4f). Administration of DSS enhanced the expressions of STAT3 mRNA and ROR- $\gamma$ t mRNA, but the level of STAT3 mRNA and ROR- $\gamma$ t mRNA in the inhibitor group was apparently decreased when compared with that in the DSS group (Fig. 4g, h). In general, our results reveal that blocking the ATX-LPA axis hampers the function of Th17 cells and inhibits the Th17 cell signaling pathway.

## DISCUSSION

Ulcerative colitis (UC) is a complicated disease characterized by a progressive or chronic remittent inflammatory condition of the intestine and its etiology is still unknown [35]. Although current treatments including 5-ASA and biological preparation have certain effects, there is quite a high rate of recurrence of UC as well as the side effect of those therapies after long-term usage [36]. So, it is urgent to elucidate the pathogenesis of chronic UC and find some more effective therapeutic methods.

Pro-inflammatory cells such as Th1 cells and Th17 cells play a pivotal role in the pathology of UC [37]. In recent years, the special role of Th17 cells on the pathology of UC has received more and more attention. Extensive evidences show that the percentage of Th17 cells is increased in various animal models of UC, and the main effector of Th17 cells, IL-17A, is upregulated in the tissue of colitis as well [37, 38]. In our model of chronic colitis, the administration of DSS also increased the percentage of Th17 cells as well as the expressions of IL-17A and IL-21 significantly. Xu et al. [6] demonstrated that knockdown miR-155 in DSS-induced mice could bring relief to the inflammatory response by inhibiting the differentiation of Th17 cells, thereby alleviating UC. Therefore, it is convincing that restraining the differentiation of Th17 cells could relieve chronic UC, and the pathways of modulating Th17 cells differentiation might be promising therapeutic targets.

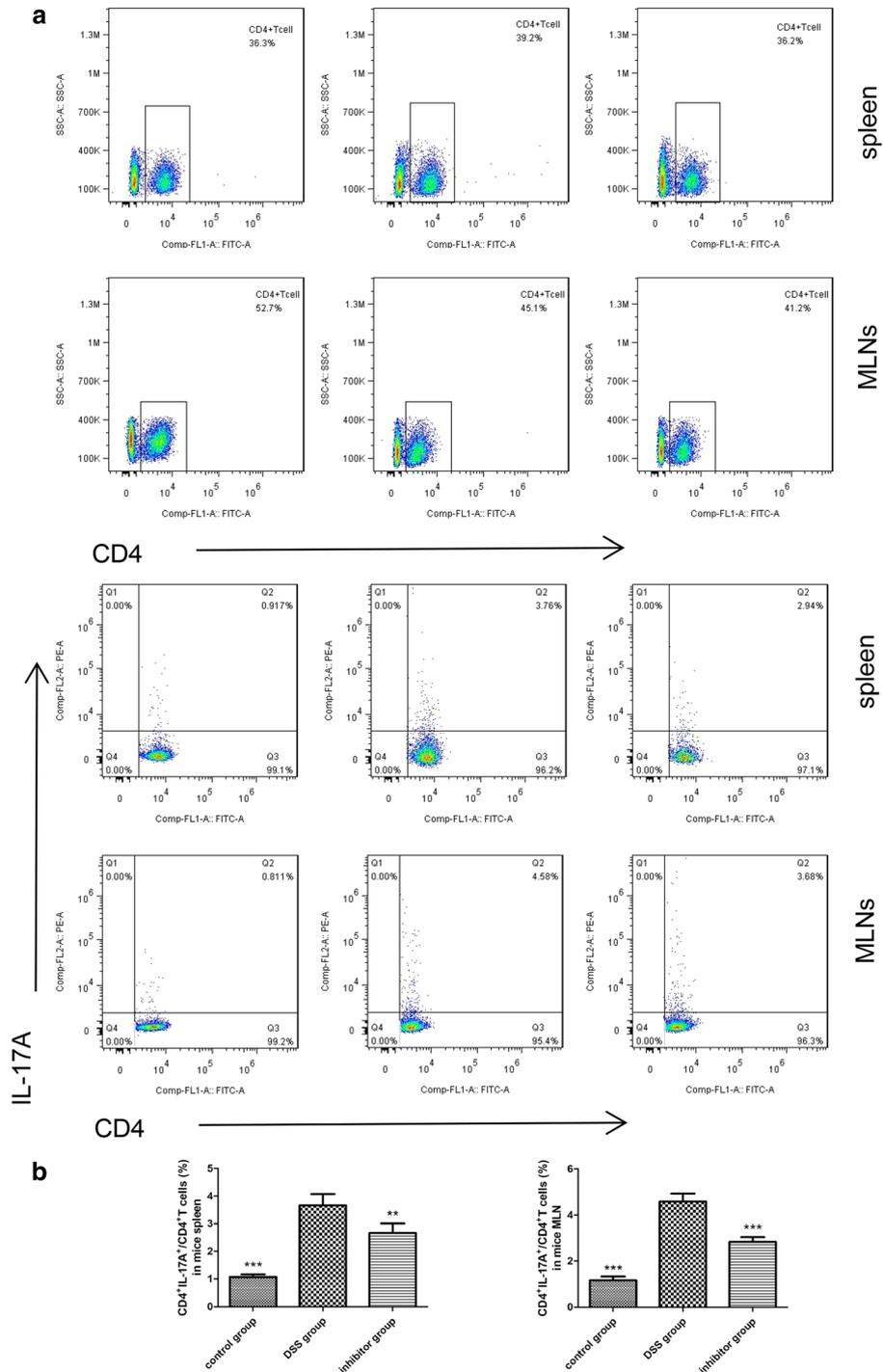
In this study, the results of our experiments indicated that the expressions of ATX and LPA receptor were increased along with the proportion of Th17 cells in mice model of chronic colitis, and ATX blockade downregulated the percentage of Th17 cells. ATX, first found in the liquid supernatant of melanoma cells and named because of its ability to accelerate the movement of cells, plays an important role in the generation of LPA [39]. ATX-LPA axis participates in several cellular processes by combining with diverse LPA receptors, which are distributed mainly on the surface of lymphocytes, fibroblasts, smooth muscle cells, and epithelial cells [20]. The activation of different LPA receptors stimulates their downstream target proteins including mitogen-activated protein kinases (MAPK), Rho kinases, and phosphoinositide-3 kinases (PI3K), respectively, which modulate the proliferation, differentiation, survival, migration, and apoptosis of cells [33]. In recent decades, the role of ATX-LPA axis has been widely researched in the pathogenesis of several diseases, such as scleroderma fibrosis [40], rheumatoid arthritis [41], cancer [42, 43], and some neoplastic diseases, which are closely related to inflammation [44]. Moreover, numerous researches have explored the therapeutic role of intervention on ATX-LPA axis. Tager et al. [45] demonstrated that ATX-LPA axis contributes to pulmonary fibrosis in bleomycin-induced mice model by modulating the activity of fibroblast, and mice with LPA<sub>1</sub> knocked suffer milder pulmonary fibrosis. In ovarian cancer (OC), ATX-LPA axis promotes the growth of OC cells and inhibits the apoptosis through ATK, ERK, and NF- $\kappa$ B signaling pathways, blocking the pathways that inhibit the proliferation of cells [46]. Hideaki et al. [25] found that ATX-LPA axis is associated with IBD patients and mice models by driving



**Fig. 2.** Expressions of ATX, MAdCAM-1, LPA<sub>2</sub>, and CD34 in colonic tissue. **a** The colonic protein levels of ATX and MAdCAM-1 measured by Western blotting. **b** Gray value of ATX normalized to  $\beta$ -actin. **c** Gray value of MAdCAM-1 normalized to GAPDH. The mRNA expression of **d** ATX, **e** LPA<sub>2</sub>, and **f** MAdCAM-1. **g** IHC ( $\times 400$ ) analysis of CD34. Each bar represents mean  $\pm$  SD ( $n = 8$ ). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , vs. DSS group.

pathological lymphocyte migration to diseased region, and ATX blockade in mice models reduces lymphocyte infiltration. Besides, ATX-LPA axis enhances the secretion of several pro-inflammatory cytokines and the activation of some transcription factors. In airway epithelial cells, LPA promotes the expression and secretion of IL-8, which contributes to lung inflammation [47], and the expression and secretion of IL-6 have been confirmed to be elevated by the promotion of LPA in several diseases including ovarian cancer and oral squamous cell carcinoma [48–

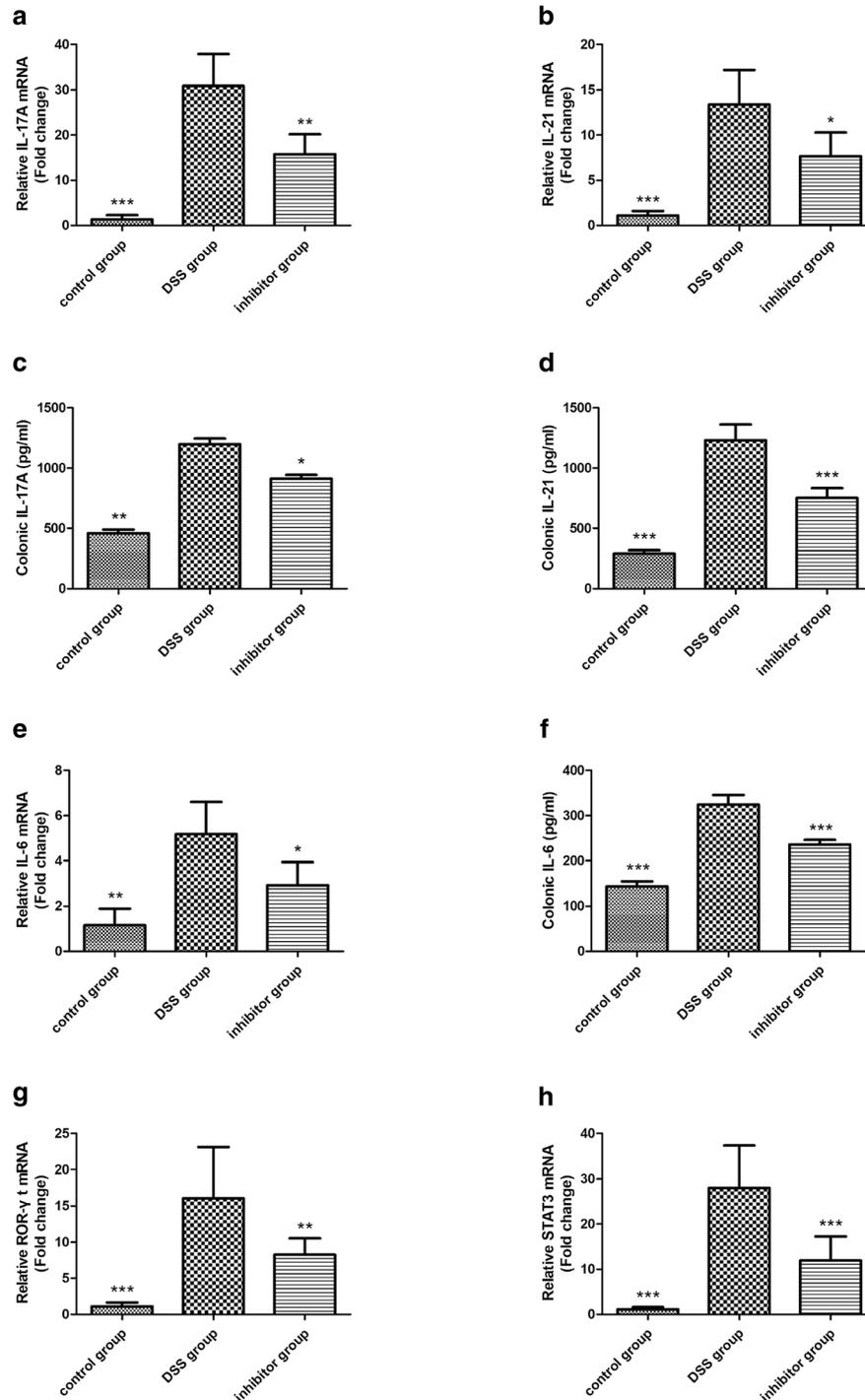
50]. LPA-LPAR signaling increases the abundance of STAT3 through its downstream factor ROCK in the conversion of primed pluripotent stem cells (PSCs) [51] and it induces STAT3 phosphorylation in ovarian cancer cells [52]. STAT3 is a key factor for Th17 cell development in chronic colitis, which is correlated with the impact of IL-6, and T cells with STAT3 deficiency tend to differentiate into regulatory T cells (Tregs) [53]. Considering the role of IL-6 and STAT3 in the differentiation of Th17 cells, we hypothesized that ATX-LPA axis could modulate the



**Fig. 3.** The proportion of Th17 cells in spleen and MLNs. **a** The proportion of Th17 cells in CD4 + T cells in spleen and MLNs detected by flow cytometry. **b** The average proportion of Th17 cells in spleen and MLNs. Each bar represents mean ± SD (n = 8). \*P < 0.05, \*\*P < 0.01, vs. DSS group.

development of Th17 cells in chronic UC. The outcomes were in agreement with our speculation and the

expressions of IL-6 and STAT3 were decreased at the intervention of ATX inhibitor.



**Fig. 4.** Detection of Th17 cells function and signaling pathway in colonic tissue. The mRNA expression of **a** IL-17A and **b** IL-21. The concentrations of **c** IL-17A and **d** IL-21. **e** The mRNA expression of IL-6. **f** The concentrations of IL-6. The mRNA expression of **g** ROR- $\gamma$ t and **h** STAT3. Each bar represents mean  $\pm$  SD ( $n=8$ ). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , vs. DSS group.

Despite the pivotal role of IL-6 and STAT3 on the development of Th17 cells, the effect of ROR- $\gamma$ t cannot be ignored. ROR- $\gamma$ t is mainly expressed in some immune cells, which could enter the nucleus to regulate the transcription of target genes directly, and it is also the essential transcription factor driving the development of Th17 cells [13]. Recently, ROR- $\gamma$ t is regarded as a therapeutic target of autoimmune diseases, especially multiple sclerosis (MS), because of its function in Th17 cell differentiation [54]. To identify the effect of ATX-LPA axis on Th17 cell signaling pathway, we also examined the expression of ROR- $\gamma$ t in colonic tissue. Similarly, DSS administration increased the expression of ROR- $\gamma$ t, whereas when ATX-LPA axis was suppressed, the indicator was decreased. In some studies, TGF- $\beta$  is also regarded as an important cytokine for Th17 cell differentiation. It is indicated that naive T cells exposed to high concentration of IL-6 accompanied by low concentration of TGF- $\beta$  are induced to Th17 cells [6, 15]. However, we did not detect it because of its complicated effects on chronic colitis. Extensive evidences indicate that the ATX-LPA axis plays an important role in tissue fibrosis, mainly by promoting the production of TGF- $\beta$  at a relatively high concentration [40]. Our previous study showed that fibrosis of colon existed in chronic colitis [27], and in this study, the colonic weight to length ratio was obviously raised in chronic UC mice, which also proves the fibrosis of colon in DSS-induced chronic colitis. Thus, the expression of TGF- $\beta$  in our study could not be precisely applied to the analysis of Th17 cell differentiation. However, our results were also able to suggest the role of ATX-LPA axis in Th17 cell signaling pathway.

Furthermore, ATX is widely distributed in many organs and body fluids with low expression [55]. Some evidences showed that ATX is released from the endothelial cells of HEV in the intestine [25, 33]. HEVs are special post-capillary venules found in the lymphoid tissue, which are channels for lymphocytes to enter the lymphatic tissue. In chronic inflammation of the gastrointestinal tract, there is a large number of hyperplasia of HEV-like vessels, the functions of which are the same as HEVs in the function and construction [25]. So, in the pathology of gastrointestinal inflammation, ATX-LPA axis, HEV-like vessel, and Th17 cells work like the amplification loop. Our results showed that during the induction of chronic colitis, inhibition of ATX decreased the expression of LPA<sub>2</sub>, HEV-like vessels, as well as ATX. Meanwhile, with the administration of an ATX inhibitor, the typical symptoms including significant weight loss, activity decrease, severe diarrhea, and bloody stools of the DSS-induced mice were obviously alleviated when compared with the model mice, and so

were the pathologic damage of colon and the expression of MAdCAM-1. Briefly, ATX-LPA axis contributed to the pathogenesis of chronic UC, and ATX blockade could relieve the inflammation.

In summary, our results proved that ATX-LPA axis was involved in the pathological process of DSS-induced chronic colitis, and the potential mechanism of aggravating inflammatory response was associated with promoting Th17 cell differentiation. Thus, ATX inhibition may be a potential therapeutic approach for the treatment of recurrent UC.

## ACKNOWLEDGMENTS

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## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest.** The authors declare that they have no conflict of interest.

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